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Floral epidermal structure and flower orientation: getting to grips with awkward flowers

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Abstract

The petal epidermis has been found to be important in mediating flower-pollinator interactions. Structures produced on the petal surface, in particular cone-shaped papillate (or conical) cells, have been shown to enhance bumblebee preference for flowers. One reason for this increase in preference is that the conical cells facilitate efficient handling of flowers. This is particularly clear when flower architecture requires bees to land on a vertical surface. We therefore tested the hypothesis that flowers that are held vertically show a greater tendency to produce conical cells. Analysis of 183 species finds that there is no significant relationship between the structures on the petal surface and flower orientation. We discuss the multifunctional properties of conical cells and other floral surface structures that may mean that other factors are of equal or greater importance in the relationship between pollinators and petal epidermal form.

Keywords: tactile structures, grip, floral orientation, plant surface, conical cell, pairwise comparisons

Introduction

Angiosperms produce an incredibly diverse range of flowers. The evolution of many floral features has been driven by co-evolution with, and adaptation to, animal pollinators. One floral feature that appears to be intricately linked to animal pollination is that of floral morphology, from the shape of the whole flower to the structure of the petal epidermis. The production of cone-shaped papillate epidermal cells (conical cells – see figure 1A) is a morphological feature that appears to have been widely selected for as it occurs in approximately 79% of petaloid angiosperm species (Kay et al. 1981).

FIGURE 1 NEAR HERE

Each flower presents a complex set of multiple cues and signals which influence the attraction and foraging efficiency of pollinators, and much research is still necessary to disentangle the influence of components of floral morphology such as colour (Chittka & Menzel 1992), symmetry (Giurfa et al. 1999), patterning (Whitney et al. 2009a) and iridescence (Whitney et al. 2009a, b). All of these visual components of floral design are not always solely dependent on floral pigmentation to produce the signal but can also, to a greater or lesser extent, be produced or enhanced by the cellular morphology of the petal epidermis (Baumann et al. 2007; Noda et al. 1994; Whitney et al. 2009b). This was first observed in the *mixta* mutant of *Antirrhinum*, which was originally isolated due to the effect that the loss of conical cells has on petal colour (Noda et al. 1994).

The cellular microstructure of the petals can also influence non-visual components of the flower. It has long been known that honeybees are able to tell the difference between surfaces based on their tactile quality alone (Erber et al. 1998; Kevan & Lane 1985; Scheiner et al. 1999; Scheiner et al. 2001; Simone-Finstrom et al. 2010), and it has been demonstrated that surface structure influences the sensory cues picked up by hawkmoths (Goyret & Raguso 2006).

We recently demonstrated that free-flying foraging bumblebees are able to discriminate between petal surfaces with different cellular structure by touch alone. Using differential conditioning with both *Antirrhinum mixta* mutants and biomimetic replicas of floral surfaces, which differed only in the presence or lack of conical cells, it was found that bumblebees could distinguish between flat celled and conical celled surfaces that produced no visual cue. Furthermore, the same live and artificial flowers were used to determine whether bees showed any innate preference for particular floral surfaces. These experiments showed an effect of

floral orientation on the response of the bees, which showed a distinct preference for a ‘rough’ cellular surface when the real and artificial flowers were oriented vertically. The observation that there was little preference when the flowers were presented horizontally suggested that a rougher surface may be preferred if it aids the bee in gripping the flower when vertical, but will offer little added value to a horizontal flower on which the pollinator can land and sit without structural aid (Whitney et al. 2009c, d).

This suggests that if rough cellular structures are found in petals in order to aid the grip of visiting pollinators, then species that orient their flowers vertically should be more likely to possess these structures. Here, we conduct a comparative analysis using a well-characterised dataset of the cellular structure of petals (Kay et al. 1981) to test this hypothesis. We examine the link between floral orientation and whether conical cells are present on the inner or outer epidermis of the petal. If conical cells are principally present to add ‘grip’ to a petal, we would expect there to be a correlation between orientation and the presence of conical cells on the inner epidermis, but would not expect any correlation with cells on the outer epidermis.

Methods

Comparative analysis

Correlating characters using data from multiple species requires phylogenetically-controlled tests in order to avoid problems of autocorrelation (Harvey & Pagel 1991; Martins 1996; Nee et al. 1996; Pagel 2000). Controlling for phylogenies when attempting to correlate discrete characters with each other raises further problems (Ridley & Grafen 1996), which we addressed using the methods described by Maddison (2000), whereby phylogenetically separate pairs of species are compared to detect whether characters are associated. This technique involves calculating the likelihood that discrete characters are correlated by considering all possible phylogenetically-separated pairings of species within a taxonomic tree (or, as we conduct here, a suitable randomly sampled subset of all pairings possible if the phylogeny is large).

We constructed a complete phylogeny as described below. Using orientation and presence/absence of cell types as binary data, we tested for paired character correlations with the *Pairwise Comparisons* 1.1 package (Maddison 2006) within *Mesquite* 1.12 (Maddison & Maddison 2006), using the ‘pairs for two characters’ option to allow for the arguments raised by Read & Nee (1995). Probabilities of correlation were calculated between plant epidermal structures and orientation (both assessed as described below), limiting our sampling to 1,000,000 of the possible sets of pairings.

Plant characters

Epidermal structures were coded according to table 2 of Kay et al. (1981). This table presents three levels for each structure: absence, ‘+’ or ‘++’: we translated this to a binary ‘absent’ or ‘present’. The cell-types described by Kay et al. (1981) as ‘lenticular’, ‘multiple-lenticular’, ‘convex-lenticular’, ‘striated’, ‘reversed-papillate’ and ‘multiple-reversed papillate’ were disregarded because their effect on pollinator behaviour is unknown. Cells coded as ‘flat’ (e.g. figure 1B) were however included as a comparison.

Floral orientation was coded independently from knowledge of epidermal structure, by searching a number of botanical image databases on the 26th February 2008: sites indexed

within the Vascular Plant Image Library (www.csdl.tamu.edu/FLORA/gallery.htm), the Plant Image Gallery (www.plant-pictures.com/), CalPhotos (<http://calphotos.berkeley.edu/>), BioPix (www.biopix.com), the Encyclopedia of Life (www.eol.org), Aluka (www.aluka.org), the Floras of North America, China, and Russia (www.efloras.org), Tropicos® (<http://www.tropicos.org>), Karlsruhe University Botanic Garden (<http://www.botanik.uni-karlsruhe.de>), www.pflanzen-im-web.de, www.agraria.org, Desert Tropicals (www.desert-tropicals.com), and Hong & Zhou (2003). From these images, flowers were scored as being ‘upward’ or ‘not-upward’. To qualify as upward, the region of the flower through which a pollinator would enter/probe in order to gain access to the nectaries had to be pointing upwards or within 45° of upright. To qualify as “not-upward”, the entrance had to be at an angle greater than 45° (so a vertically-presented flower such as a *Narcissus* and a hanging flower such as a *Campanula* would both be classified as not-upward). In the case of compound flowers, unless all florets were classifiable as upward (such as in *Daucus carota*), the species was classified as not-upward. Data are presented in Additional File 1.

Phylogenetic reconstruction

Species considered in the analysis were taken from the table presented in Kay et al. (1981): entries that were not named to the species level were discarded. After checking for synonyms using the *Integrated Taxonomic Information System* (<http://www.itis.gov>), *Flora Europaea* (<http://rbg-web2.rbge.org.uk/FE/fe.html>), *Tropicos* (<http://www.tropicos.org>) and the *Germplasm Resources Information Network* (<http://www.ars-grin.gov>), we were unable to find suitable pictorial data for the species identified by Kay et al. as “*Ranunculus petala*”, and the exact phylogenetic position of *Honckenya peploides* (L.) Ehrh. (Caryophyllaceae), *Moltkia petraea* Griseb. (Boraginaceae), *Erophila verna* (L.) DC. (Brassicaceae), *Saxifraga rosacea* Moench, and *S. cebennensis* Rouy & Camus (Saxifragaceae). Because these taxa could not be resolved from the literature, they were removed from the analysis. In total, 183 species were used in the analyses, of which 90 (46.6%) were classified as ‘upward’.

The pairwise comparisons technique used here relies on comparing dichotomous branching events within a phylogeny, and does not require any information about rates of change. We therefore drew up a phylogeny (sketched in Additional Material 2) based on the taxonomic relationships described in detail by Stevens (2007). Because the phylogeny of the Lamiales is still largely unresolved (Wortley et al. 2005), we based the ordering of the relevant families on Olmstead et al. (2001) and Oxelman et al. (2005). Table 1 lists the sources used to resolve polytomies (where possible) within families. Note that not all polytomies could be resolved (described in the legend to Table 1), but these had no effect upon the analyses conducted.

TABLE 1 NEAR HERE

Results

TABLE 2 NEAR HERE

Table 2 records confidence intervals described by the upper and lower limits of the probabilities that correlations seen between orientation and cell morphology were due to chance, given 1,000,000 samplings of possible pairwise comparisons. For all of the results described in Table 2, all of the intervals described included at least some values where $p > 0.05$ (and, because multiple comparisons are made using the same dataset, applying Bonferroni corrections should reduce the critical p value below the minimum 0.032 estimate

given in Table 2 for flat, outer epidermis cells). Therefore, we cannot demonstrate that there are any meaningful correlations between orientation and petal surface.

Discussion

The lack of correlation between floral orientation and the presence of conical cells on the upper or lower petal epidermis suggests that conical cells aren't involved solely with providing grip to visiting pollinators. However, this does not rule out the possibility that they play a large part in providing grip in some species. Whitney et al. (2009c, d) demonstrated that there were behavioural differences in bumblebees landing on 'smooth' or 'rough' flowers, where the bees scabbled for grip much more when landing on the smooth surface. For a pollinator attempting to land on a flower, the orientation of the flower may be less important than whether the flower is going to move whilst the pollinator is landing. Surface structure may well reflect the windiness of the environment, and we suggest that more work should be done to explore the influence of floral movement on pollinator choice, as little is known about this aspect of floral biology (Warren & James 2008). It would therefore be useful to assess the interaction between surface structure and the amount of movement a flower experiences in its environment.

The other functions that conical cells play within the flower may be masking a significant relationship between surface structure and floral orientation. For example, petal structure may also influence the microclimate and temperature of the flower, offering a small heat reward to the forager, in addition to the nectar, pollen or other nutritional rewards it is receiving (Dyer et al. 2006; Rands & Whitney 2008; Whitney et al. 2008, in press). There is still some debate about what currencies pollinators use when they forage (Charlton & Houston 2010), or the confounding effects on pollinator visiting behaviour of multiple concurrently-flowering species with different rewards or floral morphologies (Rands & Whitney 2010). Similarly, the tactile cue may be enhancing the cues received through other sensory pathways, such as by providing information about the location of nectaries once the pollinator has close-approached the flower (Goyret & Raguso 2006). Conical cells may therefore be providing a multimodal range of signals. As multimodal signals have been found to enhance decision making in foraging pollinators such as bumblebees (Leonard et al. 2011), a single floral feature that could potentially enhance insect pollinator constancy by providing visual, heat and tactile signals, as well as enhancing pollinator foraging by improving their grip when handling a flower, could be a significant advantage. Exploring the interactions between different signals provided by this particular floral feature in guiding a forager around the flower may therefore provide some interesting new insights into plant-pollinator interactions.

The role of floral orientation itself may also confound the relationship we were testing. Little work has yet been conducted exploring the effect that orientation has upon pollinator behaviour. Floral orientation may be linked to the pollinators a flower is adapted to attract, as well as the environment in which it grows (Fenster et al. 2009). For example, in a rainy environment, it may be optimal for a plant to have its flowers pointing downwards in order to avoid flooding out the nectar (Aizen 2003; Tadey & Aizen 2001). Similarly, heliotropic plants move their flowers to face towards the sun throughout the day, which may be an important strategy for pollinator attraction or floral development in cold climates (Kevan 1975; Kudo 1995; Patiño et al. 2002). Furthermore, field experiments have demonstrated that unusual floral orientation may have an effect upon pollinator landing behaviour (Ushimaru & Hyodo 2005) and pollination success (Ushimaru et al. 2009), and it is known that floral orientation can be used as a cue by honeybees (Lamb & Wells 1995) and beetles (Dafni & Potts 2004). As well as heliotropic movement, there is some evidence that plants may orient the angle of their flowers during growth to take advantage of environmental features (Imamura & Ushimaru 2007; Ushimaru et al. 2006). It is therefore feasible that plants could alter both the

angle and the surface structure of their flowers to best attract and accommodate visiting pollinators.

Finally, this study does not account for the pollinators involved with each of the plant species considered. ‘Grip’ structures would be useless in a flower that is principally pollinated by a hovering pollinator (or may even be detrimental, if it makes it easier for non-beneficial visitors). Equally, this study does not take into account the variety of mechanisms that pollinators use to collect pollen. Some, such as those that conduct buzz pollination (where flowers are usually down-facing) need to grip onto the anthers independently of the sepals or petals to be able to conduct pollen collection, and the use of anthers and other floral structures in flowers (such as in the Malvaceae) may also independently enhance insect grip. It could also be in the plant’s interest to have petals that are less grippable, as this then forces the pollinator to use this alternative floral architecture in order to harvest pollen, which in turn might enhance pollen transfer. It would therefore be useful to include the mode of pollinator access to the flower within the analysis. For a large-scale comparative analysis, this is no easy task: pollinator identity is rarely known, flowers are often adapted to a range of pollinators, and growing evidence suggests pollination syndromes should not be trusted (Ollerton et al. 2009). Furthermore, we used a very simple binary summary of floral structure within this study (‘upright’ *versus* ‘not upright’), and it could be the case that the structure we assume to be in contact with a visiting pollinator may not be at the orientation we recorded here (especially for the species where flowers were found to be in all orientations, which were simply treated as ‘non-upright’ in our dataset). Ideally, we would suggest that a database of plant species is compiled, recording the identity of pollinators visiting the species, the structure of the surface that they are in contact with during the visit, and the orientation of that surface. Such a database would give us a much more detailed insight into the relationship between floral structure and pollinator choices.

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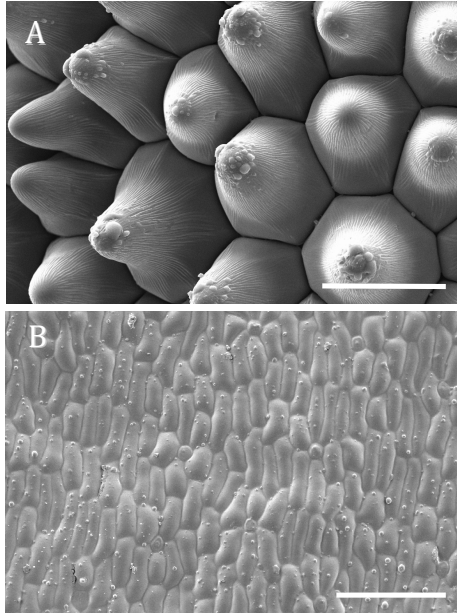
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Figure 1. Examples of petal epidermal cell shape. **A)** conical cells, from *Antirrhinum majus* (Scanning Electron Microscope image, scale bar = 50 μm); **B)** flat cells from *Nymphaea alba* (scale bar = 200 μm).



Tables

Table 1. Sources of information used to resolve polytomies in combined phylogeny. In the Apiaceae, the two sources (Downie et al. 2000; Spalik & Downie 2001) give alternative resolutions, but the four species involved have identical characters and therefore exact order has no effect on the analyses conducted. In the Hyacinthaceae, *Chionodoxa* was grouped with *Scilla*, as discussed by Speta (1971) and Pfosser and Speta (1999).

Apiaceae	Downie et al. (2000), Spalik & Downie (2001)
Asteraceae	Bayer and Starr (1998), Goertzen et al. (2003), Jansen et al. (1991), Oberprieler and Vogt (2000), Watson et al. (2000)
Boraginaceae	Långström and Chase (2002)
Brassicaceae	Al-Shebhaz et al. (2006), Beilstein et al. (2006), Warwick and Sauder (2007)
Caryophyllaceae	Desfeux et al. (1996), Fior and Karis (2007), Fior et al. (2006), Oxelman et al. (1997)
Dipsacales (= Caprifoliaceae)	Donoghue et al. (1992)
Fabales (= Papilionaceae)	Wojciechowski (2003)
Geraniaceae	Parkinson et al. (2005)
Hyacinthaceae	Pfosser & Speta (1999)
Iridaceae	Goldblatt (1990), Reeves et al. (2001)
Lamiaceae	Wagstaff et al. (1995)
Onagraceae	Hoch et al. (1993), Levin et al. (2003)
Primulaceae	Anderberg et al. (2000), Martins et al. (2003)
Ranunculaceae	Ro et al. (1997)
Rosaceae	Morgan (1994)
Rubiaceae	Natali et al. (1995)
Saxifragaceae	Vargas et al. (1999)
Scrophulariaceae	Albach and Chase (2001), Vargas et al. (2004)

Table 2 - Probabilities of correlation between flower orientation and epidermal structure

All the values in the table represent the minima and maxima for the range of probabilities that any correlation seen in characters and orientation were due to chance, coming from surveys of 1,000,000 of the possible pairings. Because we are conducting multiple tests for correlation with flower orientation, none of the epidermal cell structures correlate with orientation after Bonferroni corrections.

shape of epidermal cells	upper epidermis	lower epidermis
Papillate	0.100 – 0.181	0.416 – 0.584
Multiple-papillate	0.500 – 0.500	0.500 – 0.500
Flat	0.172 – 0.172	0.032 – 0.084

Additional files

Additional file 1 – Data used for comparative study

This file (a comma-delimited text file) presents the character states of the 183 species used in the analysis. The top line of the file details the character type. For each species on subsequent lines, the first six characters are the character states found in Table 2 of Kay et al. (1981) where '0' denotes the absence and '1' denotes the presence of the relevant epidermal structures. The final column for each species presents the orientation data collected as is described in the methods: '0' denotes upward and '1' denotes not-upward flowers.

Additional file 2 - Phylogeny used for the comparative analyses.

Drawn using *Treeweb X* (Page 1996).