The 5th Mathematics in the Plant Sciences Study Group (MPSSG V)
3-6 January 2012 at the University of Nottingham

Modelling the formation of diffraction gratings on top of iridescent petal epidermis

Beverley Glover, Edwige Moyroud, Silvia Vignolini
Department of Plant Sciences, University of Cambridge

Background/Introduction

Pigments account for most of the colours widespread in nature, however the strongest and brightest colours arise from physical structures, made of transparent material. These 'structural colours' are much more intense and pure than chemical colours because physical structures can reflect very precise wavelengths of light, allowing all other wavelengths to be absorbed or transmitted. Conversely, pigments absorb the bulk of the incident light, having much lower bandwidth selectivity.
Animals often use structural colour to create vivid effects: peacocks, butterflies or iridescent jewel beetles owe their stunning colours to the manipulation of light by minute structures organized on or just below their surfaces. Iridescence is the most complex form of structural colour because the perceived colour varies with viewing angle (**Fig. 1**).

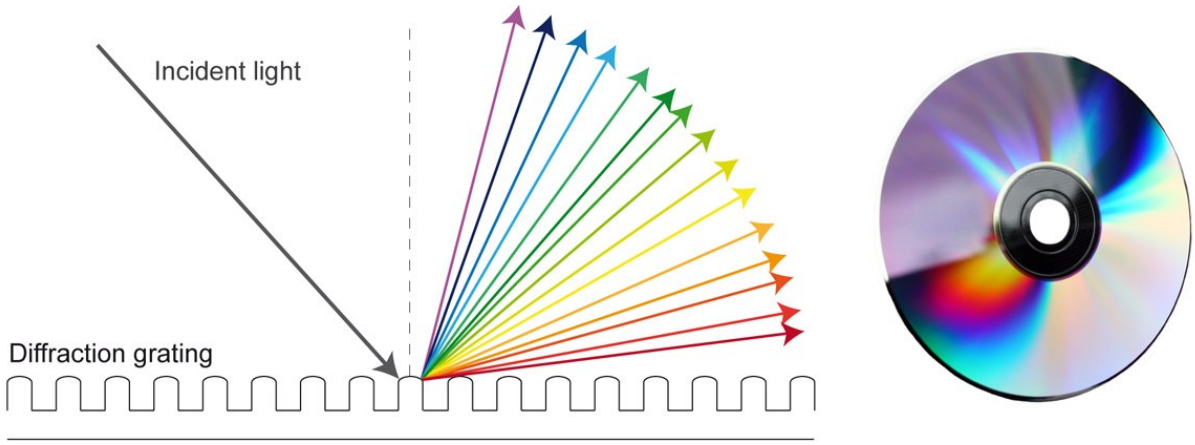

Fig. 1 | A diffraction grating (striation on top of the petal epidermis) divides white light into spectra. Rays scattered from different points on the grating interfere to give rise to an angular colour variation. The grooves on a CD act as a diffraction grating.

Our lab recently discovered that flowering plants also produce iridescence, which pollinators, such as bumblebees, can use as a cue to detect flowers (Whitney et al., 2009). We identified on the petals of several species the physical mechanism responsible for this iridescent effect: surface diffraction gratings (**Fig. 2**). Diffraction gratings are ordered striations. The particular amplitude and frequency of the ridges cause interference, giving rise to an angular colour variation (**Fig. 2**). The data grooves on a CD act as a diffraction grating, producing a familiar example of iridescence (**Fig. 1**).

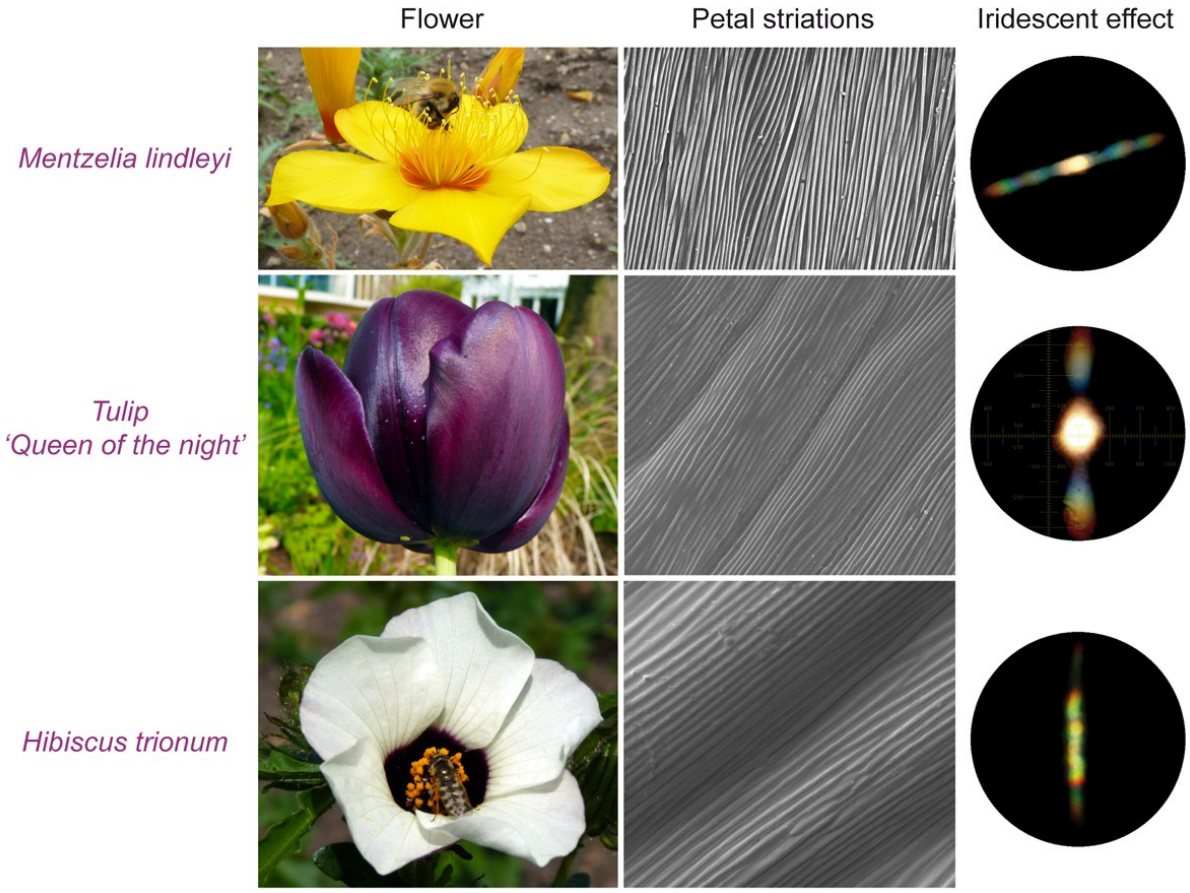

Fig. 2| Flowers of *Mentzelia lindleyii*, Tulip 'Queen of the night' and *Hibiscus trionum* (left column) produce iridescence (right column, iridescent effect recorded by transmission) due to the presence of striations on top of the petal epidermis (middle column, Scanning Electron Microscope images, each striation is < 1µm).

The presence of a diffraction grating depends on two major parameters: the shape of the epidermal cells and the patterning of their surface. First, cells must be flat, as rounded or conical cells do not allow directional reflection since they scatter light. The genetic cause of gross epidermal shape is well understood, being controlled by a family of transcription factors known as the *MIXTA*-like genes. Secondly, the epidermal cells must be striated. These striations are part of the cuticle; a protective waxy covering produced by the epidermal cells themselves. The cuticle consists of a polymer matrix that is covered with epicuticular waxes and incorporates intracuticular waxes. Its thickness, structure and chemical composition vary widely between species and between different organs of the same plant, but the potential functional consequences of such differences are poorly understood (**Fig. 3**). The biosynthesis of cuticular components (mostly cutin and waxes) within the cell and their secretion through the plasma membrane are well characterized (Kunst and Samuels, 2009; Samuels et al., 2008; Pollard et al., 2008). In addition, regulators of cutin and wax biosynthetic genes have been recently identified as *SHINE*-like transcription factors. However, the mechanisms responsible for the assembly and patterning of the cuticle remained to be understood.

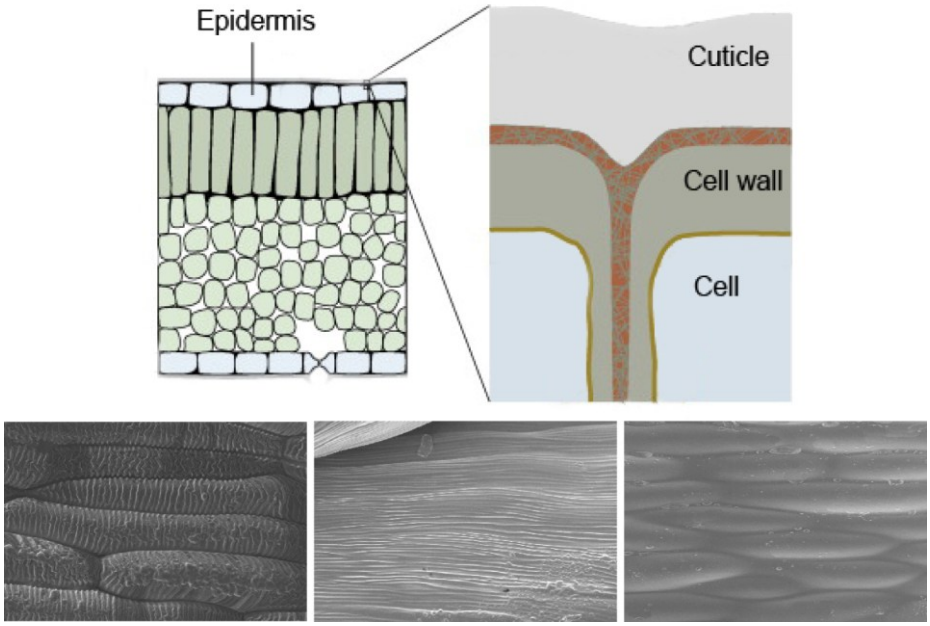

Fig. 3 | The epidermis is a single-layered group of cells that covers plants organs, including petals. The cuticle forms a boundary between the plant and its environment. Epidermal cells produce the cuticle, a polymer matrix known to contain cutin, waxes and sometimes polysaccharides of the cell wall. In addition, wax crystals are sometimes present on top of the cuticle proper. These basic components can be organized differently to create a smooth (right) or striated surface. The striation can be parallel (middle) or perpendicular (left) to the cell elongation axis.

The striations that constitute the diffraction grating could result from two processes. First, striations could be a by-product of cell elongation: the cuticular components could cross the cell wall and self-assemble on the surface as the cell elongates. However, striations often start to develop once the cell elongation has been completed or they can form perpendicularly to the cell elongation axis. Depending on environmental factors, striation can also fail to appear even while the epidermis cells elongate normally. These observations suggest that striations are not the result of mechanical forces only. Alternatively, the cell itself could control the place and time where ridges develop, for instance by regulating locally the secretion of cuticle components regularly along the plasma membrane. How the mechanical and chemical factors interact to build ridges that create constructive interference remain to be established.

Overall objective / Aim of the Study

How these ridges develop in such a regular pattern on top of the epidermis is unknown and we are interested in understanding the biomechanical principles governing their formation. We would like to generate a model that would explain how the diffraction grating is formed on top of the epidermis: what governs where the ridges appear? their spacing? their height? their regularity (which is crucial to achieve an optical effect)? what makes sure that the ridges are uninterrupted at the junction between two consecutive cells? how are the growth of the cell and the export of cuticular components coordinated? can we explain what happens at the junction between striated cells and non-striated cells in a petal with both cell morphologies? We would then be able to design experiments to test the validity of this model and refine it if necessary.

Understanding how petals develop structures to attract their pollinators is a major goal in plant biology: an estimated 35% of global crop production depends on petal-mediated animal pollination but a decrease in pollinator numbers across the world has started to limit the odds of pollination and to affect crop production rate. Unveiling the mechanisms behind diffraction grating formation would be an excellent opportunity to understand both how plants build structures that mediate interspecies communication and how epidermal cell types can regulate the morphology of plant surfaces. Once understood, the mechanisms leading to the formation of iridescent structures may be manipulated. Given the likely importance of structural colours in attracting pollinators, such knowledge will have multiple applications in crop productivity and in preservation of biodiversity in a changing climate.

Questions to be considered by the Study Group participants:

- Can a model explain where and when ridges appear?
- Is it possible to develop a model taking into account both mechanical forces and genetic data? How do we deal with integrating both physical and biological inputs in the model?
- What types of cell/ridge measurements would be the most useful? To which extant are diffraction gratings the result of a self-assembly process?
- What parameters need to be considered to describe the formation of ridges on top of the petal epidermis?
- What type of information related to the cuticle composition of our chosen species do we need to provide (thickness and structure but also chemical composition?)

Available data - What we can provide:

- We are identifying the transcription factors (genes that switch on developmental programmes) that regulate the patterning of the cuticle, and working out what their target genes.
- Data available from the literature on the transport mechanism that gets cuticular components to the top of the epidermis.
- Measurement of ridge position, timing of appearance and growth through a developmental series matched with a developmental series of general growth of the cells underneath the grating. Such data can be provided for two.
- We can compare iridescent and non-iridescent parts of the same petal, to focus on the junction between the two as the cell shape is similar but ridges present or absent.
- Ultimately, we hope to obtain mutant plants devoid of the striations but whose cell growth is unaffected and vice-versa. Such mutants will be used to test the validity of the model.

References

Kunst, L., and Samuels, L. (2009). Plant cuticles shine: advances in wax biosynthesis and export. Curr Opin Plant Biol **12,** 721-727.

Pollard, M., Beisson, F., Li, Y., and Ohlrogge, J.B. (2008). Building lipid barriers: biosynthesis of cutin and suberin. Trends Plant Sci **13,** 236-246.

Samuels, L., Kunst, L., and Jetter, R. (2008). Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu Rev Plant Biol **59,** 683-707.

Whitney, H.M., Kolle, M., Andrew, P., Chittka, L., Steiner, U., and Glover, B.J. (2009). Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. Science **323,** 130-133.