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Iridescent flowers? Contribution of surface structures to optical signaling

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Introduction

Many plants display bright flowers to distinguish themselves from their environment, thereby providing a strong signal for potential pollinators (Schiestl & Johnson, 2013; Shrestha *et al.*, 2013; Renoult *et al.*, 2014). Floral coloration is a result of light scattering by irregularly structured cell complexes and wavelength-selective absorbing pigments (Fig. 1a). The (incoherently) scattered light in the complementary wavelength range thus determines the color of the petals. For example, flowers with the blue-absorbing carotenoids are yellow, and flowers with blue-green-absorbing anthocyanins are purple (Grotewold, 2006; Lee, 2007). Pigmentary coloration commonly results in an angle-independent, diffuse distribution of the reflected light.

In addition to this pigmentary coloration, structural coloration can occur when structures exist that are (quasi-)regularly patterned with distances in the submicrometer range, that is, of the order of the light wavelength, causing coherent scattering (Kinoshita *et al.*, 2008). Structural coloration is common among animals, specifically birds, butterflies and beetles. The multilayer structures in *Morpho* butterfly scales, in beetle elytra, and in the barbules of bird of paradise feathers create striking iridescences; that is, the color shifts when the angle of illumination or

Summary

- The color of natural objects depends on how they are structured and pigmented. In flowers, both the surface structure of the petals and the pigments they contain determine coloration. The aim of the present study was to assess the contribution of structural coloration, including iridescence, to overall floral coloration.
- We studied the reflection characteristics of flower petals of various plant species with an imaging scatterometer, which allows direct visualization of the angle dependence of the reflected light in the hemisphere above the petal. To separate the light reflected by the flower surface from the light backscattered by the components inside (e.g. the vacuoles), we also investigated surface casts.
- A survey among angiosperms revealed three different types of floral surface structure, each with distinct reflections. Petals with a smooth and very flat surface had mirror-like reflections and petal surfaces with cones yielded diffuse reflections. Petals with striations yielded diffraction patterns when single cells were illuminated. The iridescent signal, however, vanished when illumination similar to that found in natural conditions was applied.
- Pigmentary rather than structural coloration determines the optical appearance of flowers. Therefore, the hypothesized signaling by flowers with striated surfaces to attract potential pollinators presently seems untenable.

observation changes (Srinivasarao, 1999; Vukusic & Sambles, 2003; Kinoshita *et al.*, 2008).

Some plants have leaves and seeds containing periodic structures, resulting in iridescence (Lee, 2007; Vignolini *et al.*, 2012a). Of specific interest are the highly structured surfaces of flower petals, which are produced by cells with regular striations (ridges) of the cuticle or conically shaped epidermal cells (Kay *et al.*, 1981). These structures can create iridescence, which has been reported to act as a cue for pollinators (Whitney *et al.*, 2009; Fernandes *et al.*, 2013). Furthermore, smooth petal surfaces create a glossy appearance, substantially adding to the flowers' visibility (Galsterer *et al.*, 1999; Vignolini *et al.*, 2012b,c). However, the relative contribution of surface reflection to the overall optical signaling of flowers has not been studied in detail.

Because of the interesting possibility of iridescence being a potential signal for pollinators, we wanted to make a quantitative assessment of the contribution of the surface structure of floral elements to the optical signal of the whole flower. Specifically, we investigated which floral surface structures produce iridescent signals and, if present, we assessed the contribution of iridescence to the optical signaling of the whole flower. A growing number of studies report floral iridescence; however, the iridescent signal is often not compared with the overall floral reflection. This is the

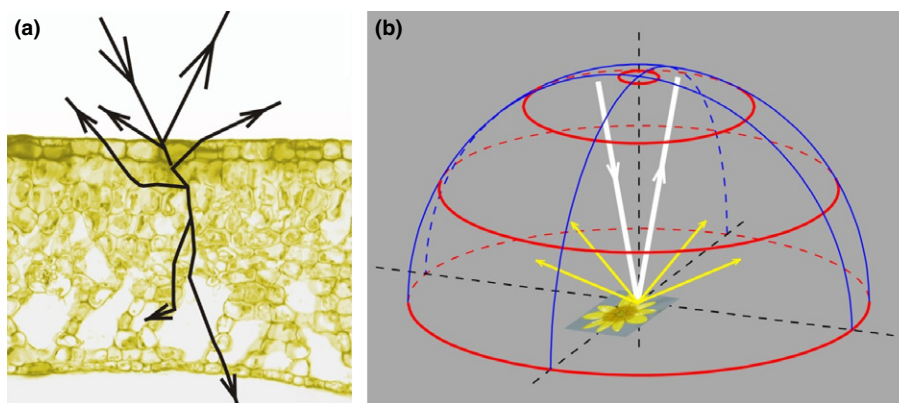


Fig. 1 Directional and diffuse reflection of light by a flat and smooth flower. (a) Simplified diagram of the propagation of incident light in a flower petal. Part of the light is reflected by the flat surface, but reflections and refractions inside the petal at the boundaries of the irregularly arranged petal cell components result in diffusely scattered light. (b) Diagram showing a *Ranunculus ficaria* flower petal of which a small area is illuminated with a narrow-aperture white-light beam. The flat surface reflects the incident light directionally, but the inner components of the petal scatter the light. The reflected and scattered light is imaged with the scatterometer as a polar plot where the angular directions of 5, 30, 60 and 90° are indicated by red circles (see Fig. 2, columns 3, 4).

first study of plant coloration which visualizes the light scattering in the complete hemisphere above a flower, providing insight into the relative contributions of surface and petal interior to flower color.

For our survey, we investigated a large number of flowers across different flower families. We selected a set of flowers with distinct petal surfaces: smooth, conically shaped, and striated. We measured the angle-dependent reflection of the selected flowers with an imaging scatterometer, which allows the immediate visualization of the difference in the spatial distribution of the structural and pigmentary colorations (Stavenga *et al.*, 2009, 2011). To separate the contribution of the flower surface to the total petal reflection, we also investigated casts of the petal surfaces with the scatterometer. We thus found that surface structures can create a minor optical signal, but a significant contribution of iridescence could not be detected. Iridescence is thus unlikely to be a component of optical signaling by flowers to attract potential pollinators.

Materials and Methods

Plant material

Flower samples from 50 plant species from 17 families (see Supporting Information Table S1) were either locally obtained from meadows around Groningen, the Netherlands, or bred from seeds (obtained from Cruydt-Hoeck, Nijeberkoop, the Netherlands), grown in a glasshouse (day : night temperature, 22°C : 17°C; light : dark, 12 h : 12 h) and watered daily. Adaxial and abaxial surface structures were investigated using casts. We selected four species with different surface structures: the lesser celandine buttercup, *Ranunculus ficaria* L. (Ranunculaceae), wild chamomile, *Matricaria chamomilla* L. (Asteraceae), the common daisy, *Bellis perennis* L. (Asteraceae), and Venice mallow, *Hibiscus trionum* L. (Malvaceae). Samples were photographed with a Nikon D70 digital camera equipped with an AF Micro-Nikkor (60 mm, f2.8) macro objective (Nikon, Tokyo, Japan).

Casts

To replicate the adaxial and abaxial surfaces of the investigated floral elements, the floral element was pressed gently into dental-impression medium of low viscosity (Provil novo; Heraeus Kulzer GmbH, Hanau, Germany). The floral element was peeled off as soon as the impression material had polymerized, leaving a negative image of the surface structures in the impression material. The resulting mold was filled with transparent nail polish and set to dry for at least 5 min. This generated a positive replica of the surface structure, that is, a cast. The quality of the cast was visually compared with the structure of the flower. No differences between the surface structure of the fresh flowers and the casts were observed.

Imaging scatterometry

Flower casts as well as freshly cut flower pieces were examined with an imaging scatterometer (Stavenga *et al.*, 2009; Vukusic & Stavenga, 2009; Wilts *et al.*, 2009). The scatterometer allows capture of the hemispherically reflected light from an object (Fig. 1b; for details, see Fig. S1). The scatterograms shown were obtained with a primary and a secondary beam, both providing spectrally broad-band, white light. The primary beam has a narrow aperture (< 5°) and focuses light onto a circular area on the object (diameter of the illumination spot: 13, 40 or 140 μm). The directional illumination of the primary beam thus is similar to that of a bright sunny day. The scatterometer's secondary beam provides full hemispherical illumination (aperture 180°) as with the omnidirectional illumination of an overcast day.

Scanning electron microscopy (SEM)

Fresh flowers contain vacuoles with water and thus do not withstand the vacuuming process necessary for scanning electron microscopy (SEM), and therefore casts of the petal surface structures were investigated, using Philips XL-30S and XL-30

ESEM scanning electron microscopes (Philips, Eindhoven, the Netherlands). Before imaging, the casts were sputtered with gold to prevent charging effects.

Reflectance spectra

Reflectance spectra of different floral elements were measured with a bifurcated optical probe. In addition, the reflectance spectra as a function of the angle of light incidence were determined with a set-up consisting of two optical fibers, positioned at two separate, co-axial goniometers. Generally three to five spectra were measured from different petal areas, which demonstrated that the shape of the measured spectra varied negligibly; the amplitude varied with the location, but by no more than 10%. The spectrometer was an Avaspec-2048 spectrometer (Avantes, Eerbeek, the Netherlands), the light source was a deuterium-halogen lamp (Avantes AvaLight-D(H)-S), and a white diffuse tile (Avantes WS-2) was used as reference.

Results

We investigated the flowers of 50 plant species from 17 families. The structures of their petal or ligule surfaces are presented in Table S1. We distinguished three surface types: smooth, conically shaped, and striated (Kay *et al.*, 1981; Lee, 2007). The surface of the conically shaped cells was mostly flat, but sometimes the cone surface featured ridges. The spacing of the striations (ridges) was regular (25% of cases) or irregular (17%; see Table 1). The regularly spaced ridges were oriented longitudinally (20%) or transversally (5%). The average distance between the regularly spaced ridges on both cones and flat cells varied between species (range 0.9–3.0 μm ; Table S1). Notably, for all striated flowers, the ridge periodicity varied within the same petal; only very locally grid-like structuring of the striations could be observed.

We selected four species with different surface structures, for which we determined the reflection characteristics in detail: *R. ficaria*, which has yellow petals with a smooth, flat surface (Fig. 2a,b); *M. chamomilla*, where the white ligules have cones that are covered by ridges (Fig. 2e,f); *B. perennis*, which also has white ligules but with longitudinal wrinkles striated transversally (Fig. 2i,j); and *H. trionum*, which has deep-red colored petal areas with similar longitudinal protrusions, striated longitudinally (Fig. 2m,n; the white petal parts have a smooth surface). Scatterograms showed the spatial reflection characteristics of the flower casts and the intact petals or ligules (Fig. 2, columns 3 and 4, respectively). Furthermore, we measured the reflectance spectra

Table 1 Frequency of different forms of epidermal cells and structure of cuticles (summary of the results listed in Supporting Information Table S1)

Surface type	Frequency (%)	Ridges		
		No	Irregular	Regular
Smooth, flat surface	23	–	–	–
Cones	35	18	9	8
Ridges, flat surface	42	–	17	25

of the flowers (Fig. 3). The spatial and spectral properties are discussed in the following sections.

Smooth petal surface of *Ranunculus ficaria*

The flowers of *R. ficaria* have yellow petals with a distinct gloss (Fig. 2a), suggesting a flat surface. Indeed, visual observation of the fresh material as well as SEM of casts revealed a very smooth surface (Fig. 2b). Accordingly, illuminating an area with diameter 13 μm (i.e. approximately one cell) on a petal cast with a narrow-aperture light beam yielded a scatterogram with a single dot (Fig. 2c), similar to a mirror (Stavenga *et al.*, 2009). The intact petal similarly yielded a bright spot (Fig. 2d), although slightly larger, presumably as a result of reflections at the surface summated with reflections from more or less parallel layers beneath the surface (Vignolini *et al.*, 2012c). The scatterogram of the fresh material showed in addition a wide-field yellow light distribution (Fig. 2d), representing diffuse scattering, as a result of light scattered by the inhomogeneities inside the petal, which are filtered by a short-wavelength-absorbing pigment (Fig. 1a). Reflectance spectra of the petals measured with a bifurcated probe indicated the presence of a blue-green-absorbing pigment, probably a carotenoid (Fig. 3).

Conically shaped surface cells of *Matricaria chamomilla*

The white ligules of *M. chamomilla* have conically shaped surface cells covered by ridges (Fig. 2f). The scatterogram of the cast showed that the surface with cones reflected the narrow-aperture incident beam into a wide angular space (Fig. 2g). The multicolored annulus with a large angular radius (at *c.* 60°) was presumably a result of diffraction effects at the more or less regularly arranged ridges adorning the cones. The central maximum represented reflections at the somewhat flattened cone top (Fig. 2f). The scatterogram of the intact ligule also showed a spatially wide-field light distribution, but here local intensity maxima occurred, which strongly varied upon slight changes of the illumination area (Fig. 2h). The scatterogram indicated rather diffuse scattering, where the spatial unevenness was created by the inhomogeneities of the cells within the ligule together with the irregularly arranged conical cells of the surface layer. The ligules appear white to the human eye, in agreement with the reflectance spectrum of Fig. 3. The low reflectance in the ultraviolet wavelength range suggests the presence of an (unidentified) UV-absorbing pigment.

Striated ligules of *Bellis perennis*

The white ligules of *B. perennis* (Fig. 2i) have distinct furrows, created by cylindrically curved cells, which have transversal striations. The striations were quite regularly spaced with ridge distances of $d = 1.0 \pm 0.1 \mu\text{m}$ (Fig. 2j, Table S1). Local illumination of the vertically oriented ridges created a scatterogram with a striking, horizontal diffraction pattern (Fig. 2k). The diffraction pattern was spread vertically, as a result of the curved surface. For light with a wavelength $\lambda = 500 \text{ nm}$, the first-order diffraction maximum occurs at an angle $\alpha = 30 \pm 3^\circ$, as expected from the

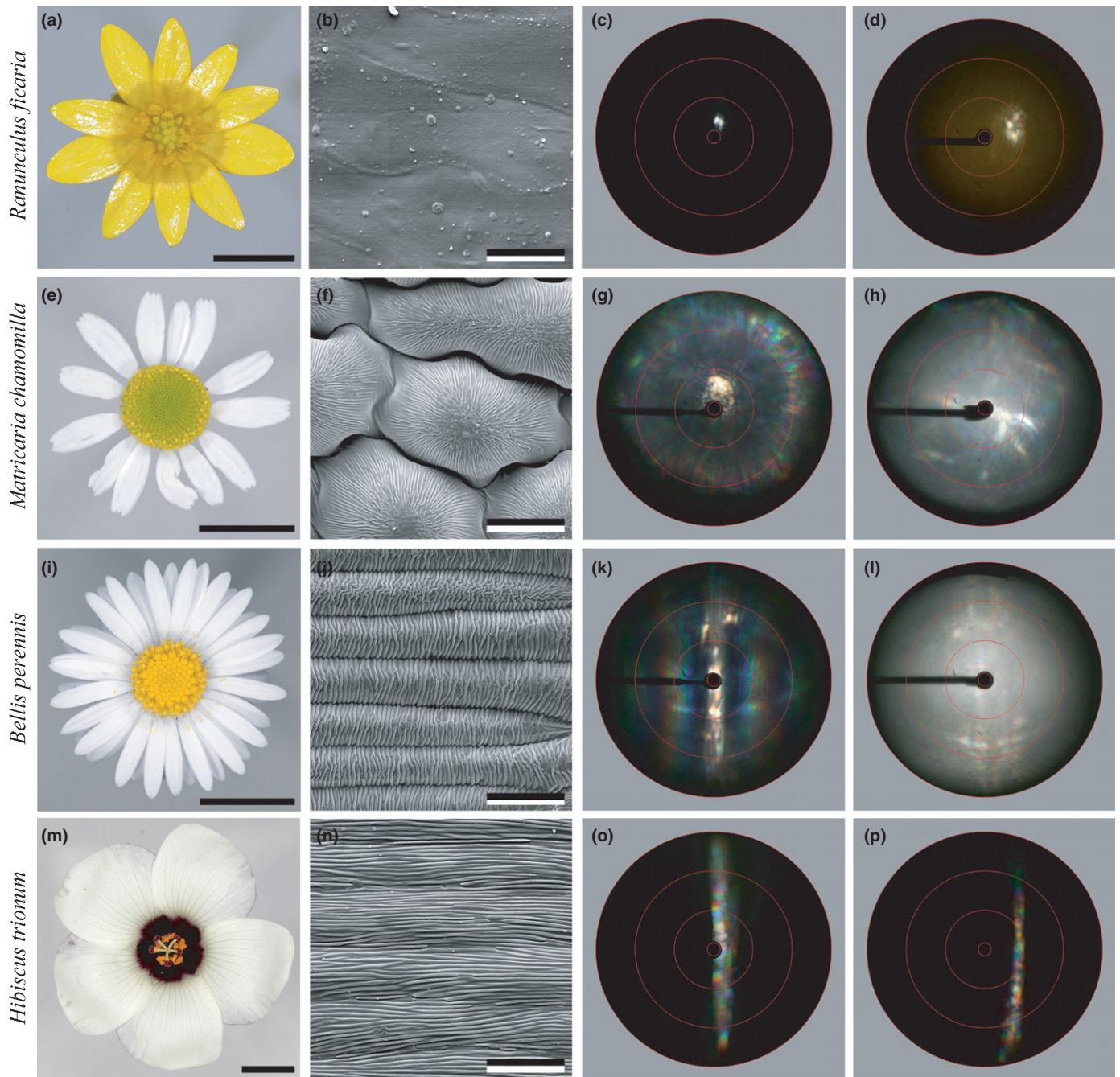


Fig. 2 Flowers with structured perianth surfaces and scattergrams. Column 1, habitus pictures of the flowers; column 2, scanning electron micrographs of casts of the flowers' surface structure; column 3, scattergrams of the casts; column 4, scattergrams of the petals. (a–d) *Ranunculus ficaria*. (e–h) *Matricaria chamomilla*. (i–l) *Bellis perennis*. (m–p) *Hibiscus trionum*. Bars: (a, e, i, m) 1 cm; (b, f, j, n) 20 μm . The red circles in the scattergrams indicate angular directions of 5, 30, 60 and 90° (see Fig. 1b). Illumination spot diameter: 13 μm (in both columns 3 and 4). The black bar at '9 o'clock' is caused by the sample holder (see Stavenga *et al.*, 2009).

diffraction formula $\sin\alpha = \lambda/d$. Yet, the scattergram of the intact white ligule showed a wide-field, diffuse scatterogram. This diffuse scattering, which originated from the irregularly arranged cellular components inside the ligule (Fig. 1a), obscured the diffraction pattern, making it virtually invisible (Fig. 2l). The reflectance of the white ligule was again low in the ultraviolet (Fig. 3), indicating that the petals also in this case contain a UV-absorbing pigment.

Striated petals of *Hibiscus trionum*

The petals of *H. trionum* are mainly white, but very proximally they have a deep-red color (Fig. 2m). In the white part of the petal, the surface was smooth, that is, there were no ridges. The scattergram of a white petal area only demonstrated diffuse white reflection, in accordance with the smooth surface (not shown). The deep-red area was more interesting, because of the

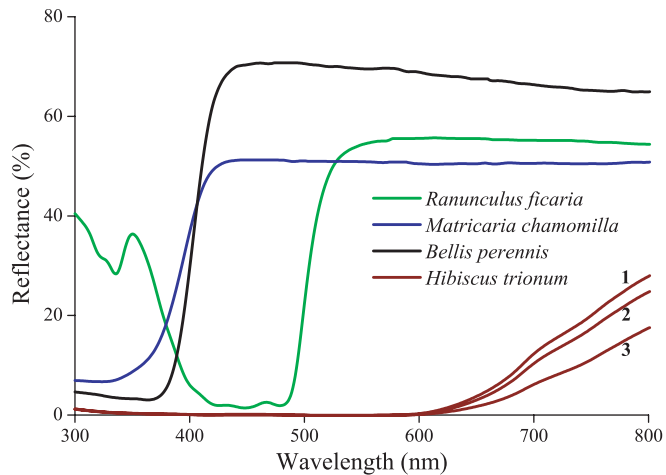


Fig. 3 Reflectance spectra of four flowers with different surface structures. The reflectance of the petals of *Ranunculus ficaria* is low between 400 and 500 nm as a result of a blue-green-absorbing pigment, possibly a carotenoid. The reflectance of both the *Matricaria chamomilla* and *Bellis perennis* ligules is low in the ultraviolet wavelength range as a result of (unidentified) pigment absorption. The reflectance of the proximal petal parts of *Hibiscus trionum* illuminated from an axial direction and measured in the angular directions 0° (1), 30° (2) and 60° (3) was only appreciable in the far-red. No evidence for iridescence was obtained. The shape of the measured spectra varied negligibly, but the amplitude varied with the location, although by no more than 10%.

petal's dense pigmentation, absorbing virtually all the light in the visible wavelength range. In addition, the surface here showed shallow, longitudinal furrows, with distinct striations parallel to the furrows (Fig. 2n). Consequently, the light reflected by the striated surface will not be drowned by the light scattered from the petal interior, as was the case with the white petals of *B. perennis*. Potentially, therefore, the coloration of the proximal parts of the petals of *H. trionum* could be dominated by the surface reflections causing iridescence.

As expected from the striations, the cast of the center of the flower revealed a clear, multicolored diffraction pattern (Fig. 2o). The pattern was almost a line, perpendicular to the striations, but no clear diffraction orders were observed. This was not only a result of the slightly curved surface, but also a result of the varying spacing of the striations (see Table S1). Scattergrams of

intact deep-red petal areas closely resembled that of the cast (Fig. 2p); however, the diffraction pattern vanished if the illumination spot size increased (Fig. 4).

Reflectance measurements showed that reflectance only became appreciable in the far-red (Fig. 3). To assess the contribution of iridescence relative to pigmentary coloration, we measured the reflectance spectra of the deep-red petal area as a function of angle. We illuminated the deep-red, central part of an intact *H. trionum* flower, keeping the illumination parallel to the flower axis, and hence normal to the plane of the flower (the on-view plane of Fig. 1m). The three reflectance spectra of *H. trionum* in Fig. 3 were obtained for detection angles 0, 30, and 60°; intermediate spectra were obtained at intermediate angles. Except for the amplitude, all spectra strongly resembled each other; that is, we were unable to find evidence for iridescent signals, partly as a result of the distinct inward curvature of the petal in the deep-red area, but mostly as a result of the blurring effects described above (Fig. 4b,c).

Discussion

The visibility of the surface reflections of flower petals with respect to the total flower display will strongly depend on both the surface structure of the petals and the scattering and absorption properties of the petal interior. The four flower species investigated in this study feature four different surface structures that occur in many plant species (Fig. 2, Tables 1, S1). The results obtained for the selected species (scattergrams in Figs 2, 4, S2) essentially hold for other flower species with similar surface structures.

With a smooth and flat surface (as in the buttercup) the petals appear glossy, which is especially apparent when the illumination is unidirectional, as occurs with direct sunlight. In the example of *R. ficaria* (Fig. 2a), the overall appearance of the flower petals is nevertheless dominated by the yellow color resulting from the presence of blue-green-absorbing pigment, such as a carotenoid (Fig. 3; cf. fig. 1 of Vignolini *et al.*, 2012c).

Petals with cone-shaped epidermal cells scatter light into a wide spatial angle, thus resulting in a matte coloration. Although some diffraction effects may occur at the cone ridges, these can be seen only with very local illuminations of casts (Fig. 2g). The diffraction phenomena vanish in intact flowers, and certainly

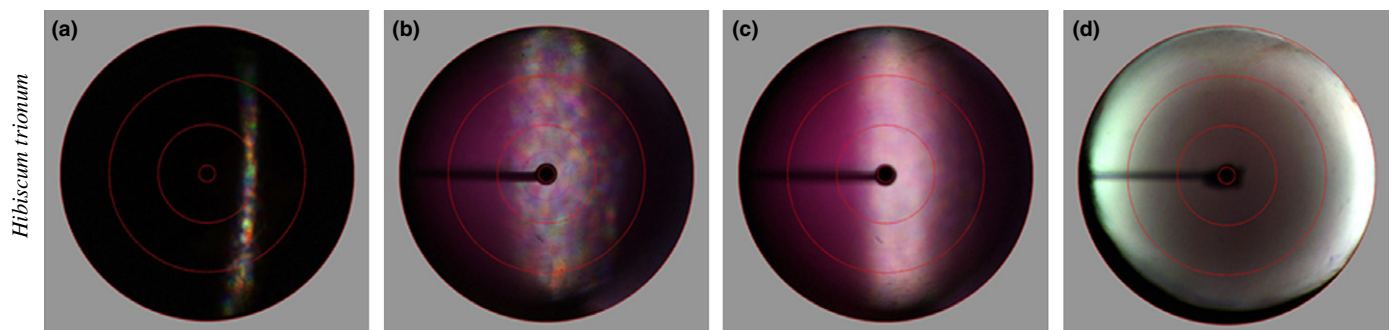


Fig. 4 Scattergrams of a *Hibiscus trionum* petal under different illumination conditions. (a–c) Primary beam illumination (aperture 5°) with spot size 13 μ m (a; same as Fig. 2p), 40 μ m (b) and 140 μ m (c). (d) Illumination with the wide-angled (aperture 180°) secondary beam.

with wide-field illuminations, and thus iridescence is invisible in cone-studded floral elements (Fig. 2h).

Diffraction phenomena, possibly associated with iridescence, can be seen on striated floral elements (Fig. 2k,o). *Hibiscus trionum* and various *Tulipa* species have been reported to be examples of iridescent flowers, and the iridescence was claimed to be a cue for animal pollinators (Whitney *et al.*, 2009; Fernandes *et al.*, 2013). The evidence provided was, however, obtained under very unnatural conditions, with bumblebees trained on artificial objects with highly reflecting, diffracting surfaces instead of flowers (see also Morehouse & Rutowski, 2009).

We have examined a large number of species, including numerous species listed as being iridescent by Whitney *et al.* (2009). However, for all species with ridge-structured surfaces investigated (see Table S1), we conclude that under natural conditions iridescent signaling is extremely unlikely. Certainly, under idealized laboratory conditions, clear diffraction patterns could be obtained from casts of flower petals with quasi-orderly striated, ridged surfaces. However, very local illumination (i.e. one or a few cells) with a narrow-aperture light beam focused on micrometer-sized spots is not representative of natural conditions. Furthermore, the ridge periodicity was never constant over a larger area, so that illumination of areas covering several cells rapidly yielded spectrally featureless reflections, resulting in a whitish gleam. Similarly, in *H. trionum* as in other orderly striated species, wide-aperture beam illumination caused blurred diffraction patterns (Figs 4, S2). Most importantly, the iridescence signal created by the structured petal surface was negligible compared with the diffuse reflection generated by the petal interior. In other words, pigmentary coloration determines the overall floral optical appearance (Figs 2l, 4, S2).

Under ideal circumstances, a colorful diffraction pattern was obtained when the petal contained a high concentration of broad-band absorbing pigment, as was the case for the proximal petal parts of the *H. trionum* flowers (Fig. 2p). However, in this exemplary case of floral iridescence, the morphology of the petal is another important point to consider. In *H. trionum* flowers, as in many flowers with striated surface structures, the potentially iridescent areas are strongly curved inwards towards the center of the flower (Fig. 2m), thus greatly reducing the possible illumination angles, so that surface reflections remain invisible to a reward-searching pollinator (Fig. 4). Yet, even in exceptional cases where the petal is flat, iridescence will be negligible, as a result of the blurring effects described above (see Fig. S2). The Queen of the Night tulip fulfills the criteria to generate a potentially strong iridescent signal (i.e. broad-band absorbing pigment, flat surface, and structured striations). However, this tulip variety is created by the tulip industry and thus does not have any evolutionary significance nor any ecological significance in natural situations.

We conclude that flowers have interesting surface structures. Our study, which included species reported to have prominent floral iridescence, demonstrated that iridescence is absent under natural light conditions. Angle-dependent coloration effects can be observed under special, idealized conditions, but it must

presently be considered unlikely that these effects function to attract pollinators.

The remaining intriguing question is why so many plants have different structured flower surfaces. Presumably these floral traits evolved for other reasons than for optical signaling to pollinators (Galen, 1999; Ellis & Johnson, 2009; Campbell & Bischoff, 2013). For instance, buckling of the cuticle occurs when the flower unfolds (Antonioni Kourouniotti *et al.*, 2013). The principal function of the flower surface structures may therefore concern the mechanical properties of the flower. Although these structures can have other optical functions, such as focusing incident light into the petal, pigmentary rather than structural coloration determines the visual appearance of flowers.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Diagram of the imaging scatterometer.

Fig. S2 Striated petal cells of *Tulipa linifolia* and *Oenothera biennis* under different illumination spots and angles.

Table S1 Investigated species and their surface structures

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