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Epidermal focusing of light and modelling of reflectance in floral-petals with conically shaped epidermal cells

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ABSTRACT

A model of floral reflectance of petals with conically-shaped epidermal cells is presented for *Nerium oleander* and *Oxalis pes-caprae*. The model was achieved by combined microscopic-scale structures and optical properties of petals; the model theory was based on concepts of physical laws, analytic geometry, vector analysis and micro-optics. The model is shown to fit experimental data of floral reflectance. Conically shaped, adaxial, epidermal cells of petals have focal regions, where incident light rays are focused on the centre of cells. Within tissues light is selectively channelled into sites containing light absorbing pigments. Particular attention was given to consequences of focusing of light within conical, epidermal cells of petals with respect to blossoming regulated by photoperiod, which acts to insure that flower opening occurs during suitable, environmental conditions.

Introduction

Flowers are among the most spectacular products of nature. Flower colours result from selective absorption of ambient light by floral tissues and light reflection at cellular particles associated with structural and biochemical components, and micro-optics (Kourounioti et al., 2013; Lee et al., 2011; Vogelmann, 1993). Optical properties of petals that represent the main floral surfaces for perception of incident light act as adverts for pollination and are associated with both light absorbing compounds and micromorphology of the tissues (Arnold et al., 2010; Kinoshita et al., 2008; Martin and Glover, 2007; Mudalige et al., 2003; Papiorek et al., 2014; Parker, 2004).

Light propagation in petal tissues is primarily dependent on the shape of epidermal cells of petals, which influence optical properties of floral tissues and light channelling within epidermal cells of petals, the location of pigments and the refractive indices of different media (Glover, 2007; Hecht, 1975; Kay et al., 1981; Lee 2007; Solovchenko, 2010; Whitney et al., 2011a; Yu et al., 2011; Zhang et al., 2008). The laws of optics allow for accurate calculation of light propagation through various tissues, which also depends on the interfaces between two media, *i.e.* the front medium (environment) and the microsculptural relief of plant tissues. Detailed visualization of plant surfaces reveals that microsculptural traits increase the surface area of the floral tissues. This surface enhancement may be of particular importance in relation to petals reflectance (Argiropoulos and Rhizopoulou, 2012a; Glover and Whitney, 2010; Ojeda et al., 2009; Rhizopoulou, 2013).

Light perceived by plant tissues is an important variable, and the subsequent path of a light beam depends on the optical properties of the plant material (Vignolini et al., 2013). In the extensive literature on leaf physiology, the importance of light perception by leaf surfaces has been recognised as a major factor of modelling foliar development (Dicker et al., 2014; Gerber et al., 2011; Kolyva et al., 2012; Martin et al., 1989; McClendon, 1984; Vogelmann and Björn, 1986; Vogelmann and Gorton, 2014; Wang et al., 2014).

The objective of the present work was to study micro-optics in floral-petals with conical epidermal cells, which occur in many plant species (Gorton and Vogelmann, 1996; Rands et al., 2011; Whitney et al., 2011a), and to model their reflectance. The model is based on datasets of micromorphology, on pigment content and optical properties of petals, on two-dimensional imaging of floral tissues, on laws of optics and the law of Lambert–Beer. Also, coordination between focal regions of incident light within epidermal cells of petals and the diurnal perception of incident solar radiation by petal micromorphology is discussed.

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Materials and methods

Plant material

Fully expanded petals from pink and white flowers of *Nerium oleander* L. (Apocynaceae) and yellow flowers of *Oxalis pes-caprae* L. (Oxalidaceae), which lack floral rewards, were collected at the campus of the University of Athens in Greece (38°57.5′N, 23°48.0′E). *Nerium oleander* is a perennial evergreen known since ancient times and a desirable ornamental, which exhibits a prolonged flowering period in the Mediterranean Basin, *i.e.* from May to October (Argiropoulos and Rhizopoulou, 2013; Elkiey et al., 1970; Herrera, 1991; Meletiou-Christou et al., 2011; Meletiou-Christou and Rhizopoulou, 2012; Niu et al., 2008; Rhizopoulou and Pantazi, 2015). *Oxalis pes-caprae* is a native weed to South Africa that was brought to Europe in the 18th century and has been widespread across the Mediterranean region (Costa et al., 2014; Lambdon, 2009; Marshall, 1987; Rottenberg and Parker, 2004; Signorini et al., 2014). In the Mediterranean region *O. pes-caprae* blossoms during early spring.

Microscopy

Samples from petal blades of *N. oleander* and *O. pes-caprae* were cut carefully into square pieces (2 mm × 2 mm) and fixed in 3% glutaraldehyde in a Na phosphate buffer (pH 7.0), at room temperature, for 2 h. The plant material was washed three times by immersion in a buffer for 30 min each time. Then, it was post fixed in 1% OsO_4 in the same buffer at 4 °C and dehydrated in acetone solution. The dehydrated tissues were embedded in SPURR resin (Serva, Heidelberg, Germany). Semithin sections of resin-embedded tissue (LKB Ultratome III microtome, Bromma, Sweden), stained in Toluidine Blue "0" in 1% borax solution, were digitally recorded with a light microscope (Zeiss Axioplan, Carl Zeiss, Thornwood, New York, USA) that was equipped with a video camera (Zeiss AxioCam MRc5). Digitally photographed, transverse sections of petals have been used in order to select spots to be entered in the simulation. Therefore, approximately 4000 spots have been selected from digitised sections of petals, which precisely reconstruct the original sectioned segments (*i.e.* 3999 sequential spots); adjacent spots on petals surfaces correspond to sequential vectors (→). Morphological traits of epidermal cells introduce tools required to successfully incorporate measurements and principles into simulation of light distribution in floral tissues (Arnold et al., 2010; Johnsen, 2012; Parker, 2004; van der Heijden et al., 2007; van der Kooi et al., 2014). Petals from 20 corollas of each of the considered species and flower colour variant were sampled for anatomical measurements; 20 measurements were made for each species and each anatomical attribute, and 20 epidermal cells were assessed for measurements per species.

In situ measurements of optical properties of fresh petal tissues

Reflectance, transmittance and absorbance were measured *in situ* in fresh expanded petals of *N. oleander* and *O. pes-caprae* adjacent to those sampled for anatomical measurements, using a UV–vis spectrophotometer (PerkinElmer Lambda-950) equipped with an integrating sphere (Stratakis et al., 2009).

Spectrophotometric measurements of absorption

Fresh expanded petal-tissues were collected from the same corollas sampled for *in situ* measurements of reflectance of *N. oleander* and *O. pes-caprae*; then, they were frozen in liquid nitrogen, crushed into fine powder and pigments were extracted with 80% ethanol. Spectrophotometric measurements of absorption bands of solutions of purified petal ethanol extracts were estimated in triplicate samples using Novaspec II spectrophotometer (Pharmacia Biotech, Cambridge, England).

Refractive indices

Incident light is propagated through the waxy epicuticular layers, within epidermal cells, mesophyll cells and the intercellular space of petals (Lee, 2007; van der Kooi et al., 2014). Refractive indices of light rays encountered in the path *via* different plant media vary between 1.30 and 1.40 (Charney and Brackett, 1961; Lee, 2007; Sun et al., 2013). The refractive index of the waxy epicuticular layers depends on the wavelength (λ) of incident radiation, while the refractive indices of mesophyll and intercellular space are independent of wavelengths (Lee, 2007). The different refractive indices of nanostructures of cell interfaces are still under consideration and interfere with fluxes within tissues (Darvishzadeh et al., 2012; Lee, 2009). The mean value of the refractive indices of interfaces, varying between 1.30 and 1.40 (Lee, 2007; Sun et al., 2013), was considered to be the best approximation of the refractive index of the cells, and was used as such in our simulation.

Modelling framework

In order to simulate channelling of light into tissues laws of optics, vector analysis, geometry and Monte Carlo techniques were used (Cieslak et al., 2008; Govaerts and Verstraete, 1998; Johnsen, 2012; Riddle, 1995), and the code was written in Fortran language. The simulation is deterministic with only one stochastic element, *i.e.* the choice of either propagated or reflected light (Cieslak et al., 2008; Durrett and Levin, 1994). The energy of propagated and/or reflected light, after the perception on the plane of incidence, was estimated according to Fresnel equations. The path of a light beam was estimated according to the law of Snell, and depends on incident angle and refractive indices of media (Yu et al., 2011). Each incident ray that falls on epidermal cells of flower-petals is unique and independent of other rays, and interference phenomena do not occur, while the probability of Rayliegh and Mie scattering is negligible (Pfündel et al., 2006; Zhang et al., 2008). Since scattering phenomena and propagation of light tracing through the fine structure of petals occur on the same tissue-level and in accordance with the laws of electromagnetism (Young, 1992), a two-dimensional (2D) presentation is sufficient in order to approximate the three-dimensional (3D) structure of living plant tissues, which is associated with environmental conditions and physiological circumstances, and is able to produce striking visual and functional effects (Dauzat et al., 2008; Fleck et al., 2003; Kumar and Silva, 1973; Matsushima et al., 2007; Prusinkiewicz and Runions, 2012; Sinoquet et al., 1998; Sonohat et al., 2002). Recently, 2D and 3D representation of plant structure has become an important aspect of various biological applications in science and opened up new possibilities for the calculation of light absorption (Lin et al., 2014; Pound et al., 2014; Pradal et al., 2009; Vos et al., 2010). The simulation and calculations were based on cross section geometry (2D) of petals of *N. oleander* and *O. pes-caprae* (Prusinkiewicz and Runions, 2012) and visualization of random rays falling on epidermal cell edges, propagated within adaxial epidermal cells, and focused at a central point of the cell.

Incident solar radiation received by floral surfaces was obtained using a portable spectroradiometer LI-1800 (Li-Cor) and a quantum sensor (Li-190SB) attached to a Li-6400 portable system (Li-Cor Lincoln, NE, USA) that sampled incident irradiance with the sensor being placed at the same level as the petals of the considered species in the field (Coskun and Oktay, 2011; Demetriades-Shah et al., 1994; Yates and Steven, 1987). Data were collected daily from