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Directional scattering from the glossy flower of *Ranunculus*: how the buttercup lights up your chin

Silvia Vignolini^{1,†}, Meredith M. Thomas^{2,†}, Mathias Kolle^{1,‡}, Tobias Wenzel¹, Alice Rowland³, Paula J. Rudall³, Jeremy J. Baumberg¹, Beverley J. Glover^{2,*} and Ullrich Steiner^{1,*}

> ¹Cavendish Laboratory, University of Cambridge, JJ Thomson Avenue, Cambridge CB3 0HE, UK

²Department of Plant Sciences, University of Cambridge, Downing Street,

Cambridge CB2 3EA, UK

³Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond, Surrey TW9 3AB, UK

The bright and glossy appearance of the flowers of *Ranunculus repens* was investigated spectroscopically and the optical results were correlated with the layered anatomy of the petal. The highly directional reflected light arises from the partially transparent, pigment-bearing epidermal layer, while a more diffused yellow colour is the result of scattering from the lower starch layer. This directionality of the light reflections causes the unusually intense gloss of the buttercup flower and the strong yellow reflection evident when holding the flower under the chin.

Keywords: gloss in plants; optical spectroscopy; Ranunculus

1. INTRODUCTION

The buttercup species Ranunculus repens (figure 1a) is widely recognized by its glossy yellow flowers and is commonly known for the children's game of holding a buttercup under the chin: the yellow reflection onto the chin is said to mean that the person likes butter (figure 1b). More seriously, scientific interest has focused on the function of the optical appearance of flowers in plant-pollinator interactions [1-5]. The observation made during the children's game therefore raises interesting questions concerning both the origin and the biological function of the peculiar optical response.

Literature, dating back to 1883 [6] and until relatively recently [7–11], emphasizes the optically striking nature of *Ranunculus* flowers. A detailed study by Parkin in 1928 described the morphology and anatomy of both glossy and non-glossy flowers of *Ranunculus* species [9]. Most flowers have a papillate epidermis [12,13], while many yellow-flowered *Ranunculus* species have a planar non-papillate epidermal surface. Moreover, in glossy species, the adaxial epidermis contains an oily pigment and the underlying adaxial hypodermal mesophyll is densely packed with starch grains [9]. The respective roles in the petal optics of the pigment and starch layers are poorly understood [9–11]. Galsterer *et al.* [7] conducted a careful optical analysis of four *Ranunculus* species. They found gloss across the entire spectral range, independent of anatomical differences in the starch layer, and a pronounced signal in the ultraviolet (UV) region. However, their study stopped short of correlating the optical response with the unique morphological layers of the *Ranunculus* petal, and a detailed experimental characterization of the flower optical response linked with the petal anatomy is still missing.

Here, we analyse the strong directionality of the reflection of light from petals of R. repens. The anatomy of the petal is studied using different electron microscopy techniques and is correlated with spectroscopic measurements. In particular, using angular resolved spectroscopy, we are able to correlate the peculiar optical response of the flower with the anatomical structure of its petals. Our investigation revealing the characteristic light reflection of *Ranunculus* petals is the result of the interplay between directional scattering and pigmentary absorption, similar to that of some bird feathers [14].

The UV reflection of the *Ranunculus* flower was also investigated because it may play an important role in plant–insect signalling [7,15,16] and could have a defensive function in terms of the prevention of tissue damage [17–19].

2. MATERIAL AND METHODS

2.1. Sample preparation

For the optical measurements, *R. repens* plants were cut in the meadows around Cambridge. Stalks were kept in water and measurements were taken within a

^{*}Authors for correspondence (bjg26@cam.ac.uk; u.steiner@phy.cam. ac.uk).

 $^{^{\}dagger} \mathrm{These}$ authors contributed equally to this work.

[‡]Present address: Harvard School of Engineering and Applied Sciences, Cambridge, MA 02138, USA.

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Figure 1. (a) Picture of a R. repens flower. (b) Chin illumination by the flower in ambient sunlight. (Online version in colour.)

few hours after collection. The ability to mount Ranunculus petals onto optically flat surfaces is important for optical microscopy and spectroscopy. The shape of the petals itself is particularly flat and the entire petal can be easily fixed to a glass microscope slide using double-sided tape. For some experiments, the epidermis of a *Ranunculus* petal was peeled off in order to investigate the properties of the individual epidermal layer and to access the properties of the starch layer. In order to maintain the epidermal layer flat and preserve it, the layer was floated on a droplet of water on top of a glass slide. Upon water evaporation, the epidermis was transferred onto the glass substrate. Optical spectroscopy was performed immediately after mounting the petal or petal parts, minimizing dehydration during the measurements.

2.2. Imaging

Optical imaging was performed with a standard Olympus BX-51 optical microscope equipped with a $5\times$ objective (Olympus, MPLFLN-BD 5X). For scanning electron microscopy (SEM) imaging, flowers were fixed in formalin acetic alcohol and stored in 70 per cent ethanol (EtOH). Each specimen was passed through an EtOH series up to 100 per cent EtOH followed by critical-point drying using a Tousimis Autosamdri 815B. Specimen were then mounted on aluminium stubs, coated with platinum using a sputter coater (Emitech K550) and examined under a Hitachi S-4700 SEM at 2 kV. For cross-sectional imaging by transmission electron microscopy (TEM), petals were fixed in Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2) overnight at 4°C. Sections of $1 \times 2 \text{ mm}^2$ were cut, rinsed in buffer and post-fixed in a 1% osmium tetroxide (Agar) phosphate buffer solution for 2 h. They were then rinsed in buffer and dehydrated in absolute ethanol solutions in steps from 30% to 100% EtOH. The material was embedded in resin (Agar) and left for one week at 4°C to allow the resin to infiltrate. The resin was changed daily. The samples were cured under vacuum at 400 mbar for 20 h at 58°C. Thin sections were cut using an ultramicrotome (Reichert-Jung Ultracut) and stained automatically with uranyl acetate and lead citrate using an Ultrostainer (Leica EM Stain) before examination by TEM.



Figure 2. (a) SEM image of an entire Ranunculus petal: in the adaxial epidermis, the cells are flat everywhere. (b) SEM image of the petal adaxial surface in the centre region with high magnification. (c) Light microscopy image of a transverse section of a Ranunculus petal, from the adaxial (top) to the abaxial (bottom) epidermis. (d) TEM transverse section of a single R. repens petal. The double arrows 1 and 2 span the adaxial epidermal and starch layers, respectively. The arrows in (c,d) point to the discontinuous air gap separating the two layers. (Online version in colour.)

2.3. Spectroscopic characterization

Both transmittance and reflectance spectra of Ranunculus petals were measured macroscopically (i.e. by averaging



Figure 3. Light microscopy images obtained with a $5 \times$ objective (numerical aperture: 0.15) in epi-illumination of (a) the entire petal, (b) the epidermal layer and (c) the starch layer. (d,e) The BF and DF spectra corresponding to (a-c). (d,e) Red solid line, entire petal; black solid line, epidermal layer; green solid line, starch layer. (Online version in colour.)

over a 1 mm^2 area) and on the microscopic scale (spot size: approx. 20 µm). The macroscopic spectroscopic measurements were performed with an angular resolved set-up, schematically shown in figure 5a. Sample and detector stages were rotated in 1° steps, allowing independent adjustment of the incidence and detection angles. Spectrally accessible wavelengths ranged from 250 to 850 nm (light source: Ocean Optics DH-2000, 215–2000 nm and USB spectrometer: Ocean Optics QE65000, 200-880 nm). In particular, the light from the lamp was coupled into a 1000 µm core-size multimode fibre, collimated and used to illuminate the sample at a fixed angle (set by sample stage rotation). The scattered/reflected light from the sample was then collected in the detection arm, focused onto another $1000 \,\mu\text{m}$ core-size optical fibre coupled to the spectrometer. Macroscopic measurements at normal incidence were instead obtained with a Standard Reflection/Backscattering Probe (Ocean Optics) connected to the light source and to the spectrometer. The spectra were normalized with respect to a white Lambertian reflectance standard (Lab-sphere Spectralon SRM-99).

The microscopic characterization was carried out in confocal configuration using a modified optical microscope (Olympus BX-51). Reflectance and transmittance measurements were obtained with a confocal microscope both in bright field (BF) and dark field (DF) configurations. The microscope halogen lamp (spectral range: 400–800 nm) served as light source and a 50 μ m core optical fibre mounted in the conjugate plane of the objective focal plane acted as a confocal pinhole. A 5× objective (Olympus, MPLFLN-BD 5X, numerical aperture: 0.15) was used. The BF spectra were normalized with respect to an aluminium mirror and the DF spectra were normalized with respect to the white Lambertian reflectance standard.

3. RESULTS

The structure of the *Ranunculus* petal is shown in figure 2. The SEM images in figure 2a, b show the tile



Figure 4. Pictures of the *Ranunculus* petals taken with a quartz lens camera (a) without filters and (b) with a bandpass filter that transmits only UV light. The arrows point to glossy spots on the petals. (c) Reflection spectra of different petals of the same flower at normal incidence ($\theta_{\rm D} = -\theta_{\rm I}$). (Online version in colour.)

pattern of predominantly flat (non-papillate and nonstriated) cells on the epidermal surface. The transverse sectional images in figure 2c, d visualize the layered structure of the petal. Glossy *Ranunculus* petals generally consist of three layers of mesophyll parenchyma cells enclosed by an adaxial and abaxial epidermis, interspersed with vascular bundles (figure 2c). In particular, the pigment-bearing epidermal layer above the mesophyll is approximately 5 µm thick, and it is covered with a cuticle that has homogeneous thickness of about 500 nm. This layer is separated by an approximately $0.5 \,\mu$ m wide discontinuous air gap from an approximately 7 µm thick starch layer. A similar morphology has been reported in earlier studies [8], but has not been properly related to the flower's optical behaviour.

3.1. Microscopy

Ranunculus petals were optically characterized in three configurations: (i) the entire petal, (ii) the peeled-off epidermal layer, and (iii) the underlying starch layer. BF reflection microscopy images are shown in figure 3a-c. This image series clearly demonstrates that the yellow pigment is contained within the epidermal layer (figure 3b). Figure 3c shows the colourless appearance of the starch layer. The BF and DF spectra of the three images are shown in figure 3d, e, respectively. The UV part of the spectrum was not measured in this configuration. The white starch layer yields a low intensity in the normal (specular) direction and shows instead the properties of a good quality broadband white diffuser. The intact petal and the epidermal layer have high BF reflectivity down to approximately 520 nm, where the absorption of the carotenoid pigment sets in [20]. In figure 3d, e (since the imaged spot-size was comparable with the size of a single cell), some of the observed oscillations stem from thin layer interference coming from the epidermal

layer (see also electronic supplementary material). Finally, the DF spectrum of the epidermal layer confirms the transparency of this layer above 500 nm and the absence of significant scattering in to the 16° cone-angle of the DF objective.

3.2. Ultraviolet signature

Figure 4 investigates the spectral reflectivity of the Ranunculus flower including the UV region. In particular, figure 4a shows a picture of a group of *Ranunculus* flowers obtained with a camera equipped with a quartz lens objective. The same camera equipped with a UV band pass filter (Baader U-Filter HWB 325–369 nm) was then used to take the photograph in figure 4b. The bright spots in both pictures correspond to the specular reflected light from the sun into the camera (indicative of the glossy appearance of the petal). In figure 4c, we show the spectra (obtained on a macroscopic area at normal incidence) of four different petals from the same Ranunculus flower. The reflectivity of different petals of the same flower is similar. The reflectivity in the UV region (250-400 nm) is comparable with that in the 550-800 nm range. The dip in reflectivity in the approximately 400-500 nm wavelength band arises from carotenoid pigment absorption [20].

3.3. Spectroscopic measurements

The detailed interplay of reflection and scattering from the three samples is better characterized by a spectroscopic measurement, which allows both illumination and detection angles to vary (figure 5*a*). The measurements shown in figure 5b-e were obtained by illuminating the sample at an angle $\theta_{\rm I} = -45^{\circ}$ and varying the detection angle $\theta_{\rm D}$ between -90° and 20° . Note that $\theta_{\rm D} = 45^{\circ}$ is the specular direction and that gloss corresponds to a high signal at this angle. The



Figure 5. (a) Schematic of the optical goniometer set-up used in the experiment. Angle-resolved measurements of (b) the entire *Ranunculus* petal, (c) the epidermal layer and (d) the starch layer. For comparison, (e) shows the angular resolved measurement for a yellow garden rose (for greater than 400 nm). (Online version in colour.)

off-specular signal at all other angles arises from the light scattered from the petal or its individual layers.

Figure 5c, d confirms the results of figure 3. The epidermal layer in figure 5c exclusively shows broadband specular reflection above 520 nm with a marginal amount of background scattering.

The starch layer in figure 5d behaves as a characteristic white scattering medium, yielding a nearly homogeneous signal level across all wavelengths and angles.

By comparison, we report the spectral response of a petal of a yellow rose representing generic, non-glossy yellow flowers (figure 5e). As in the majority of flowers, the adaxial epidermis of the rose is characterized by conical papillate cells [13,21] (electronic supplementary material). For the rose, we observe a diffusive scattering of light without an increase in signal in the specular direction.

3.4. Directional reflection

The redistribution of the signal in space is more clearly visualized in figure 6a, where the scattered intensity is averaged over the 500–600 nm interval and plotted in polar coordinates as a function of the detection angle. The pointed narrow shape of the epidermal layer is characteristic of specular reflection, while the nearly semi-circular shape of the starch layer is indicative of scattering.

Before discussing the signal of the entire *Ranunculus* petal, it is instructive to make a comparison with a wellunderstood optical medium: coated and uncoated white paper (figure 6b). The optical signature of uncoated white paper is very similar to the *Ranunculus* starch layer. Paper can be made glossy by coating it with a transparent layer. The resulting optical signature in figure 6b is a direct superposition of the pointed trace of specular reflection and the semicircular scattering curve of uncoated paper at the centre of the graph. The *Ranunculus* petal shows a similar spectral trace (figure 6a,b) caused by the presence of the epidermal layer on top a diffusive material. In contrast, within the 500–600 nm interval, the rose in figure 6b,d has the optical signature of a good scatterer, similar to the *Ranunculus* starch layer or uncoated white paper.

Reflectivity was further investigated in the specular direction by simultaneously varying $\theta_{\rm D} = -\theta_{\rm I}$ in figure 6c, d, averaging again over the 500–600 nm wavelength band. Figure 6 compares *Ranunculus* petal reflection with the two paper types and the yellow rose. The starch layer and the rose exhibit similar flat responses, which are characteristic of the multiple scattering process as that shown by the white paper. The *Ranunculus* petal shows a similar gloss to the coated paper.

4. DISCUSSION

The results of figures 1-4 paint a clear picture of the *Ranunculus* optical response, schematically shown in figure 7*a*. The gloss arises from the highly transparent, pigment-bearing adaxial epidermal layer. The overall specular signal has two contributions: (i) the reflection of the planar top (pigment-free) cuticle layer and (ii) a second yellow reflection from the lower interface of the epidermal layer.

The yellow colour of the specular signal arises from the fact that the light path (ii) traverses the carotenoid pigment-bearing epidermal layer twice, where light in the 400–500 nm range is strongly absorbed. The air gap separating the epidermal from the starch layer is instrumental for the high directional reflectivity because it provides a second planar interface from which light is specularly reflected. The overall reflectivity from the epidermal layer is approximately 0.04.

The light transmitted through the epidermal layer (approx. 80%) enters the starch layer, which is a





Figure 6. (a) Reflectivity averaged over the 500–600 nm wavelength range of the entire Ranunculus petal, the epidermal layer and the starch layer. (a,c) Red circle, entire petal; black circle, epidermis; green circle, starch layer. The illumination angle was 45° and the radial intensity scale uses arbitrary units. (b) Averaged reflected intensity (on a logarithmic scale) of the entire Ranunculus petal compared with uncoated and coated paper and a yellow rose ((b,d) red circle, Ranunculus; blue circle, rose; blue solid line, coated paper (/10); magenta solid line, uncoated paper) for an illumination angle of 45°. (c,d) Averaged reflected intensity in the specular direction as a function of angle. The intensity of the coated (glossy) paper is divided by a factor of 10 in (b,d). (Online version in colour.)



Figure 7. (a) Schematic of the *Ranunculus* light response. (b) Sketch of the mechanism responsible for the chin-illumination effect. (Online version in colour.)

broadband white-scattering film. Its backscattering efficiency is approximately 0.6 when compared with a white standard.

Overall, this leads to the *Ranunculus* spectral distribution of figures 5 and 6 consisting of (i) broadband specular reflection from the clear cuticular surface, (ii) a yellow specular reflex stemming from light reflecting from the epidermal interface with the air gap, and (iii) a yellow diffuse background of the backscattered light from the starch layer. The two latter contributions traverse the carotenoid epidermal layer twice, thereby giving rise to the intense yellow appearance of the flower.

The interplay of back-reflected light from the two layers explains the striking colour appearance of *Ranunculus*. Although the overall back-reflected light, when measured with an integrating sphere, of *Ranunculus* and the rose do not differ (in the wavelength region above 500 nm), the *enhancement* of the intensity in the specular direction gives rise to the exceptionally bright and glossy appearance by which the buttercup is recognized. See electronic supplementary material for the integrating sphere measurement.

This interpretation finally provides an explanation for the chin-illumination game, schematically indicated in figure 7b. When held correctly, the directionality of back-reflection efficiently redirects the incident light, generating an intense yellow spot that is visible even on a relatively poor screen such as the human skin.

It is likely that the spectral features of *Ranunculus* fulfill a similar purpose as other striking optical signatures of petals in attracting animal pollinators. The directionality of light reflection creates a dynamic intensity pattern that could potentially be recognized by pollinators. The reflected light would appear as a flash, which could initially capture their attention. Additionally, brightly illuminated buttercup flowers are warmed by the Sun and could act as basking spots; bees are attracted to warmer flowers, which offer the best chance of a metabolic reward [5].

5. CONCLUSIONS

Our study provides a detailed correlation between the *Ranunculus* petal structure and its optical signature. Although several aspects of the Ranunculus gloss and its spectral response have been reported before, this study shows experimentally the detailed optical interplay of the layers in the petal that produces the characteristic *Ranunculus* optical signature. The salient features of the buttercup spectral response are well reproduced by a transfer matrix calculation (electronic supplementary material), but an optical model of complex petal structure is still missing. The semitransparent, pigment-bearing epidermal layer that is bounded by two planar surfaces, the paper-white starch layer and the air-gap between these two layers yield a combined optical response that is highly directional. This directionality of reflection is responsible for the intense gloss and the chin-illumination game, both of which have intrigued scientists and laymen alike for centuries.

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