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DOI: 10.1016/j.cub.2011.04.005

Pollinator Attraction: The Importance of Looking Good and Smelling Nice

Flowers entice animal pollinators using a complex array of attractions. Reciprocal transfer of traits between *Petunia* species now shows that colour and scent are equally important to hawkmoths in choosing between different flowers.

Beverley J. Glover

The spectacular diversity of flower colour, shape and scent has long been interpreted as part of a dynamic conversation between plants and pollinating animals, particularly insects. However, the very complexity, both of individual flowers and of the ecological networks in which they function, has made it difficult to do much more than describe floral traits and observe correlations between flowers and the animals that frequent them. In recent years the first *in vivo* characterisations of the functions of individual floral traits have been made, using genetic and transgenic approaches to isolate particular characters. In this issue of *Current Biology* the Kuhlemeier group [1] investigate the interaction between two key floral traits — colour and scent. By recombining the scent of one species with the colour of a second, and *vice versa*, they show that colour and scent are equally weighted by hawkmoths when selecting between different flowers.

The flowers of many plants are very complex, featuring a diverse range of colour, scent, shape, symmetry, organ number, surface texture and other traits that are generally assumed to attract animal pollinators. The enormous diversity of flower types, and indeed of angiosperm species, is often seen as a reflection both of the myriad ways in which these different traits can be combined and of the immense number of animal species that can potentially be recruited as pollen vectors. Of course, insect pollinators can be attracted to apparently simple flowers, such as the *Cistus* flower in Figure 1A, although even this flower advertises its nectar and pollen rewards through a combination of yellow pigment, purple pigment, a glossy surface, and an exaggerated number of yellow stamens. When interactions with pollinating animals become more specialised then the complexity of the flower can increase enormously. For example, the flower of *Ophrys speculum* (Figure 1B) attracts only males of a single pollinator species, the wasp *Campsoscolia ciliata*, by mimicking females of the same species. This mimicry extends to the production of wasp-specific pheromones [2], modification of the exaggerated lower petal (the labellum) to be wasp-shaped, the apparent development of eye spots on that petal by pigmentation, the differentiation of long hairs at the edges of the petal, and the production of the smooth mirror-like blue speculum in the middle of the petal, thought to mimic the sheen on the wasp's wings [3]. Disentangling the relative contribution of complex floral traits, and their interactions, has seemed so difficult that, until recently, most papers focussed on real flowers and their pollinators were understandably limited to description and correlation. At the same time, groups interested in insect learning have provided useful information about the likely function of some traits [4], including the suggestion that combining multiple floral traits within a single artificial stimulus enhances the ability of animals to identify target flowers [5,6].

In recent years genetic and transgenic approaches have at last allowed analysis of the function of individual floral traits in the background of an otherwise normal flower. The classic paper in this field describes the introgression of the *YUP* locus, responsible for yellow carotenoid-based pigmentation, from orange/red hummingbird-pollinated *Mimulus cardinalis* into pink bee-pollinated *M. lewisii*, and the reciprocal transfer of the *M. lewisii* allele into *M. cardinalis* [7]. The resulting orange/red *M. lewisii* flowers were now visited by hummingbirds, but less frequently by bees than their wild-type pink relatives. Meanwhile the pink *M. cardinalis* flowers were now visited by bees, although hummingbirds were apparently not deterred by the lack of carotenoids. These data show that carotenoid pigmentation is important for pollinators in distinguishing between species in this genus, and therefore important to the plants in limiting interspecific hybridisation. Studies using the *mixta* mutant of *Antirrhinum majus* have similarly

Figure 1. Attracting pollinators.

(A) Honeybee feeding from a simple *Cistus* flower. Photo by Alison Reed. (B) The complex flower of *Ophrys speculum*. Photo by Sam Brockington. (C) Hawkmoth feeding from white, scented *Petunia axillaris* flower. Photo by Cris Kuhlemeier.

shown that the conical epidermal cells common to many petals are important in the bumblebee pollination of this species. Mutants lacking the conical petal cell morphology receive fewer visits from pollinators, primarily because flat petal cells are harder for insects to grip onto, resulting in less efficient foraging [8,9]. The role of anthocyanin pigmentation in pollinator attraction was quantified by the Kuhlemeier group using a transgenic approach in petunia [10]. The *AN2* gene, encoding a MYB transcriptional regulator of anthocyanin synthesis, was introduced from pink-flowered *Petunia integrifolia* into white-flowered *P. axillaris*. When pollinators were offered a choice between the pink-flowered transgenics or the white-flowered wild-type plants, hawkmoths chose the white flowers while bumblebees preferred the pink flowers. All of these studies have provided novel insight into plant–pollinator interactions by isolating single traits, with known genetic bases, and investigating their effect on animal behaviour.

However, to date none of these studies has considered the intersection between two or more pollinator-attracting traits. In this issue the Kuhlemeier group investigate the interaction between petunia flower colour and floral scent [1]. The main focus of their paper is the identification of major loci responsible for the difference in floral scent between perfumed hawkmoth-attracting *P. axillaris* and unperfumed hummingbird-pollinated *P. exserta*. One of these loci encodes the MYB transcription factor ODO1, a known regulator of production of the methylbenzoate that is a major component of petunia perfume [11]. However, *P. axillaris* and *P. exserta* differ in flower colour, as well as in scent. Like many bird-pollinated species, *P. exserta* produces red flowers, while *P. axillaris* produces the white flowers common to plants pollinated by night-flying moths. To investigate the interaction between scent and colour in pollinator discrimination, introgression of *ODO1* was used to generate scented, red *P. exserta*-like flowers, and unscented, white *P. axillaris*-like flowers. When presented with a choice of scented or unscented white flowers, hawkmoths consistently chose the scented flowers, as expected. Similarly, they chose scented red flowers over unscented red flowers. However, the unique component of this study was the ability to offer the moths a choice between scented red flowers, and unscented white flowers, both flowers presenting a conflicting combination of cues. Given this choice the moths initially flew forwards in the plume of scent from the red flowers, confirming that scent is the more important of the two cues at long range. However, on arriving at the flowers the moths chose to feed from the two types equally, demonstrating that colour and scent are of equal importance in hawkmoth foraging decisions.

This study provides the first controlled analysis of how multiple floral traits combine to produce the complex signals that pollinators recognise. In its use of near isogenic lines, differing in traits characterised by molecular genetic approaches, it provides an elegant example of how plant–pollinator interactions can be dissected using animal-pollinated model systems. This paper sets the stage for the challenges to come — a full understanding of how the very many floral cues in more complex flowers, like that of *Ophrys speculum*, interact to generate beneficial animal behaviours.

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DOI: 10.1016/j.cub.2011.03.061

Mechanotransduction: Getting Morphogenesis Down Pat

Embryonic morphogenesis requires the coordination of forces across multiple tissues and their associated extracellular matrices. A new study reports a mechanical feedback loop in the *Caenorhabditis elegans* embryo between muscle and epidermis that may provide a model for understanding how tissues coordinate morphogenetic events in the embryo.

Jeff Hardin

Morphogenesis in animal embryos is a complicated business, both in time and in space. Cells in the embryo must coordinate movement and force production, and do so across multiple germ layers with intricate spatial relationships, often separated by multiple layers of extracellular matrix [1]. If the embryo is a quintessential example of D'Arcy Thomson's dictum that the organism is a "diagram of forces" at work within it [2], then understanding the mechanical interplay between multiple tissues during morphogenesis is a key challenge of current research at the interface between cellular and developmental biology. One difficulty is finding discrete morphogenetic events that are amenable to genetic and cellular analysis and that are sufficiently simple to begin to tease apart the rules that underlie this interplay. Examples exist, including the three germ layers of the amphibian embryo as it elongates [3] and the multiple tissues that contribute to dorsal closure in the *Drosophila* embryo [4]. Recent work by Zhang *et al.* [5] in the *Caenorhabditis elegans* embryo represents a fresh entry in this area.

The *C. elegans* embryo undergoes a dramatic elongation along its anterior–posterior (A–P) axis during late embryogenesis, becoming roughly four times longer along the A–P axis during this time. Unlike many organisms, in which convergent extension movements drive such dramatic elongation, elongation of the *C. elegans* embryo is accomplished largely by a coordinated change in shape of its epidermal (or hypodermal) cells [6]. This explosive cell shape change requires the construction of a remarkably ordered set of circumferential actin filament bundles in dorsal and ventral epidermal cells, and carefully tuned, cell-specific activation or suppression of non-muscle myosin activity within epidermal cells (Figure 1, left). Previous experiments showed that lateral (or seam) epidermal cells require elevated activity of the myosin regulatory light chain, MLC-4, presumably downstream of Rho and activated ROCK/LET-502 [7,8], whereas myosin activity is downregulated in other epidermal cells via a RhoGAP, RGA-2 [8], and the myosin phosphatase MEL-11 [7].

During the early steps of elongation, the cadherin–catenin complex is required for transmitting forces generated within the epidermal cells to adherens junctions at their surfaces. Zygotic loss of *hmp-1*/α-catenin function or the function of the other core components of the cadherin–catenin complex severely affects elongation, leading to dorsal folds in the epidermis and failure of morphogenesis early in elongation [9,10]. Recent work indicates that the actin-binding function of HMP-1 is crucial for these events; embryos that produce a carboxy-terminally deleted form of HMP-1 that lacks the actin-binding domain can form circumferential filament bundles, but fail to form a mechanically robust junctional-proximal actin network, leading to physical ripping of circumferential filaments away from the cell periphery [10].

Although actomyosin-mediated contractile forces, transmitted through the cadherin complex, are crucial for the early steps of elongation, later stages curiously depend on underlying muscle cells. Muscle cells are mechanically connected to the overlying epidermal cells, and ultimately to the cuticular exoskeleton through an elaborate set of epidermal attachment structures known as fibrous organelles (FOs). FOs consist of hemidesmosome-like attachments on the apical and basal surfaces of epidermal cells that are spanned by intermediate filaments (Figure 1, right). FOs transmit the forces produced by muscle contractions through the basal lamina to the epidermis and ultimately to the cuticle. Several clues gleaned over a period of many years indicated an intimate mechanical and functional connection between muscle and epidermis. Embryos that lack muscle activity, or in which attachment of muscle cells