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Intraspecific variation in the petal epidermal cell morphology of *Vicia faba* L. (Fabaceae)

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ABSTRACT

At a microscopic scale, the shape and fine cell relief of the petal epidermal cells of a flower play a key role in its interaction with pollinators. In particular, conical shaped petal epidermal cells have been shown to have an important function in providing grip on the surface of bee-pollinated flowers and can influence bee visitation rates. Previous studies have explored interspecific variation in this trait within genera and families, but naturally-occurring intraspecific variation has not yet been comprehensively studied. Here, we investigate petal epidermal cell morphology in 32 genotypes of the crop *Vicia faba*, which has a yield highly dependent on pollinators. We hypothesise that conical cells may have been lost in some genotypes as a consequence of selective sweeps or genetic drift during breeding programmes. We find that 13% of our lines have a distribution of conical petal epidermal cells that deviates from that normally seen in *V. faba* flowers. These abnormal phenotypes were specific to the ad/abaxial side of petals, suggesting that these changes are the result of altered gene expression patterns rather than loss of gene function.

1. Introduction

The majority of flowers rely on animals, particularly insects, for their pollination. This has led to a magnificent array of flower colours, scents and shapes which maximise the reproductive fitness of species with diverse pollinators (Faegri and van der Pijl, 1979). One trait that is less well known with respect to its influence on pollinator visitation rates is that of the fine scale surface structure of the petal. The epidermis of plants is highly variable in morphology, with different cell shape and cell surface textures resulting from cuticle folding and ornamentation with other compounds such as epicuticular waxes (Koch et al., 2008). These different cell structures influence the interaction of plants with pathogens, pests and mutualists by altering the grip and accessibility of the surface, as well as its optical properties (Gorton and Vogelmann, 1996; Comba et al., 2000; Whitney et al., 2009; Alcorn et al., 2012).

One particular cell morphology that influences the interaction of a flower with its pollinators is the presence of conical petal epidermal cells. These cone-shaped cells are found on the petals of 75–80% of angiosperms analysed (Kay et al., 1981; Christensen and Hansen, 1998). Bees have been shown to have a preference for flowers with conical epidermal cells (Glover and Martin, 1998), especially when flowers are more difficult to manipulate, because they improve grip on the surface (Whitney et al., 2009; Alcorn et al., 2012). This increased grip will reduce the energy expenditure required to feed from a flower. Conical cells have been suggested to increase the temperature of flowers (Comba et al., 2000), although there is debate about the extent and significance of this effect (Whitney et al., 2011a). Therefore, conical cells may further reduce the energy expenditure of bees by reducing their need to use muscle shivering to maintain their body temperature (Heinrich and Esch, 1994). From an advertising perspective, conical cells are also known to benefit a flower by enhancing its colour by focusing light onto the floral pigments (Noda et al., 1994; Gorton and Vogelmann, 1996). It has also been suggested that conical cells, which reduce the wettability of the flower surface, act as a self-cleaning mechanism to keep flowers free of dust and other particles which may make their surface less attractive to pollinators (Whitney et al., 2011b).

Bilaterally symmetrical flowers such as those found in most legumes are particularly interesting when investigating the function of petal epidermal cell morphology because of the specific way pollinators interact with these petals. Fabaceae flowers are generally organised into three petal types: the dorsal standard, lateral wing and ventral keel petals. The wing and keel petals are joined at their base by petal folds. During a legitimate visit, a bee alights on the wing petals and pushes

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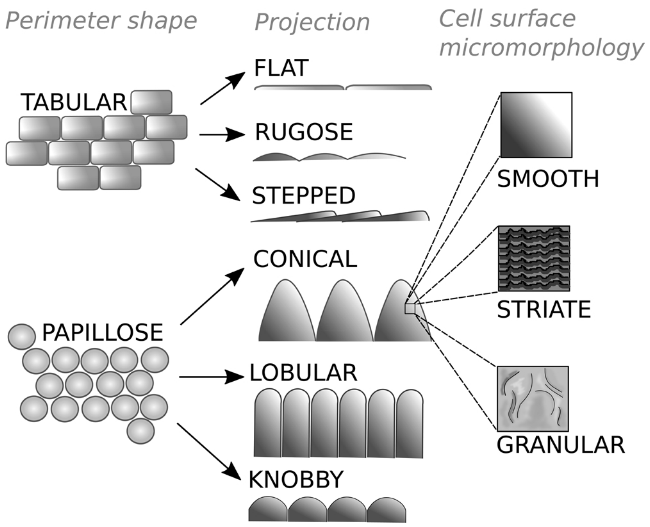

Fig. 1. The classification of the protruding parts of epidermal cell morphology. Epidermal cells can be classified based on three levels, the shape of the cell perimeter (Perimeter Shape), the amount of projection from the cell surface (Projection), and the micromorphology of the cell surface (Cell surface micromorphology).

downwards on the wing petals to allow access to the nectar at the base of the flower and pollen contained on the anthers and within the keel petals (Stoddard, 1991). The standard predominantly acts as an advertisement to pollinators.

A large-scale analysis of flower epidermal cell morphology in the Fabaceae identified six main categories of cell types (Fig. 1) based on both their primary (cell shape) and secondary structure (cell wall fine relief); tabular rugose granular, tabular rugose striate, tabular flat striate, papillose conical striate, papillose knobby rugose, and papillose lobular striate (Ojeda et al., 2009). This study suggested that certain cell types are associated with the standard, wings and keel petals in Fabaceae. For example, papillose conical striate cells (conical cells) are generally a feature of the standard and wing but not keel petals in the most derived subfamily, the Papilionoideae (Ojeda et al., 2009). Given that the keel petal plays more of a functional role in containing the pollen of the flower rather than directly interacting with or attracting pollinators, this distribution of cell morphology within the flowers of the Papilionoideae is therefore not surprising.

Previous investigations into the distribution of petal epidermal cell morphology have largely focused on differences between broad taxonomic groups (Kay et al., 1981; Christensen and Hansen, 1998; Papiorek et al., 2014) or within specific families (Baagøe, 1977, 1980; Ojeda et al., 2009). A handful of studies have also examined differences in petal epidermal morphology within genera, particularly in genera with more than one functional group of pollinators (Di Stilio et al., 2009; Çildir et al., 2012; Ojeda et al., 2012, 2016). From these previous studies we know that substantial variation can occur in the petal epidermal cell types present between flowers of different species, even within a genus. This is particularly true when species evolve associations with non-insect pollinators. For example, in all five cases of shifts from insect to bird pollination in Macaronesia (in *Lotus* spp. L. (Fabaceae), *Anagyris latifolia* Brouss. ex Willd. (Fabaceae), *Navaea phoenicea* Webb & Berthel. (Malvaceae), *Isoplexis* spp. (Lindl.) Loudon (Plantaginaceae), and *Canarina canariensis* (L.) Vatke (Campanulaceae)) the transition is associated with the loss of conical cells (Ojeda et al., 2016). More generally, bird pollination is associated with quantitatively flatter petal epidermal cells than in bee pollinated flowers, which may help to deter nectar robbing bees (Papiorek et al., 2014). Similarly, in *Thalictrum* Tourn. ex L. transitions from insect to wind pollination are also associated with the loss of conical cells (Di Stilio et al., 2009). However, despite these investigations into the distribution of petal epidermal cell types across flowering plant families, there is little discussion of intraspecific variation. Those studies that have examined multiple individuals from a single species have generally found no significant variation between individuals (Ojeda et al., 2009; Çildir et al., 2012), with the exception of two subspecies of *Echium wildpretii* H.Pearson ex Hook.f. with different functional groups of pollinators (Ojeda et al., 2016). However, these studies had limited sample sizes (2–6 individuals per species), and no explicit intention to sample across the genetic diversity of a species, and may therefore underrepresent the diversity of epidermal phenotypes found within a species.

Crop plants present an ideal opportunity to explore the presence of intraspecific variation in petal epidermal morphology, because many independent genotypes are retained in stock centres for commercial breeding. Crops such as the field bean *Vicia faba* L. are dependent on pollinators for maximum yield (Klein et al., 2007; Cunningham and Le Feuvre, 2013; Garratt et al., 2014). However, floral traits are rarely selected for in breeding programmes and therefore may have become suboptimal for maximizing pollination through genetic drift (Kobayashi et al., 2010; Bailes et al., 2015). Previously it has been reported that the major epidermal cell type present on *V. faba* standard and keel petals are tabular cells, whereas the wing petals mainly display conical cells (Ojeda et al., 2009). We were interested in determining whether this phenotype was consistent between different genotypes of *V. faba* or whether intraspecific variation, potentially providing an opportunity for selective breeding to improve pollinator attraction, was present. We examined the petal epidermal cell morphology of the apical (pollinator-contacting) portion of cells for 32 genetically distinct lines of *V. faba* and asked (i) which cell types are present within *V. faba* flowers? and (ii) is there variation in the distribution of conical petal epidermal cells between genetically distinct lines of *V. faba*?

2. Methods

2.1. Plant material

To determine the level of variation in epidermal cell morphology within *V. faba* we randomly selected 32 lines from the seed collections at the National Institute of Agricultural Botany (Sources in Table S1). These lines had been self-pollinated for at least 5 generations and therefore should be homozygous at the majority of loci. The majority of the lines were white with black wing-petal spots, as is typical for field bean flowers. However, lines NV175, NV643, NV644, NV676 and NV868 lack wing-petal spots, and are pure white. Line NV706 had a crimson flower with dark wing-petal spots. Vouchers for specimens of a plant from each line used in the study were deposited in the University of Cambridge herbarium (Cambridge, UK), with the voucher numbers CGE33556 – CGE33587 (Table S1).

2.2. Sampling strategy

For each line, one flower was analysed to represent that genotype, as petal epidermal cell type has never been shown to be influenced by environment. The pollinator-interacting wing and standard petals were imaged for all 32 lines, focusing on the distribution of conical cells in these petals. For a subset of five of these lines a more in depth analysis of the cell types present was undertaken, including of the keel petals.

2.3. Imaging

Dental wax (Zhermack Elite HD + Dental wax, Light body) casts of fresh fully open flowers were made for both the adaxial and abaxial surface of all petals of interest by pressing each petal into freshly mixed wax then peeling the petal away once the wax was set. This method preserves the native structure of the petal surface and reduces the risk of introducing artefacts compared to tissue preparation processes that use dehydration. From these, epoxy-resin replicas were produced using