The Physics of Pollinator Attraction

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Contents

Summary

This Tansley Insight focuses on recent advances in our understanding of how flowers manipulate physical forces to attract animal pollinators and ensure reproductive success. Research has traditionally explored the role of chemical pigments and volatile organic compounds as cues for pollinators, but recent reports have demonstrated the importance of physical and structural means of pollinator attraction. Here we explore the role of petal microstructure in influencing floral light capture and optics, analysing colour, gloss and polarization effects. We discuss the interaction between flower, pollinator and gravity, and how petal surface structure can influence that interaction. Finally, we consider the role of electrostatic forces in pollen transfer and pollinator attraction. We conclude that this new interdisciplinary field is evolving rapidly.

Key words: flower, pollination, light focusing, structural colour, gloss, polarization, grip, electrostatic force

Introduction

Attracting animal pollinators is essential to the reproductive success of the great majority of angiosperm species. The angiosperm flower, particularly the corolla, has been described as a sensory billboard, catching the attention of potential pollinators and advertising the presence of nectar and pollen rewards within (Raguso, 2004). This billboard is often visibly arresting. It may also smell nice, or offer easy-grip textures or other inducements to visiting animals. Plants achieve this diverse array of attractive properties in a variety of ways, but the approaches that generated early interest and have been best studied can be thought of as chemistry-based. The best known examples of these are the use of chemical pigments to generate colours and the use of volatile organic compounds to produce scent. In this Tansley Insight we focus instead on recently described ways of attracting pollinators that rely on the physical properties of flowers and their ability to influence physical forces in the world around them. We summarise recent advances in our understanding of the optical properties of flowers arising from their surface structuring, the physical means by which flowers influence their direct contact with animal pollinators, and the ways in which flowers use electrostatic forces to manipulate their pollination success.

Playing with the light

Most objects appear colourful because of chemical pigments that selectively absorb certain wavelengths of light. Flowers use pigments such as anthocyanins or carotenoids to provide contrast with the surrounding green foliage. However, there are other means to generate colour or, at least, to modify the colour that would be produced by a pigment alone, using physical properties instead of chemistry: these "structural" mechanisms generate optical effects by exploiting the interaction of light with microscopic structures of the flower and are increasingly regarded as playing a key role in mediating plant-pollinator interactions.

<u>Light focusing</u>

When Noda et al. (1994) identified the *mixta* mutant of *Antirrhinum majus* they expected the causal mutation to affect the production of anthocyanin pigments as the flowers appeared paler than those of wild-type (Fig. 1(a)). However, the gene affected instead controlled the shape of the epidermal cells: in the absence of a functional MIXTA protein, the pigments remain unchanged but the cells in the petal epidermis are flat rather than the characteristic conical shape found in the wild-type (Noda et al., 1994). The conical geometry (Fig. 1(b)) has

a double effect: it acts as a lens by focusing light into the vacuole (containing the anthocyanin pigment), which increases the intensity of the colour, but it also scatters the light reflected from the mesophyll more efficiently than a flat surface. As a result, the colour saturation is increased and the petal acquires a sparkling appearance (Gorton and Vogelmann, 1996).

Is this optical trick part of the flower's arsenal to attract pollinators? Conical cells are present on the petal epidermal surface of the vast majority of angiosperms, and generally on the section that is directly exposed to pollinators (Kay et al., 1981, Papiorek et al., 2014). Wild type *Antirrhinum* with conical cells receive more pollinator visits and produce more fruits than mutants with flat cells (Glover and Martin 1998, Comba et al., 2000; Dyer et al., 2007) and bumblebees can perceive the difference in hue generated by cell shape (Dyer et al., 2007). Thus, by "playing with the light" to modify the appearance of the flower, conical cells can generate a cue that can be detected at distance by pollinators. This cue could help pollinators to distinguish flowers with different handling properties (see below for role of conical cells in providing grip) (Dyer et al., 2007, Whitney et al., 2011).

Gloss

Having a smooth, lustrous surface is an efficient way of being conspicuous and gloss is another optical effect, produced by physical means, which has been described in flowers of multiple species (Fig. 1(c)) (Parkin 1928, Gaisterer 1999; Vignolini 2012a,; Whitney et al., 2012; Papiorek et al., 2014). Petal gloss can be defined as the specular reflection of light from the surface of the petal and this effect is maximised when the petal surface is flat (Gaisterer 1999; Whitney et al., 2012). However, most flowers do not have flat cells in the visually-active part of their petal epidermis and are thus not expected to be very glossy (Kay 1981; Papiorek et al., 2014). Instead, gloss appears only at the tips of the conical cells commonly found on petal epidermis, generating a pattern of regularly arranged angle-dependent highlights (Papiorek et al., 2014). Alternatively, gloss can occur in defined regions of the petal, for example in the green psuedonectaries of some *Solanum* species.

Whether gloss acts as an attractant for insects or birds remains an open question: Gaisterer et al (1999) found that although gloss reduced the colour contrast of the petal of *Ranunculus lingua* against the background, the bright flashes arising from floral gloss provided a dynamic visual display that could attract pollinators (Gaisterer et al., 1999). However, gloss is only expected to be effective as an attractant if the leaves are not also glossy, and, in the case above, not moving. Glossiness is hard to assess in a reproducible manner, and therefore hard

to compare between leaves and petals, although recent attempts have been made (Whitney et al., 2012, Papiorek et al., 2014). There are inherent limitations to measuring gloss, such as the multidimensional nature of gloss perception and the fact that glossiness varies hugely with ambient light conditions (Chadwick and Kentridge, 2015). In addition, the amount of gloss does not depend solely on the geometry of the epidermis. For instance, micropatterning of the cell surface can greatly diminish the reflectivity of the surface while the anatomy of the underlying cell layers can, in contrast, enhance the optical effect (Vignolini et al., 2012a; Papiorek et al., 2014).

Structural colour

The physical features of the petal can not only modify the appearance of pigments but they can also generate colour directly. These 'structural colours' are produced by regular microscopic structures positioned just on or below the surface, which generate colour by diffraction and interference phenomena. The appearance of structural colours often changes with the observation angle, an effect known as 'iridescence'.

Structural colouration was first reported in the leaves of a handful of plant species (Lee and Lowry, 1975) and more recently described in fruits and flowers (Fig. 1(d)) (Vignolini et al., 2012c; Whitney et al., 2009a; Kolle et al., 2013; Vignolini et al., 2014, 2015). So far, all described examples of structural colours in petals are produced by pseudo-regular striations of the cuticle, acting as disordered diffraction gratings (Whitney et al., 2009a). Like gloss, iridescence is challenging to measure (Vignolini et al 2014, 2015; Van der Kooi et al., 2014) and floral iridescence is not common. However, it is a widespread trait, as iridescent species can be identified in all major groups of angiosperms (Vignolini et al., 2015).

We know that bumblebees, like other animals tested, can perceive iridescence (Whitney et al., 2009a) and they can also use it as a cue to detect flowers more efficiently (Whitney et al., 2016). However, like many biological photonic structures, floral diffraction gratings are not perfect as the spacing, height and width of the cuticle striations vary across the surface. Whether this disorder is biologically significant remains to be established, but recently Whitney et al. (2016) demonstrated that, unlike a perfectly regular grating, "imperfect" petal iridescence enhances flower detectability without compromising correct identification. Thus, the disorder observed could have adaptive value.

Polarization

Insects use sensitivity to polarized light for a range of purposes, from general navigation to detection of food sources or nesting sites (Kraft et al., 2011; Wehner, 2014). Intriguingly, the flowers of angiosperms often display polarization patterns that probably emerge from differences in petal surface structure (i.e., different types of epidermal macro/microgeometry next to each other on a surface) (Foster et al. 2014). Although the polarization-sensitive dorsal rim of the bumblebee eye has long been thought to function only in navigation, Foster et al. (2014) showed that bees could distinguish between two different polarization patterns and identify correctly a rewarding target. Thus, polarization could act as a cue produced by physical rather than chemical means to attract pollinators, although whether this is the case in nature remains to be established.

Finally, it is worth noting that light focusing, gloss, structural colour and polarisation all emerge from the same set of physical properties (cell shape and micro/macropatterning). These different cues are probably interdependent and it will be important for future studies to consider their combined, rather than separate, effects.

Floral mechanical tricks

<u>Conical cells, grip and slip</u>

Landing on a flower that is moving in the wind, and finding the correct angle and approach to access the reward, is not always simple. Many plant species have evolved strategies that take advantage of the interplay between pollinator, flower surface and gravity, to limit access to certain groups of animals. A simple way in which many flowers improve the grip and handling efficiency of their flowers is to develop conical epidermal cells on the petal (Fig. 1(b)). Various studies have shown that these improve foraging efficiency for bees by providing an interlocking surface for their tarsal claws, which is of particular importance when flowers have to be handled at difficult angles or in windy or slippery conditions (Kevan and Lane 1985; Whitney et al. 2009b; Alcorn et al. 2012).

Several recent reports have shown that some flowers manipulate pollinators by selective loss of these cells. Ojeda et al. (2016) analysed petal epidermal micromorphology for plants on the Macaronesian islands, and found that in all 5 independent cases of transition to bird pollination, conical epidermal cells were lost. This evolutionary change is postulated to minimise nectar robbing by insects, by making the petal surface more slippery. Similarly,

Papiorek et al. (2014) analysed petal epidermal morphology on all surfaces of 58 independent species. They observed that the parts of flowers which were vulnerable to nectar robbing, often the base of the corolla tube, generally had flat epidermal cells. These authors concluded that even bee-pollinated flowers were selectively distributing petal epidermal cell types in a way which maximised the advantages to the flower of controlling the physical forces acting on foraging insects.

Triggering pollen transfer

Physical forces can be recruited by plants to aid pollen transfer, and particularly pollen placement on specific parts of the pollinator's body. Controlling pollen placement is considered particularly important for plants that flower in the same habitat as other species that use the same pollinator, as accurate pollen placement may limit the chances of interspecific pollen transfer. A recent report demonstrated that *Impatiens frithii*, flowering alongside four related species on Mount Cameroon, achieves specific pollen placement on the ventral part of the head of its pollinating sunbird, as opposed to on the more usual dorsal surface (Bartos and Janecek, 2014). This clever trick is achieved by a developmental twist in the shape of the flower's nectar spur, which curves upwards. When the bird inserts its bill to feed, the physical force it exerts causes the flower to rotate 180 degrees, placing pollen below the beak. Here, the flower is using a combination of physical force from the bird with a flexible pedicel that can tolerate rotation, to achieve optimal pollen placement.

An even cleverer method of managing pollen placement through physical force is used by species of the trigger plant genus, *Stylidium*. In these flowers, from a family in the Asterales, the stamens and style are fused to make a single reproductive column which is held upwards and behind the corolla (Fig. 1(e)). This position is achieved over several minutes by changes in volume and length of cells, driven by potassium ion transport. When an insect brushes against the flower the energy stored in the column is released and it snaps forward and down (Fig. 1(f)), in a matter of milliseconds, to deposit pollen on the back of the insect (Joyeux, 2011). The column is then slowly pulled back to its original position over the next few minutes. This example of the use of physical forces is similar to the mechanisms used by many carnivorous plants, but in this case has been recruited to ensure successful pollination.

Slower movements can also prove highly effective at pollen placement. The stamens of the cactus *Opuntia polyacantha* move inwards over the course of 2-20 seconds after being touched by an insect of sufficient weight (Cota-Sanchez et al., 2013). This movement may

force pollinators to push through the stamens to exit the flower, increasing their pollen uptake.

Electrostatic forces

Electrostatic forces have long been suspected to play a significant role in pollination because charged pollen settles more effectively on stigmas than uncharged pollen (Buchmann and Hurley 1978; Hardin 1976). Early discussions proposed that electrostatic charges accumulate on the bodies of flying pollinators and that these charges could induce an electrical field between the flower, also electrically charged, and the approaching insect. This electrical field, which gets stronger as distance decreases, would facilitate the transfer of pollen from anther to pollinator but also from the pollinator to the stigma (Hardin 1976, Corbet et al., 1982, Ericsson and Buchmann, 1983, Colin et al., 1991). This theory is well supported experimentally: electrostatic attraction of pollen onto bees has been experimentally observed (Corbert et al., 1982) and the accumulated charge on the bee body has been measured and shown to be sufficient to trigger non-contact pollen transfer (Gan-Mor et al., 1995). Wild Anna's hummingbirds (*Calipte anna*) have recently been shown to accumulate enough electrical charge to attract free pollen grains and even to trigger bending of the stamens over a short distance (Badger et al., 2015).

But are flower visitors aware of the electric charges on flowers? Recent studies have demonstrated that bumblebees can directly sense the electrical field between them and a flower, using their mechanosensory hairs (Sutton et al., 2016) to make foraging decisions (Clarke et al., 2013): various attempts to model electrostatic interaction between flowers and their incoming visitors had already suggested that floral electric fields directly depend on the geometry of flowers (Dai and Law, 1995; Bechar et al., 1999). Clarke et al (2013) confirmed this idea by spraying electrically charged paint on flowers that revealed in detail the structure of their individual electric fields (Fig. 1(g)). They showed that bumblebees could discriminate between flowers with electrical fields of different shapes. These experiments revealed that the electric field of a flower starts changing just before a bee lands and that this effects lasts for several minutes. One intriguing possibility is that this could be used by pollinators to distinguish which flowers have recently been visited. Thus, flower could have evolved specific forms and sizes that maximize or at least influence pollen exchange with their pollinators (Vaknin et al., 2001) but that also produce dynamic cues such as electrostatic forces to broadcast "live" information about their identity and their current reward status.

Conclusions

All living organisms function in a world dominated by physical forces, so it is perhaps not surprising that some have evolved ways of taking advantage of physics to attract pollinators and ensure their reproductive success. The new findings we have outlined all require considerable further research, to establish how important these physical cues are in natural pollination scenarios. The study of physical forces and pollinator attraction is a field that depends on interdisciplinary working and interdisciplinary funding models. It is an exciting research area that provides great breadth of training and opportunities for the next generation of plant scientists.

Acknowledgements

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Figure Legends

Figure 1 – Examples of physical properties of flowers that contribute to pollinator attraction. (a) A flower of the *mixta* mutant in Snapdragon (*Antirrhinum majus*) appears paler than a wild-type flower because its epidermal cells are flat instead of conical. **(b)** Example of petal conical epidermal cells observed under scanning electron microscope

(adaxial petal of *Hibiscus sabdariffa*, scale bar = 20µm). **(c)** Petals of *Linum grandiflorum* are characteristically glossy because of their smooth surface. **(d)** Close-up image of a tepal of tulip cultivar Queen of the Night demonstrating the iridescent effect caused by striations of the cuticle. **(e)** Flower of *Stylidium turbinatum* before and after **(f)** being triggered. Note the change in position of the column, indicated by a white arrow (images by Holger Hennern, CC BY-SA 3.0, Wikimedia commons). **(g)** Capitulum of *Gerbera hybrida* before (left half) and after (right) spraying with electrostatic coloured powder to reveal the pattern of the floral electric field in this species (modified from Clarke et al., 2013; picture courtesy of Dominic Clarke).

(a) *mixta*   Wild type