Petal cell shape and flower-pollinator interaction in *Nicotiana*

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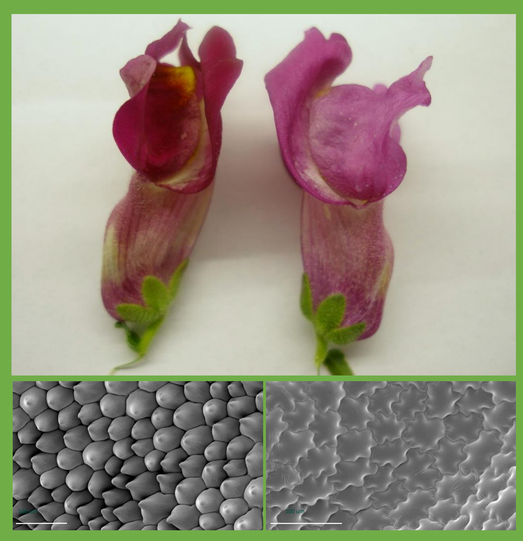
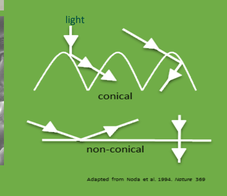
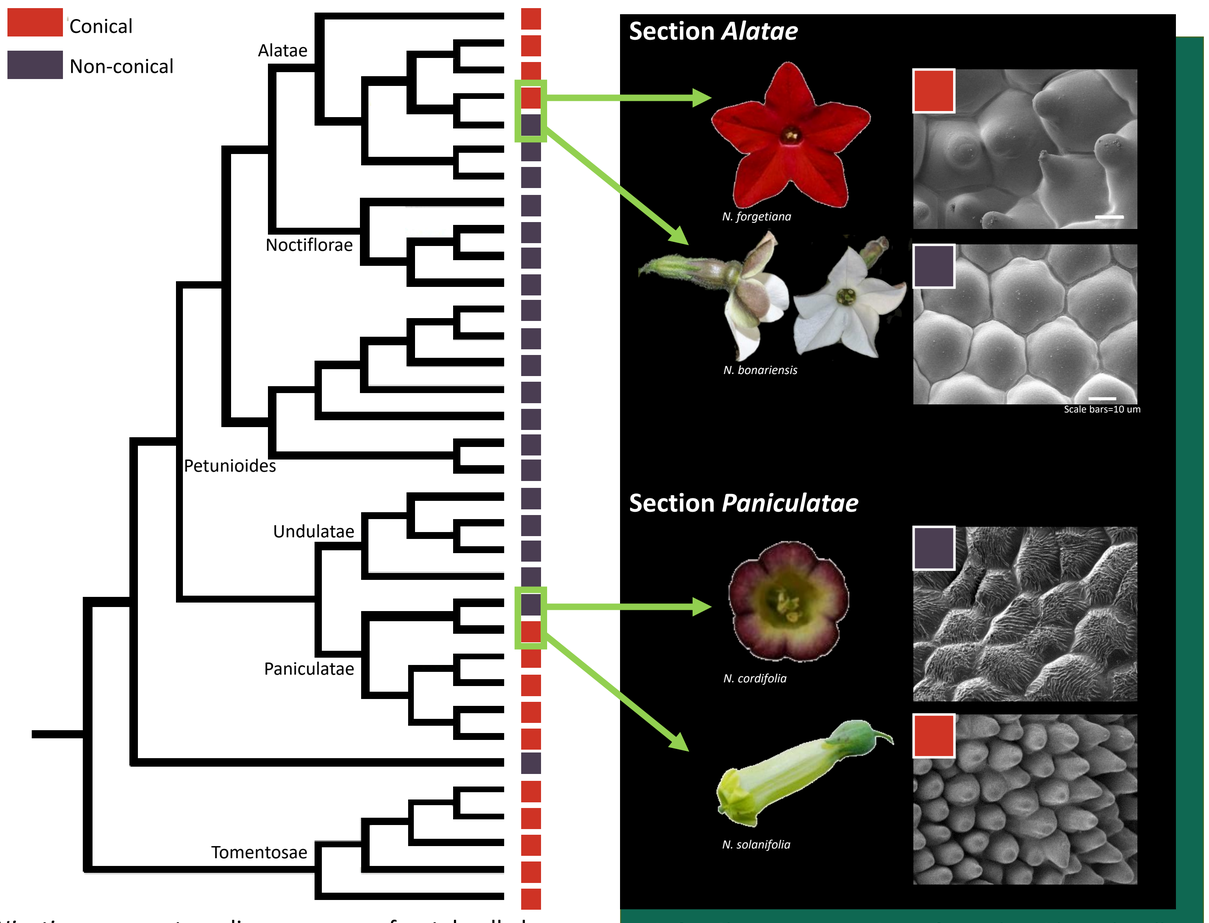
Petal epidermal cell shape plays a crucial role in plant-pollinator interaction. Conical cells are known to enhance petal colouration, regulate petal temperature and wettability, and increase grip for pollinators[1]. This research looks to understand the developmental and genetic mechanisms that regulate petal cell shape in ***Nicotiana*** by comparing sister species with contrasting characteristics (conical■ vs. non-conical■). It also explores the impact petal cell shape has on the interaction between flowers and pollinators in the genus.



Petals with conical cells in wild type *Antirrhinum majus* (left) present enhanced colouration and provide increased grip for small pollinators (*e.g.* bees) compared to petals with non-conical cells in mutants (right).



Convergent evolution of petal cell shape in *Nicotiana*

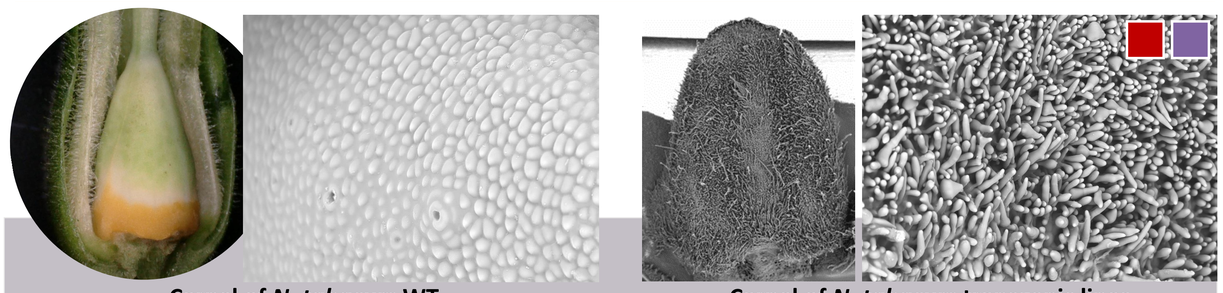


Nicotiana presents a diverse range of petal cell shapes. Interestingly, sister species in at least two phylogenetically distinct clades of the genus have contrasting petal epidermal cell shapes (**conical vs. non-conical**). This provides an exceptional opportunity to study the evolution of petal epidermal cell shape and explore convergent evolution of this trait in the genus.

Left: Character mapping of petal epidermal cell shape in the diploid species of *Nicotiana.* Phylogeny modified from [2] and [3].
Right: Flower morphology and petal epidermal cell micrographs of sister species *Nicotiana forgetiana* and *N. bonariensis* (Section *Alatae*) and *N. solanifolia* and *N. cordifolia* (Section *Paniculatae*).

Molecular control of petal cell development

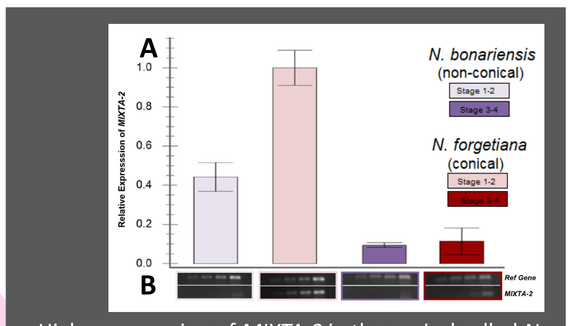
The R2R3 MYB subgroup 9 transcription factors are known to be involved in the regulation of epidermal cell outgrowths in plants. A candidate gene approach demonstrated that differences in gene expression rather than nucleotide substitutions in the gene sequences might be responsible for the differences in petal cell shape between sister species of *Nicotiana.*



Carpel of *N. tabacum* WT **Carpel of *N. tabacum* transgenic lines**

Alternative versions of the candidate genes, those from the conical celled species as well as those from the non-conical celled species, were ectopically expressed in *Nicotiana tabacum* via *Agrobacterium tumefaciens* genetic transformation. All versions of the candidate genes, regardless of their origin (conical or non-conical celled species), had the ability to develop epidermal outgrowths, including conical cells.

Quantification of the relative expression of candidate genes across developmental stages of petals, using real-time quantitative PCR (**A**) and semiquantitative PCR (**B**), revealed significant differences between the conical celled and the non-conical celled species of *Nicotiana.*



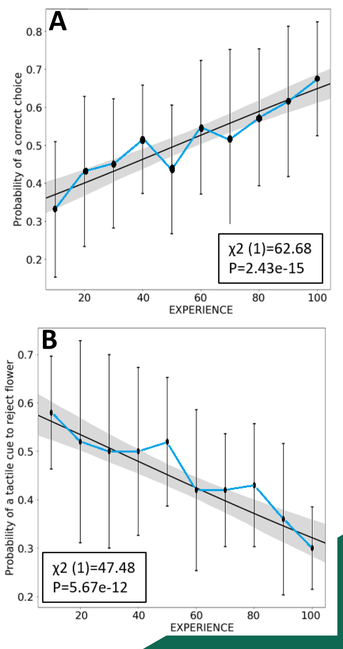
Higher expression of *MIXTA-2* in the conical celled *N. forgetiana*, compared to the non-conical celled *N. bonariensis,* suggests this gene might be involved in conical cell development in this species.

Development of petal epidermal cells in *Nicotiana*

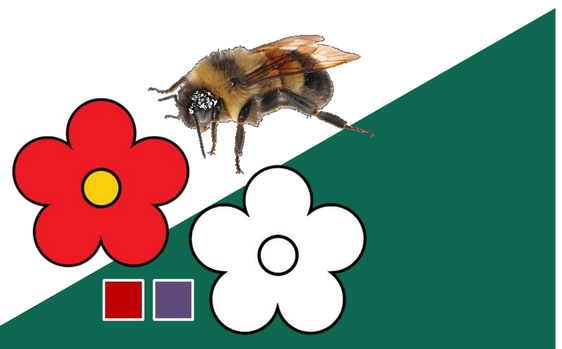
Similar stages of cell development are reached at similar stages of floral development in *N. forgetiana* (conical) and *N. bonariensis* (non-conical). Both species start with flat cells (stage 1). The cells expand and become more rounded (stage 2-3). By stage 4 the cells of both species are dome shaped. The differences in final cell shape of these two species do not become apparent until late in floral development (stage 5).

Flower morphology and cryo-SEM characterization of *N. forgetiana* (A) and *N. bonariensis* (B). Numbers indicate successive developmental stages. Scale bars for morphology series = 10 mm; scale bars for SEM series = 10 µm

Exploring the function of petal cell shape in pollination

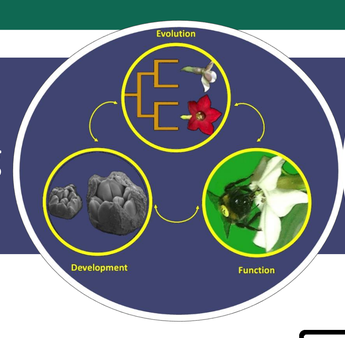


Learning curves for 20 bees choosing from six discs, three with conical and three with non-conical celled texture. The probability of a bee choosing a rewarding flower **(A)** increases with experience, while the probability of a bee using tactile clues to reject a punishing flower **(B)** decreases with experience.



Differential conditioning experiments with artificial flowers indicate that the bumblebees can discriminate flowers with conical from flowers with non-conical surfaces, on a red and on a white background, using visual cues alone as well as tactile cues alone.

***Nicotiana* (Solanaceae)** as a system to further understand the **EVOLUTION, DEVELOPMENT and FUNCTION** of petal cell shape in flowering plants and its implications in pollination systems, by combining tools of molecular biology, morphology and pollinator behaviour experiments.



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References: [1]Whitney et al., 2009. Curr. Biol. 19: 948–53; [2]Knapp, S. et al. 2004. *Taxon* 53: 73–82; [3]Clarkson, J.J. et al. 2010. *Mol. Phylogenet. Evol.* 55: 99–112.

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