**NanoPetals - Publishable summary**

**Introduction**

Living organisms use nanoscale structures arranged periodically to produce vibrant colours that change with viewing angle, a phenomenon known as iridescence. These structural colours have been intensively studied in animals and the morphological structures responsible for these colour effects have been described, but **we know nothing about the mechanisms underlying their development and patterning.** Flowering plants also produce iridescence, which can be used as a cue by insect pollinators to detect flowers. The physical mechanism responsible for this effect is a surface diffraction grating formed by ordered striations on flat petal cells (like that produced by the data grooves on a CD). The particular amplitude and frequency of the striations cause interference, giving rise to an angular colour variation. These striations are part of the cuticle (a hydrophobic layer consisting of a polymer matrix (cutin) impregnated with waxes) that covers the surface of all plant organs.

**Summary description of the Project Objectives**

This project aims to understand **how a living organism can produce a nanopattern with sufficient accuracy that it interferes with light to generate an iridescent signal.** Specifically, it aims to understand how an optically accurate diffraction grating can develop on the surface of a flower petal, by integrating the tools of **mechanics** and **optics** with modern **genetic analysis**, live-tissue **imaging** and **behavioural ecology**. The objectives of the proposed project are organized in three main sections:

**Part 1.** Identification of the genes controlling the formation of diffraction gratings, using a candidate gene approach

**Part 2.** Understanding how diffraction gratings are built by isolating the targets of the master regulator(s) identified in Part 1. This will be achieved using an original combination of biochemical techniques, modelling and next-generation sequencing technologies.

**Part 3.** Exploring the biological impact of diffraction gratings by answering two questions:

1. Is iridescence a widespread feature of flowering plants?
2. Do diffraction gratings make the flower more attractive to pollinators?

**Work performed during the project and Main results**

Parts 1 and 2 of the project use *Hibiscus trionum* as a model system: this plant is easily grown in the lab, has a rapid life cycle and produces numerous large white flowers with a striking purple iridescent centre. Since the beginning of the project, **we have developed an efficient protocol to perform *Agrobacterium*-mediated direct transformation of *Hibiscus trionum*** allowing us to modify directly *in planta* the expression of any gene of interest to analyse its effect on the formation of the diffraction grating. To identify which mechanisms could be responsible for the formation of regular striations on the petal epidermis, we have collaborated with mathematicians from the Universities of Nottingham and Manchester to **produce a biomechanical model that rationalises the development of surface nanopatterns**. According to this model, the formation of a diffraction grating depends on the rate of cuticle production and the rate and directionality of cell expansion. To test this model **we have isolated homologs of genes controlling cuticle production** (*HtSHINE1*,*2* and *3*) **or cell expansion** (*HtMIXTA*, *HtMIXTA-like1,2* and *HtCIN1,2*) in *Hibiscus trionum* and **analysed their expression through petal development** using qRT-PCR. Next, we have **generated a series of plasmid constructs to modify the expression of these genes *in planta*** and determined, using SEM and TEM imaging, whether a perturbation of cuticle production or cell growth triggers a modification of the nanopattern produced. **Transgenic lines of *H. trionum* expressing a fluorescent YFP protein targeted to the plasma membrane or a fluorescent mCherry protein fused to tubulin have also been generated.** These lines allow us to monitor in real time cell division and expansion or cytoskeleton organisation in the different mutant backgrounds using confocal imaging. Subsequently, we have collaborated with chemists from the University of Cambridge to take into account the composition of the cuticle and refine our biomechanical model. The results we obtained suggest that **the cuticle forming the diffraction grating could present a different degree of cutin polymerisation and could contain more waxes than a smooth cuticle**. Thus, we isolated the *Hibiscus*homologs of *DEWAX* and*CD1*, (two genes recently identified as controlling wax production and cutin polymerisation in model species) and we are currently generating transgenic *Hibiscus* plants with a modified expression of these genes **to test the contribution of waxes and cutin polymerisation to the production of petal iridescence.**

To understand how the *SHINE* and *MIXTA* families control cuticle production and cell expansion respectively, **we have produced and purified a recombinant version of the corresponding proteins** and used these proteins **to perform SELEX experiments to identify the DNA motifs that are recognised by these master regulators**. Using the MIXTA-motif to screen the promoters of potential targets, **we have been able to identify three members of the expansin family (a family of enzymes involved in cell wall remodelling) as targets through which MIXTA homologs act to regulate cell shape**.

Finally, to address Part 3 of the project, we conducted a large-scale survey using the living collections of Cambridge University Botanic Garden and the Royal Botanic Garden Kew to evaluate the occurrence of diffraction gratings in angiosperms. **Our results indicate that iridescence is a common phenomenon among flowering plants** as petal diffraction gratings can be found in all main sections of the flowering plant phylogeny except for the most basal angiosperms (ANA grade + Magnoliids), indicating that **petal iridescence probably originated multiple times independently in angiosperm evolution**. During this survey we also made some unexpected discoveries: **we identified a striking example of structural colour in the fruit of *Pollia condensata***, a flowering plant from West Africa and we **analysed the interplay between pigment and structure responsible for the glossy appearance of *Ophrys speculum* (the mirror orchid)**.

**Final results and their potential impact and use**

* The molecular mechanisms underpinning the development of structural colour have never been investigated in plants or indeed in animals; therefore, **this project brings the first characterisation of the genetic circuit behind the iridescence of any organism**.
* Petal diffraction gratings are part of the cuticle. The cuticle is a fundamental layer that covers plant surfaces but the assembly of the various components required to make a functional cuticle is still poorly understood. Understanding the molecular mechanisms that control the development of diffraction gratings **will help to elucidate how cuticle components become organized to generate extracellular three-dimensional features**.
* 40% of global crop production depends on animal pollination but a decrease in pollinator numbers affects the crop production rate. Structural colours attract pollinators. **Discovering the structures flowers use to attract insects and the developmental pathways at work to sculpt plant surfaces has the potential to improve crop productivity.**
* Plants have been neglected bioinspirations: this project **will generate new synthetic applications as nanotechnologies can now replicate biological structures**. Food and cosmetics companies have expressed interest in using the structures we identify to manufacture new non-toxic coloured materials.

**Website:** <http://www.plantsci.cam.ac.uk/research/beverleyglover>

 http://www.plantsci.cam.ac.uk/directory/moyroud-edwige