

Letters

Is floral iridescence a biologically relevant cue in plant–pollinator signaling?

Flowers allure potential pollinators by displaying attractive color patterns, which are generally created by their petal arrangement and pigmentation. In addition to the pigmentary coloration, in certain cases reflecting surface structures may contribute to the flowers' appearance. For instance, a flower petal surface with periodic striations, acting as a grating reflector, can create an angle-dependent colored reflection, that is iridescence.

A striking demonstration of this phenomenon is reported by Vignolini *et al.* (2014, in this issue of *New Phytologist*, pp. 97–101). They show that with specific illumination of a *Hibiscus trionum* L. flower, the proximal part of the petals, where the surface is regularly striated, displays an iridescent, bluish shine in addition to the deep-red pigmentary color. Inspired by a previous study by (largely) the same team on floral iridescence across various plant species, entitled 'Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators' (Whitney *et al.*, 2009), we investigated the surface reflections of numerous plant species and confirmed the widespread presence of striations that have diffractive properties in certain controlled conditions. However, the iridescent signal vanished under natural illumination, and therefore we concluded that in no case did surface reflections add a noticeable iridescent



Fig. 1 A few flowers with distinct surface striations, which potentially could create noticeable iridescence, illuminated from a fixed position (40° from the normal) and observed from three different angular directions (0° , 25° , 40°). (a) *Nolana paradoxa*; (b) *Tulipa kolpakowskiana*; (c) *Tulipa linifolia*; (d) *Hibiscus trionum*. *N. paradoxa* and *H. trionum* have striated epidermal cells in the corolla tube and proximal part of the petal, respectively. *T. kolpakowskiana* and *T. linifolia* have striated epidermal cells over the whole tepal. Bars: (a) 1 cm; (b–d) 2 cm.

signal to the pigimentary coloration that could be utilized by an insect pollinator in a biologically relevant scenario (van der Kooi *et al.*, 2014).

Here, we substantiate our previous statement that floral iridescence acting as signaling cue to pollinators is presently untenable by presenting photographs of a number of different flowers with striated epidermal cells and a new set of angle-dependent reflectance measurements on *H. trionum* flowers. Additionally, we discuss the contribution of (minor) visual signals to the overall floral coloration as perceived by insects under natural conditions.

To further illustrate that striated surface structures do not visibly contribute to the coloration, we show a few key flowers that were previously reported by Whitney *et al.* (2009) to display iridescence created by ordered surface striations: *Nolana paradoxa* Lindl. and *Tulipa kolpakowskiana* Regel (Fig. 1a,b). Naturally, their display size is angle-dependent, but their coloration is fully determined by the flowers' pigmentation. Similarly, *Tulipa linifolia* Regel is a tulip with periodically ordered epidermal striations that create clear surface gloss, but its angle-dependent reflections do not contribute a color signal (Fig. 1c).

In our previous study, we paid specific attention to the proximal part of the petals of *H. trionum*, because we considered that its long-wavelength (deep-red) pigimentary color could be combined with a short-wavelength (blue) structural color, similar as occurs in the brightly colored wings of some butterflies (Pirih *et al.*, 2011). Yet, combined angle-dependent scatterometry and spectral measurements yielded no appreciable iridescence (van der Kooi *et al.*, 2014). The new, conflicting findings of Vignolini *et al.* (2014) motivated us to repeat the detailed inspection of the *H. trionum* flowers. We examined many (>50) flowers of the different *H. trionum* plants from multiple origins (reared in our department's glasshouse, a botanical garden and a private collection), but under neither natural nor artificial illumination did the petals display noticeable structural reflections (Fig. 1d).

We also repeated the measurements as reported by Vignolini *et al.* (2014) under the identical conditions, but we obtained severely different reflectance spectra (Fig. 2). Furthermore, angle-dependent reflectance measurements with varying angles of illumination and detection, keeping mirror conditions, and measuring in planes perpendicular as well as parallel to the surface striations also yielded no appreciable iridescent signal (Supporting Information Fig. S1). The deviations between our results and those of Vignolini *et al.* (2014) can possibly be reconciled by assuming that we have studied different subspecies, meaning that the iridescence found by Vignolini *et al.* (2014) is not generalizable to all *H. trionum* flowers, and also not to other species.

Vignolini *et al.* (2014) challenge our previous study by stating that we studied four species, whilst we actually investigated 50 different plant species (supporting information online of van der Kooi *et al.*, 2014), including many species listed to be iridescent by Whitney *et al.* (2009). Furthermore, Vignolini *et al.* (2014) state that 'optical scatterometry provides in only one measurement the directionality of the reflected light in all directions (i.e. an image of the scattered light in the entire hemisphere), while with our optical goniometer we acquire spectrometric data'. Actually, our scatterometer allows both spatial and spectral measurements (Stavenga

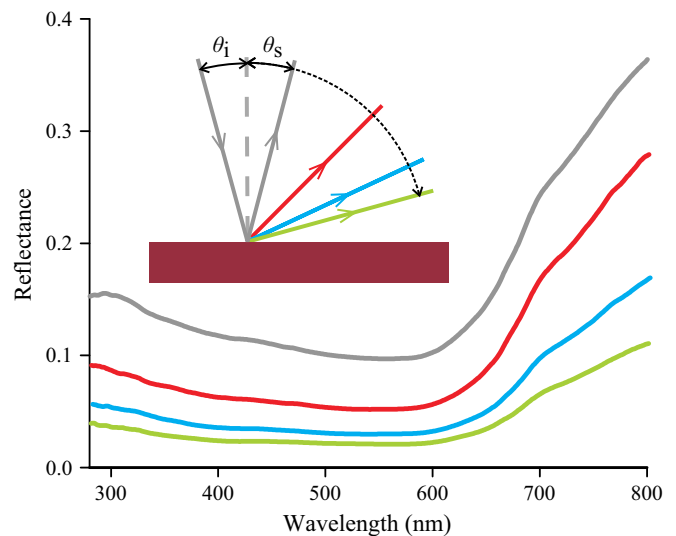


Fig. 2 Angle-dependent reflectance spectra of the proximal part of a *Hibiscus trionum* petal, using two optical fibers positioned at two separate, co-axial goniometers (for details: see van der Kooi *et al.*, 2014). The angle of light incidence, θ_i (fixed at 15°), and angles of reflected and scattered light detection, θ_s (15° , 45° , 65° and 75° ; gray, red, blue, and green curves, respectively), were chosen to be identical to the measurement conditions reported by Vignolini *et al.* (2014, in this issue of *New Phytologist*, pp. 97–101).

et al., 2009; Vukusic, 2011; Constant *et al.*, 2013), and we did include angle-dependent spectra (Fig. 3 of van der Kooi *et al.*, 2014).

The exceptional finding of structural coloration of *H. trionum* flowers reported by Vignolini *et al.* (2014) is certainly interesting, but its relevance for pollination remains questionable, considering the low visibility of the proximal flower parts due to limitations in the spatial angle (Fig. 1d). Furthermore, when discussing whether a visual signal will be distinguished by insects, it is important to consider the limited spatial acuity of insect vision. Bee compound eyes have interommatidial angles of *c.* 1° and therefore millimeter-sized visual patterns will only be discriminable at very short range, that is within a few centimeters distance (e.g. Hempel de Ibarra *et al.*, 2014), especially when considering that bees need to pool signals from several ommatidia to detect and discriminate visual stimuli (Giurfa *et al.*, 1996; Dyer *et al.*, 2008). The visual signal will be severely diminished if the structures are rather 'hidden' at the proximal end of the petal, as is the case with many striated parts on flowers (Fig. 1a,d). Vignolini *et al.* (2014) nevertheless conclude 'that a role for cuticular folding and iridescence in floral signalling is highly plausible'. How they reach this conclusion remains unclear however, and it is somewhat contradictory with the added proviso that 'its distribution and significance remains to be explored'.

Studies presenting spectacular optical phenomena as being of major importance in plant–pollinator signaling are often widely perpetuated in both the popular and scientific press. Experiments performed with extraordinary experimental stimuli in comparison to the natural levels of the optical signal can easily cause a biased image for the general audience, however. Future studies on the biological significance of floral iridescence and structural coloration in flowers should therefore focus on whether pollinator visitation indeed depends on the structural coloration of real flowers, rather than only on laboratory-based psychophysics evidence using

artificial stimuli that display highly constrained visual signals (also see Morehouse & Rutowski, 2009). Indeed, it is now well known that insects like honeybees and bumblebees have sufficient plasticity to learn a wide range of perceptually difficult visual tasks, like navigating mazes, complex rule learning, recognizing faces or categorizing painting styles if afforded the opportunity in controlled conditions (Zhang *et al.*, 1996; Chittka & Walker, 2006; Avarguès-Weber *et al.*, 2010, 2012; Wu *et al.*, 2013). Whilst such studies are of high comparative value for understanding the cognitive abilities of insects, it is not necessarily possible to directly link such laboratory-based studies to how bee pollinators actually interact with flowers in very complex natural conditions (Dyer, 2012; Avarguès-Weber & Giurfa, 2014).

The hypothesis of iridescent signaling of flowers is very interesting. Currently however, there is no evidence to suggest that structural coloration and its angle-dependence (i.e. iridescence) of flowers act as a signaling cue to pollinators under natural conditions. Future studies should focus on whether pollinator visitation indeed depends on structural coloration and possibly even iridescence. These studies should be performed using wild flower species, rather than artificial objects with extreme visual signals or flower varieties (e.g. the tulip variety 'Queen of the Night') as varieties created by the plant industry lack ecological and evolutionary significance. Before that is achieved, caution with claiming that plant–pollinator signaling occurs through floral iridescence seems to be in order.

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Supporting Information

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

Fig. S1 Angle-dependent reflectance spectra of the proximal part of *Hibiscus trionum* flowers measured parallel and perpendicular to the epidermal striations.

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