Supplementary material

Imaging scatterometry

To visualise the spatial reflection properties of the flowers, we examined freshly cut flower petals with an imaging scatterometer (Stavenga *et al.*, 2009). A piece of petal was therefore glued to a glass micropipette mounted on a micromanipulator, which was used to position the petal piece in the focal point of the scatterometer. A narrow-aperture (<5°) illuminating beam provided spectrally broadband, white light, focused on a circular area with diameter ~30 µm. The hemispherically reflected light from the petal was recorded by the scatterometer's camera, an Olympus DP70 (Olympus, Tokyo, Japan).

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, $m = 2$, $\sigma = 0$ 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, $m = 2$, $\sigma = 100$ 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

Light propagation in a stack of n layers, where each layer $i = 1, 2, \ldots n$ has a characteristic reflectance r_i and transmittance t_i for both forward incident light I_i and backward incident light J_i, can be described by (Yamada & Fujimura, 1991)

$$\begin{pmatrix} I_{i+1} \\ J_{i+1} \end{pmatrix} = M_i \begin{pmatrix} I_i \\ J_i \end{pmatrix}, \text{ with } M_i = \frac{1}{t_i}\begin{bmatrix} t_i^2 - r_i^2 & r_i \\ -r_i & 1 \end{bmatrix} \quad (1)$$

For the total stack of n layers

$$\begin{pmatrix} I_{n+1} \\ J_{n+1} \end{pmatrix} = M_s \begin{pmatrix} I_1 \\ J_1 \end{pmatrix}, \text{ with } M_s = M_n M_{n-1} \ldots M_1 = \begin{bmatrix} m_{11} & m_{12} \\ m_{21} & m_{22} \end{bmatrix} \quad (2)$$

When a unit light flux is only incident from one side $I_1 = 1$ and $J_{n+1} = 0$, and the stack reflectance and transmittance then are

$$R = J_1 = -m_{21}/m_{22} \qquad \text{and} \qquad T = I_{n+1} = m_{11} + m_{12}R \qquad (3)$$

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 slighty reflecting layer. The reflectance and transmittance (*r* and *t*) of tissues that scatter and contain pigment can be derived from Kubelka-Munk theory (Kubelka & Munk, 1931) when the tissue's thickness (*d*) as well as the scattering (*S*) and absorption (*K*) coefficients are known (Yamada & Fujimura, 1991)

$$r = (S^* \sinh B^*)/(A^* \sinh B^* + B^* \cosh B^*) \qquad (4a)$$

$$t = B^*/(A^* \sinh B^* + B^* \cosh B^*) \qquad (4b)$$

where $S^* = Sd, \qquad K^* = Kd, \qquad A^* = S^* + K^*, \qquad B^* = \sqrt{K^{*2} + 2K^*S^*}$ (4c)

In the absence of scattering ($S = 0$), the reflectance $r = 0$ and the transmittance $t = \exp(-K^*)$, which is Lambert-Beer's law. Without pigment ($K = 0$) it follows that $r = S^*/(1 + S^*)$ and $t = 1/(1+S^*)$. We calculated the reflectance and transmittance spectra of the buttercup *R. acris* with the above combined Kubelka-Munk-layer-stack model (Fig. 6d).

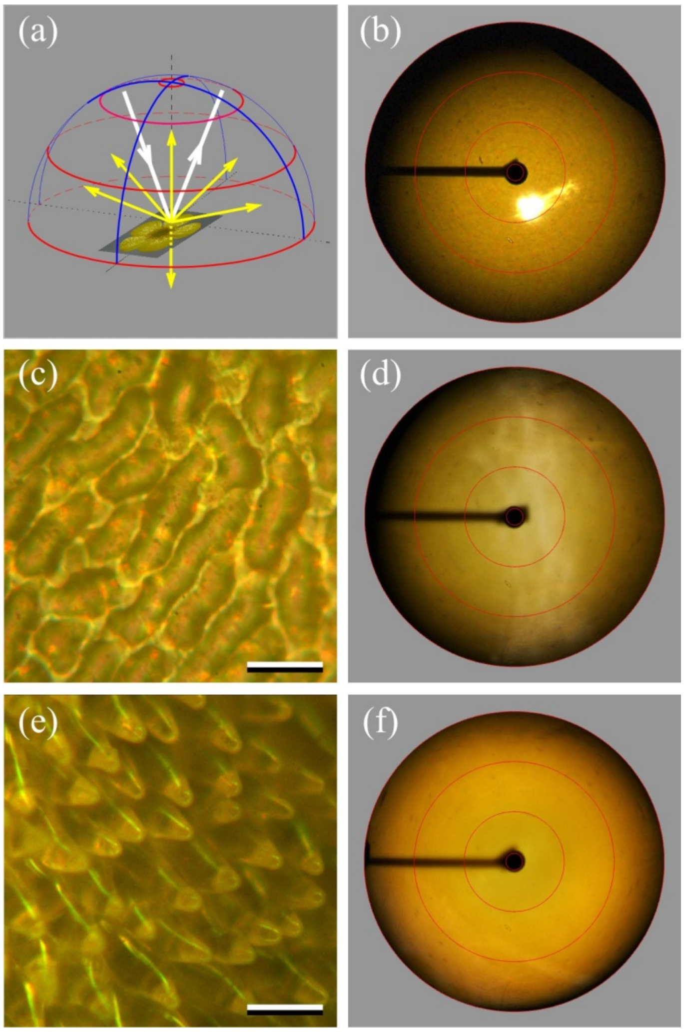

Fig. S1. Imaging scatterometry of the buttercup *Ranunculus acris* and the kingcup *Caltha palustris*. (a) Diagram of a reflecting and scattering buttercup petal. Incident light is partly specularly reflected (white arrows) and diffusely scattered and transmitted (yellow arrows). (b) Scatterogram of 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) and (b) indicate angular directions of 5, 30, 60 and 90º; the black bar at 9 o'clock in (a) is caused by the sample holder and the central black circle with angular size ~5º is due to a central hole in the ellipsoidal mirror of the scatterometer (for details see Stavenga *et al.*, 2009).

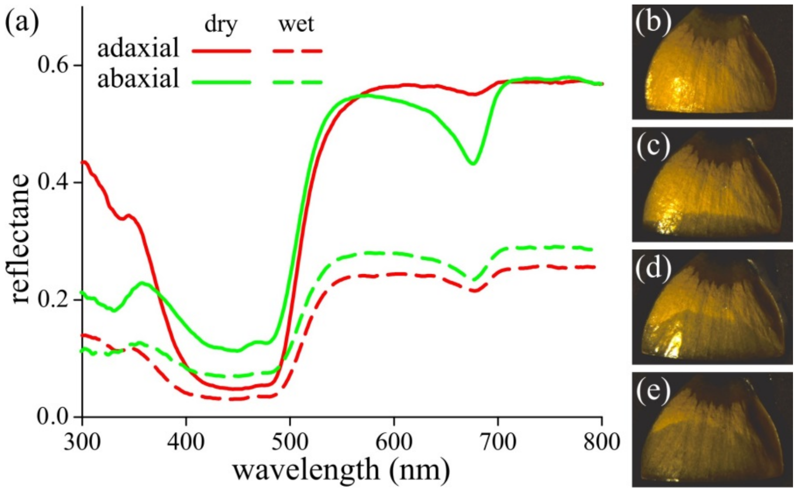

Fig S2. Reflection changes induced by wetting cut petals. (a) Reflectance spectra measured with an integrated sphere from the adaxial and abaxial sides of a dry and fully internally wetted *R. lingua* petal. (b-e) Progressive capillary water uptake by a cut petal of *R. repens* after putting a water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

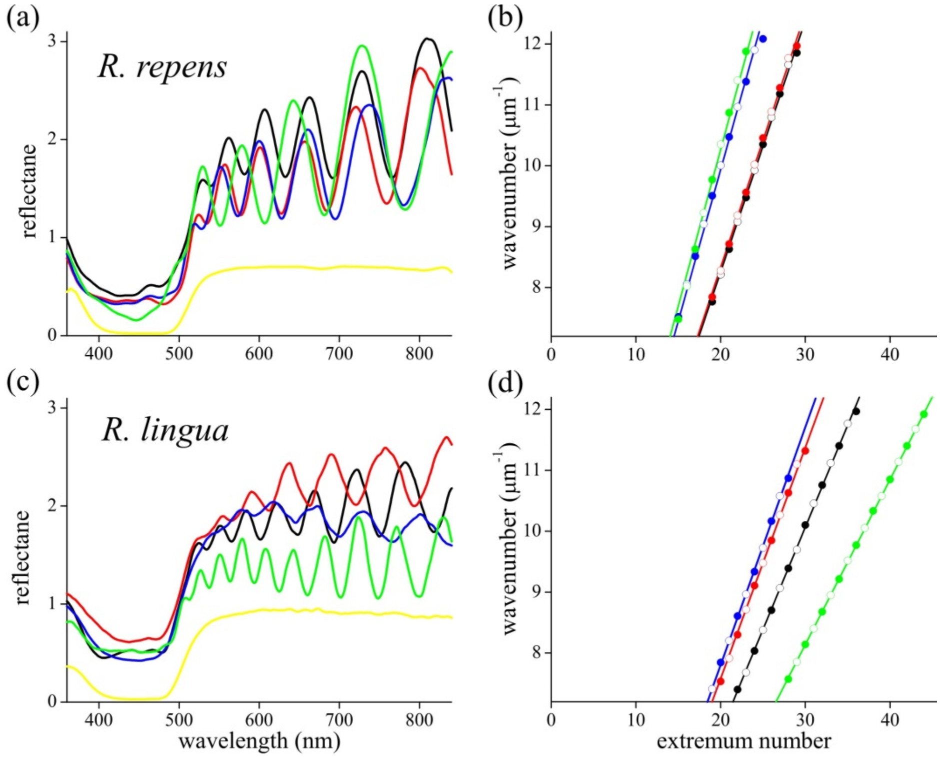

Fig. S3. Reflectance spectra measured in the main distal area of buttercups with a microspectrophotometer (MSP) and wavenumber values of the oscillation extrema. (a, b) *Ranunculus repens*; (c, d) *R. lingua*. The oscillating spectra are from areas with gloss. The yellow curves were obtained from matte areas, that is, where the surface reflections were outside the aperture of the MSP's objective. The reflectance spectra were measured relative to a white diffuser. The linear fits to the extrema wavenumbers yielded thickness values of the upper epidermis (see Materials and Methods).

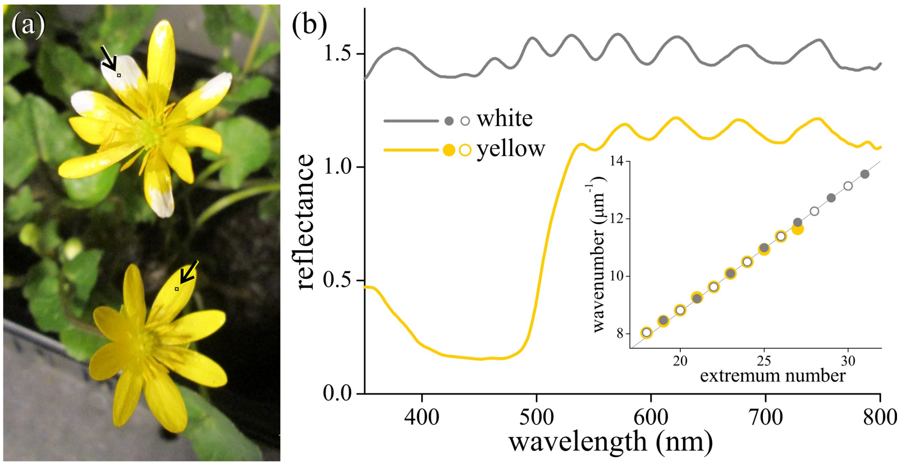

Figure S4. Thin film optics of the upper epidermis of *Ficaria verna*. (a) Two *F. verna* flowers, the upper flower having petals with white areas. (b) Reflectance spectra measured with an MSP from a small area of a white and yellow petal area (small squares indicated by arrows in panel a). Inset: the wavelengths of the reflectance extrema converted into wavenumbers (closed symbols: maxima; open symbols: minima) fitted with a linear function, which yields a thin film thickness of ~2.6 μm.

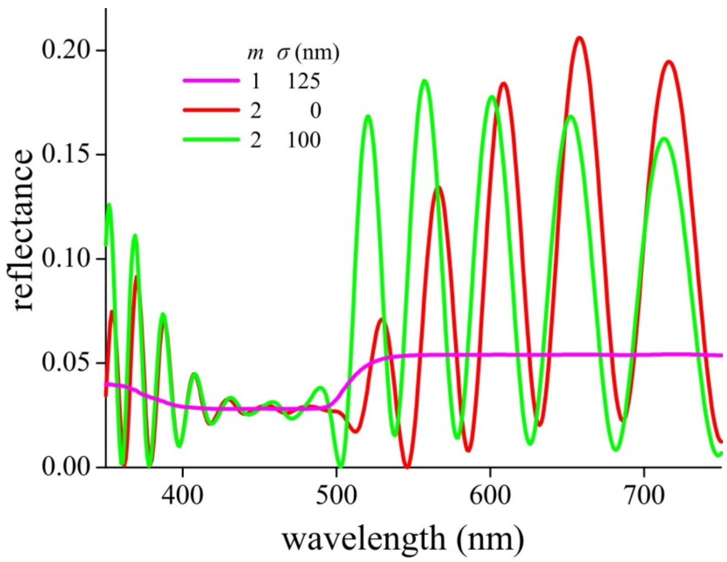

Fig. S5. Reflectance spectra of: *i*) a thin film (m = 1) with Gaussian varying thickness (σ = 125 nm) and mean thickness 2.7 µm; *ii*) a multilayer consisting of a thin film with fixed thickness 2.7 µm and an air gap with thickness 5.0 µm facing an infinite (starch) layer with refractive index 1.36 (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).

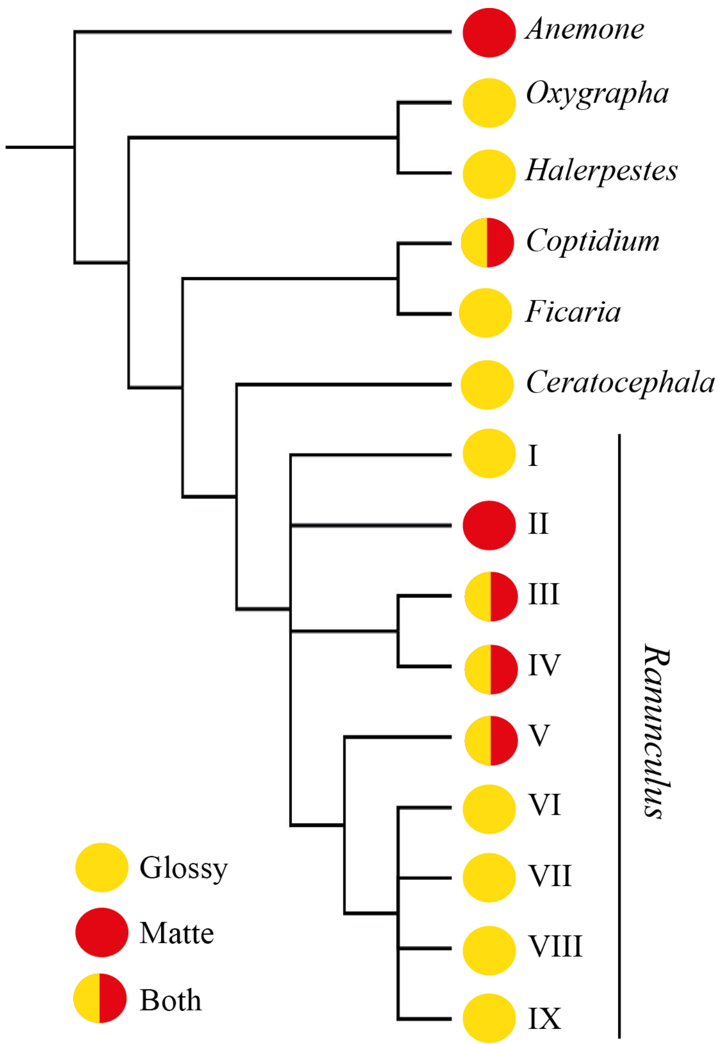

Fig. S6. Phylogenetic reconstruction of glossy flowers in *Ranunculus* and related genera. Species with glossy flowers largely outnumber those with non-glossy, matte flowers. In several taxa or clades glossiness was lost. Information on petal glossiness was obtained from Parkin (1928), Lauber and Wagner (2001) and personal observations. Phylogeny and *Ranunculus* section names (denoted by Roman numerals) are taken from Hörandl and Emadzade (2012). I: Thora; II: Acanitifolii, Epirotes, Leucoranunculus, Ranuncella; III: Batrachium, Hecatonia, Pseudanonis; IV: Auricomus; V: Flammula; VI: Ranunculus; VII: Echinella, Trisecti; VIII: Polyanthemos; IX: Ranunculastrum.

References

Hörandl E & Emadzade (2012) Evolutionary classification: a case study on the diverse plant genus *Ranunculus* (Ranunculaceae). *Persp. Plant Ecol. Evol. Syst.* 14:310-324.

Lauber K & Wagner G (2001) Flora Helvetica. Aarau: Haupt Verlag.

Kubelka P & Munk F (1931) Ein Beitrag zur Optik der Farbanstriche. *Zeit. Techn. Phys.* 12:593-601.

Parkin J (1928) The glossy petals of *Ranunculus*. *Ann. Bot.* 42:739-755.

Stavenga DG, Leertouwer HL, Pirih P, & Wehling MF (2009) Imaging scatterometry of butterfly wing scales. *Opt. Exp.* 17:193-202.

Yamada N & Fujimura S (1991) Nondestructive measurement of chlorophyll pigment content in plant leaves from three-color reflectance and transmittance. *Appl. Opt.* 30:3964-3973.