

Review

Sculpting the surface: Structural patterning of plant epidermis

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SUMMARY

Plant epidermis are multifunctional surfaces that directly affect how plants interact with animals or microorganisms and influence their ability to harvest or protect from abiotic factors. To do this, plants rely on minuscule structures that confer remarkable properties to their outer layer. These microscopic features emerge from the hierarchical organization of epidermal cells with various shapes and dimensions combined with different elaborations of the cuticle, a protective film that covers plant surfaces. Understanding the properties and functions of those tridimensional elements as well as disentangling the mechanisms that control their formation and spatial distribution warrant a multidisciplinary approach. Here we show how interdisciplinary efforts of coupling modern tools of experimental biology, physics, and chemistry with advanced computational modeling and state-of-the art microscopy are yielding broad new insights into the seemingly arcane patterning processes that sculpt the outer layer of plants.

INTRODUCTION

Humans have tried to see beyond their sight for as long as ~1000 BC. The first cells observed by Hook were plant cells. One of those cells, a hair on a stinging nettle, is beautifully illustrated in the book *Micrographia* (Hooke et al., 1665), and microscopic structures on the surfaces of plants have been an object of fascination ever since. The plant epidermis is a single-cell layer that covers all plant organs and consists of a handful of characteristic cell types: pavement cells are the most common and form the bulk of the epidermis; guard cells work in pair to control gas exchange, whereas trichomes, or hairs, made of one or several cells constitute highly specialized structures that fulfill a wide range of functions. Other less common cell types can be found in specific species or tissues: the epidermis of grasses for instance often displays species-specific distribution patterns of silica cells, a type of biomineralized cell, paired with cork cells (Kumar et al., 2017). Epidermal cells are topped with a waxy waterproof layer, the cuticle. The cuticle combines a polymer of C16 and C18 fatty acids known as cutin (although another biopolymer, cutan, is sometimes present) with a mixture of waxes made of very long-chain fatty acids (C20 to C34), terpenoids, sterols, and flavonoids. Waxes can be embedded within or deposited on top of this cuticular matrix. We now have a good understanding of the biosynthetic pathways involved in the synthesis of the cuticle “building blocks” (reviewed in Fich et al., 2016; Leide et al., 2020; Lewandowska et al., 2020, Table 1). Recent studies have also shed light on the mechanisms involved in the delivery and assembly of cuticular components (Stępiński et al., 2020, Table 1) as well as on the origins and evolution of associated pathways (Kong et al., 2020; Leide et al., 2020; Li and Chang, 2021). However, the processes that sculpt the cuticle and give the plant epidermis its texture are not well understood. The cuticle varies in thickness (from a few nm to a few μm), ultrastructure, and composition between species, organs, or during development, but together with the epidermis it creates a multifunctional interface between the plant and its environment, participating in protection, communication, gas exchange, and water retention.

Colorful markings such as those found on the petals of many flowers can easily catch the eye, but local accumulation of chemical pigments is by no means the only way to pattern plant surfaces. Indeed, a multitude of cell shapes combined with cuticular elements of various geometries produce intricate tridimensional patterns on the epidermis of aerial organs. In this review we will refer to those patterns as “structural patterns” to distinguish them from the colorful “chemical patterns” produced by pigments only. Those microscopic patterns can confer remarkable physicochemical properties to plant surfaces. Scientists from all disciplines are working together to explore their biological functions and the roles played by physics and chemistry in their formation. Engineers, designers, architects, and artists alike use them as a source of inspiration

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Table 1. Recent reviews covering in details specific aspects relevant to plant epidermis sculpting

Topic	Reference	DOI
Plant epidermis	Robinson, D.O. and Roeder, A.H., 2015. <i>Current Opinion in Genetics & Development</i> , 32, pp.55–65.	https://doi.org/10.1016/j.gde.2015.01.008
Cuticle biosynthesis	Bhanot, V., Fadanavis, S.V. and Panwar, J., 2021. <i>Environmental and Experimental Botany</i> , 183, p.104364.	https://doi.org/10.1016/j.envexpbot.2020.104364
	Fich, E.A., Segerson, N.A. and Rose, J.K., 2016. <i>Annual review of plant biology</i> , 67, pp.207–233.	https://doi.org/10.1146/annurev-arplant-043015-111929
	Lewandowska, M., Keyl, A. and Feussner, I., 2020. <i>New Phytologist</i> , 227(3), pp.698–713.	https://doi.org/10.1111/nph.16571
	Stępiński, D., et al., 2020. <i>Cells</i> , 9(8), p.1778.	https://doi.org/10.3390/cells9081778
Cuticle origins & evolution	Li, H. and Chang, C., 2021. <i>Plant Signaling & Behavior</i> , p.1943921.	https://doi.org/10.1080/15592324.2021.1943921
	Kong, L., et al., 2020. <i>Plant Physiology</i> , 184(4), pp.1998–2010.	https://doi.org/10.1104/pp.20.00913
Plant cell wall synthesis	Lampugnani, E.R., Khan, G.A., Somssich, M. and Persson, S., 2018. <i>Journal of Cell Science</i> , 131(2), p.jcs207373.	https://doi.org/10.1242/jcs.207373
	Hoffmann, N., King, S., Samuels, A.L. and McFarlane, H.E., 2021. <i>Developmental Cell</i> .	https://doi.org/10.1016/j.devcel.2021.03.004
	Polko, J.K. and Kieber, J.J., 2019. <i>The Plant Cell</i> , 31(2), pp.282–296.	https://doi.org/10.1105/tpc.18.00760
	Lampugnani, E.R., et al., 2019. <i>Trends in Plant Science</i> , 24(5), pp.402–412.	https://doi.org/10.1016/j.tplants.2019.02.011
	Zhang, B., Gao, Y., Zhang, L. and Zhou, Y., 2021. <i>Journal of Integrative Plant Biology</i> , 63(1), pp.251–272.	https://doi.org/10.1111/jipb.13055
Cell growth	Bidhendi, A.J. and Geitmann, A., 2016. <i>Journal of experimental botany</i> , 67(2), pp.449–461.	https://doi.org/10.1093/jxb/erv535
	Chebli, Y. and Geitmann, A., 2017. <i>Current opinion in cell biology</i> , 44, pp.28–35.	https://doi.org/10.1016/j.ceb.2017.01.002
	Cosgrove, D.J., 2018. <i>Plant Physiology</i> , 176(1), pp.16–27.	https://doi.org/10.1104/pp.17.01541
	Cosgrove, D.J., 2018. <i>Current opinion in cell biology</i> , 46, pp. 77–86	https://doi.org/10.1016/j.pbi.2018.07.016
	Franciosini, A., Rymen, B., Shibata, M., Favero, D.S. and Sugimoto, K., 2017. <i>Current opinion in plant biology</i> , 35, pp.98–104.	https://doi.org/10.1016/j.pbi.2016.11.010
Cell shape	Sapala, A., Runions, A. and Smith, R.S., 2019. <i>Current Opinion in Plant Biology</i> , 47, pp.1–8.	https://doi.org/10.1016/j.pbi.2018.07.017
	Eng, R.C. and Sampathkumar, A., 2018. <i>Current opinion in plant biology</i> , 46, pp.25–31.	https://doi.org/10.1016/j.pbi.2018.07.002

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Table 1. Continued

Topic	Reference	DOI
Plant surface & environment	Heredia-Guerrero, J.A., et al., 2018. <i>Global change biology</i> , 24(7), pp.2749-2751.	https://doi.org/10.1111/gcb.14276
	Fernández, V., et al., 2017. <i>Journal of Experimental Botany</i> , 68(19), pp.5293–5306.	https://doi.org/10.1093/jxb/erx302
	Aragón, W., Reina-Pinto, J.J. and Serrano, M., 2017. <i>Journal of Experimental Botany</i> , 68(19), pp.5339–5350.	https://doi.org/10.1093/jxb/erx327
	Arya, G.C., Sarkar, S., Manasherova, E., Aharoni, A. and Cohen, H., 2021. <i>Frontiers in Plant Science</i> , 12, p.1264.	https://doi.org/10.3389/fpls.2021.663165
Plant Biomimetics	Barthlott, W., Mail, M., Bhushan, B. and Koch, K., 2017. <i>Nano-Micro Letters</i> , 9(2), p.23.	https://doi.org/10.1007/s40820-016-0125-1
	Almeida, A.P., et al., 2018. <i>Advanced Materials</i> , 30(19), p.1703655.	https://doi.org/10.1002/adma.201703655
	Speck, O. and Speck, T., 2021. <i>New Phytologist</i> .	https://doi.org/10.1111/nph.17396
Imaging plant surfaces & measuring their properties	Zhao, Y., Man, Y., Wen, J., Guo, Y. and Lin, J., 2019. <i>Trends in plant science</i> , 24(9), pp.867–878.	https://doi.org/10.1016/j.tplants.2019.05.009
	Komis, G., Novák, D., Ovečka, M., Šamajová, O. and Šamaj, J., 2018. <i>Plant physiology</i> , 176(1), pp.80–93.	https://doi.org/10.1016/j.tplants.2019.05.009
	Grossmann, G., et al., 2018. <i>Journal of Cell Science</i> , 131(2), p.jcs209270.	https://doi.org/10.1242/jcs.209270
	Urban, M.A., Barclay, R.S., Sivaguru, M. and Punyasena, S.W., 2018. <i>Microscopy research and technique</i> , 81(2), pp.129–140.	https://doi.org/10.1002/jemt.22667
	Otegui, M.S., 2021. <i>Recent Advances in Polyphenol Research</i> , 7, pp.281–295.	https://doi.org/10.1002/9781119545958.ch11
	Farber, C., Wang, R., Chemelewski, R., Mullet, J. and Kourouski, D., 2019. <i>Analytical chemistry</i> , 91(3), pp.2472–2479.	https://doi.org/10.1021/acs.analchem.8b05294
	Otegui, M.S. and Pennington, J.G., 2019. <i>Microscopy</i> , 68(1), pp.69–79.	https://doi.org/10.1093/jmicro/dfy133
	Ovečka, M., et al., 2018. <i>Nature Plants</i> , 4(9), pp.639–650.	https://doi.org/10.1038/s41477-018-0238-2
	Vignolini, S., Moyroud, E., Glover, B.J. and Steiner, U., 2013. <i>Journal of The Royal Society Interface</i> , 10(87), p.20130394.	https://doi.org/10.1098/rsif.2013.0394
	Vogler, H., Felekis, D., Nelson, B.J. and Grossniklaus, U., 2015. <i>Plants</i> , 4(2), pp.167–182.	https://doi.org/10.3390/plants4020167
Bidhendi, A.J. and Geitmann, A., 2019. <i>Journal of Experimental Botany</i> , 70(14), pp.3615–3648.	https://doi.org/10.1093/jxb/erz281	

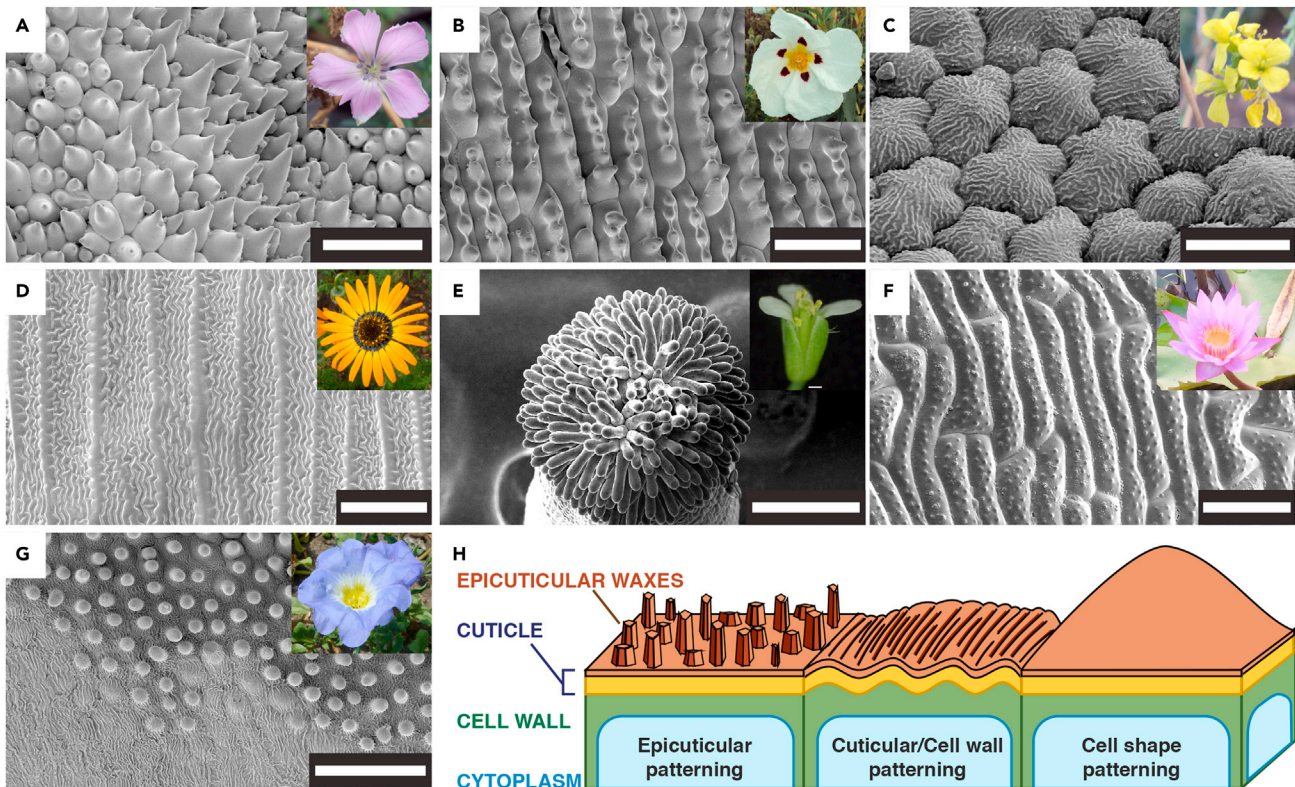


Figure 1. Diversity of tridimensional features patterning the surface of plant epidermis

Examples of patterns created by distinct cell shapes and cuticular features on the surface of flower epidermis, imaged by scanning electron microscopy (SEM).

(A) Conical cells on the petal of *Dianthus fruticosus* (adaxial side, distal region).

(B) Multiple papillate cells on the petal of *Cistus cyrius* (rock rose).

(C) Puzzle-shaped cells of *Sinapis alba* (white mustard) petal ornated with disordered cuticular striations.

(D) Flat elongated and striated cells of *Ursinia speciosa* petal.

(E) Elongated and smooth stigmatic cells of *Arabidopsis thaliana*.

(F) Tabular cells of the petal of *Nymphaea micrantha*.

(G) The corolla of *Nolana paradoxa* displays a structural pattern made of smooth papillate cells in the blue region of the petal limb contrasting with flat cells covered with prominent cuticular ridges in the white portion of the corolla tube.

(H) Schematic representations of plant cell displaying various examples of hierarchical structural patterns: epicuticular waxes forming nanoscopic patterns, nano/microscopic features involving cuticular and/or cell wall sculpting and micropatterning relying on the formation of specific cell shape. Scale bars in (A), (B), (F), and (G), 50 μm ; scale bars in (C) and (D), 20 μm ; and scale bars in (E), 100 μm . Picture credit: (F) Yercaud-elango and (G) Magnus Manske via Wikimedia Commons.

to build novel materials with advantageous properties. However, the mechanisms underpinning the formation and organization of those microscopic patterns remain largely unexplored. Here we review our current knowledge of physical features that pattern the plant epidermis. We focus in particular on recent studies that illustrate how interdisciplinary approaches can transform our understanding of how plants sculpt their surfaces.

STRUCTURAL PATTERNS: DIVERSITY OVERVIEW AND BIOLOGICAL FUNCTIONS

Uncovering and characterizing structural patterns

Plants can display a huge range of patterns on their epidermis, yet we are only just starting to “scratch the surface” of the diversity that populates the microscale. Most flowering plant species remain to be examined with cellular resolution, and not all plant parts have received equal attention. The surface of petals, leaves, seeds, and pollen grains have been examined most frequently. One of the earliest large-scale study of petal epidermal structures recognized six distinct basic cell shapes, including conical/papillate (Figure 1A) and multiple papillate cells, which exhibit one or several small projections on their surface, respectively

(Figure 1B) (Kay et al., 1981). Already, the authors acknowledged that basic cell types can vary enormously in the geometry of their base (e.g., jigsaw in Figure 1C or rectangular in Figure 1D) or in the scale and proportions of the projection. Indeed, cell expansions can involve the entire apical side of a cell or can stem from localized deformations and range from extremely acute (Figures 1E and 1A) to stumpy (Figure 1F). Although such studies provided an unprecedented window into the huge morphological diversity that lays on the surface of plants (Barthlott, 1981; Kay et al., 1981), they also had some shortcomings. We use below a selection of recent examples to illustrate how latter studies have attempted to overcome those limitations by applying a more comprehensive framework, which deploys interdisciplinary and quantitative approaches benefiting from technological advances, to explore plant “skins.”

First, tridimensional features do not fit well into discrete categories but rather belong to a geometrical continuum. Thus, providing qualitative information is often insufficient. As the physical properties of epidermal cells depend on their geometry, a quantitative characterization of cell proportions and dimensions is necessary to investigate their role (e.g., Koch et al., 2013; Schulte et al., 2019) and understand the mechanisms accounting for their formation. The outlines of leaf epidermal cells from more than 270 species of land plants were recently extracted and quantified using morphometric descriptors, ranging from cell area to solidity and circularity (Vofely et al., 2018). The results revealed that most species only exhibit mild undulations of leaf cell margins unlike the prominent interdigitations of the jigsaw pavement cells found in the leaf epidermis of the classic plant model *Arabidopsis*. The significant diversity of leaf cell shapes exposed by this study hints at the possible existence of different programs regulating cell shape in distantly related species. Alternatively, different cell outlines could easily emerge from modifications of the molecular mechanisms known to regulate jigsaw cell formation in *Arabidopsis*. Such changes could involve modifications of the cytoskeleton behavior but also differences in the biochemical and mechanical properties of the cell wall itself (Altartouri et al., 2019).

Second, cell shapes can be combined with a diversity of overlaying nanostructures (Figure 1H) involving components of the cuticle and sometimes the cell wall. Cells apical surfaces can be smooth (Figures 1A and 1B) or decorated with minute structures (such as striations seen in Figure 1D) that must be accounted for to understand epidermis properties. By deploying a 3D surface profiler to quantify precisely leaf roughness in various species, a team of engineers showed that the ability of leaves to wet is highly dependent on the height and relative spacing between the micro/nanofeatures that generate the coarseness (Abbott and Zhu, 2019). Similarly, measuring accurately the geometry of conical cells from flowers displaying UV-absorbing and UV-reflecting sectors revealed that the UV-absorbing regions tend to have higher, more acute, cellular profiles. As transparent replicas of the petal surface retained the UV motifs, this further showed that UV patterns are not solely due to the local accumulation of UV-absorbing pigments but they also have a structural cause (Schulte et al., 2019).

Finally, cell shape and cuticular ornamentation not only differ between species but can also vary in both space and time across an epidermis. The surface of developing organs changes as cells differentiate, and cells located in different regions can acquire distinct fates, creating higher-order patterns (Figure 1G). Detailed examinations of petal epidermis micromorphology in Lotus and Broad bean showed that distinct cell types can be found in the different petal types that make the complex flowers of legumes: in bee-pollinated species, the jigsaw-like flat cells that cover the bottom surface of the lateral petals are often replaced by conical cells in the top region directly contacted by pollinators (Bailes and Glover, 2018; Ojeda et al., 2009, 2012, 2016). A papillate shape is thought to provide better grip, as surfaces with conical cells are preferred by visiting bumblebees when flowers are difficult to handle, either because presented vertically (Whitney et al., 2009) or because of movement (Alcorn et al., 2012). Other cell types are also restricted to very specific regions of the epidermis. In Broad bean, overlapping rows of flat striated cells associate with folds of the lateral petals that act as hinges and could play an essential role in the opening mechanism of the flower (Bailes and Glover, 2018).

Physical properties emerge from hierarchical sculpting

The link between surface properties and structural patterning is far from being completely understood. However, an increasing number of studies led by interdisciplinary teams are uncovering some of the remarkable qualities those structural patterns confer to various plant parts, which have been extensively reviewed elsewhere (Barthlott et al., 2017; Fernández et al., 2016), and we only discuss here selected examples to illustrate key conclusions.

Optical and mechanical properties are most commonly reported. For example, the disordered wax platelets that cover the surface of Purple heart leaves (*Tradescantia pallida* “purpurea”) can act as light scatterers producing enhanced long wavelength reflection visible as a characteristic golden shimmer (Kerkhof et al., 2020), whereas silica deposits on the epidermis of rice leaves can form ladder-like structures at the junction between two cell walls, preventing torsion and keeping the thin leaf flat (Yamanaka et al., 2009). The sculpting of plant surfaces can provide other interesting physical properties (air retention, water repellent etc.) and even account for thermal and electrical attributes of the epidermis: cell shape can modulate the temperature locally on petals (Whitney et al., 2011), and variations in temperature across a leaf surface are mostly due to the tridimensional microtopography of the leaf that impacts both air flow and light interception (Saudreau et al., 2017).

The properties of a surface rarely stem from one single element but rather originate in the hierarchical combination of tridimensional features at different scales. Cells geometry and texture need to be captured at the micro- and nanoscale, respectively, but the topography of the whole organ or tissue at the macroscale must also be accounted for, as they all contribute to the overall physical effect. Dimensions and hierarchical organization matter too: the margins of pitcher plant trap (a modified leaf) and the petals of many tulips are covered with cuticular ridges; however, those striations operate at different scales and confer very distinct properties to the epidermis of each species. The edges of the pitcher trap exhibit cuticular folds with 100–200 μm periodicity, overlaid with microscopic striations spaced by 10–20 μm . This hierarchical structure, when wet, renders the rim extremely slippery to preys: the macroscopic grooves act as slippery tracks by stopping water to spread laterally, whereas the microscopic folding stabilizes water films, preventing the surface from drying out (Labonte et al., 2021). The petals of many Tulip species also display long pseudo-parallel cuticular striations but those are only a few hundreds of nanometers apart from each other, interfering with visible light and producing a visible iridescent effect, particularly pronounced in the blue-UV part of the spectrum (Moyroud et al., 2017).

It remains extremely challenging to predict the properties of an organic surface from experimental observations alone. Theoretical modeling plays an essential role in disentangling the relative contribution of the different structural elements and in understanding the origins of plant tissues behaviors. The vast majority of flowering plants have conical cells on their petals, and modeling explains how these cells can act as lenses and light trap for incident light, refracting and focusing the rays to the cell vacuole that contains the red to blue anthocyanin pigments—the end result being a striking enhancement of pigment visual display (Gorton and Vogelmann, 1996). California poppy petals owe their emblematic orange hue to the presence of carotenoids pigments. A cross-section of California poppy petals revealed that epidermis cells also have a triangular appearance; this can be surprising at first, as unlike anthocyanins, carotenoids are not water soluble and reside in chromoplasts instead of the vacuole. Further investigations showed that the conical projection of those cells does not involve the cell membrane and content but instead corresponds to an accumulation of cell-wall material on top of an otherwise flat apical membrane, creating a prism-like structure (Figure 2D). Computational simulations demonstrated that this device focuses the incident light on the basal portion of the cell content, where the carotenoid-rich chromoplasts accumulate, creating the intense color and silky effect characteristic of poppy petals (Wilts et al., 2018).

Finally, physical properties are not mutually exclusive, and plant epidermis often behave as multifunctional surfaces. The wax crystals behind the golden appearance of Purple heart leaves, when combined with other structural elements of the epidermis, also provide para-hydrophobic behaviors, allowing efficient harvest of water droplets (Suvindran et al., 2018). Such a behavior could facilitate self-irrigation and might contribute to survival in drought habitats. Whether both optical and water-adhesion properties play a role or whether the optical effect is a by-product of the evolution of a water-retention device is unclear. To answer such questions, functional investigations by behavioral ecologists and physiologists are vital.

Biological roles of structural patterns

A myriad of biological functions has been attributed to the features that pattern plant surfaces. More often than not, those are hypotheses that still remain to be rigorously tested experimentally. However, as hierarchical sculpting equips the plant epidermis with a broad range of physical properties, it is not surprising that structural patterns participate in an equally large catalog of functions: from enhancing flower salience (Moyroud et al., 2017; Whitney et al., 2011; Wilts et al., 2018), seed dispersal (Middleton et al., 2020; Vignolini et al., 2012, 2016), or prey capture by carnivorous plants (Bohn and Federle, 2004; Scholz et al., 2010) to

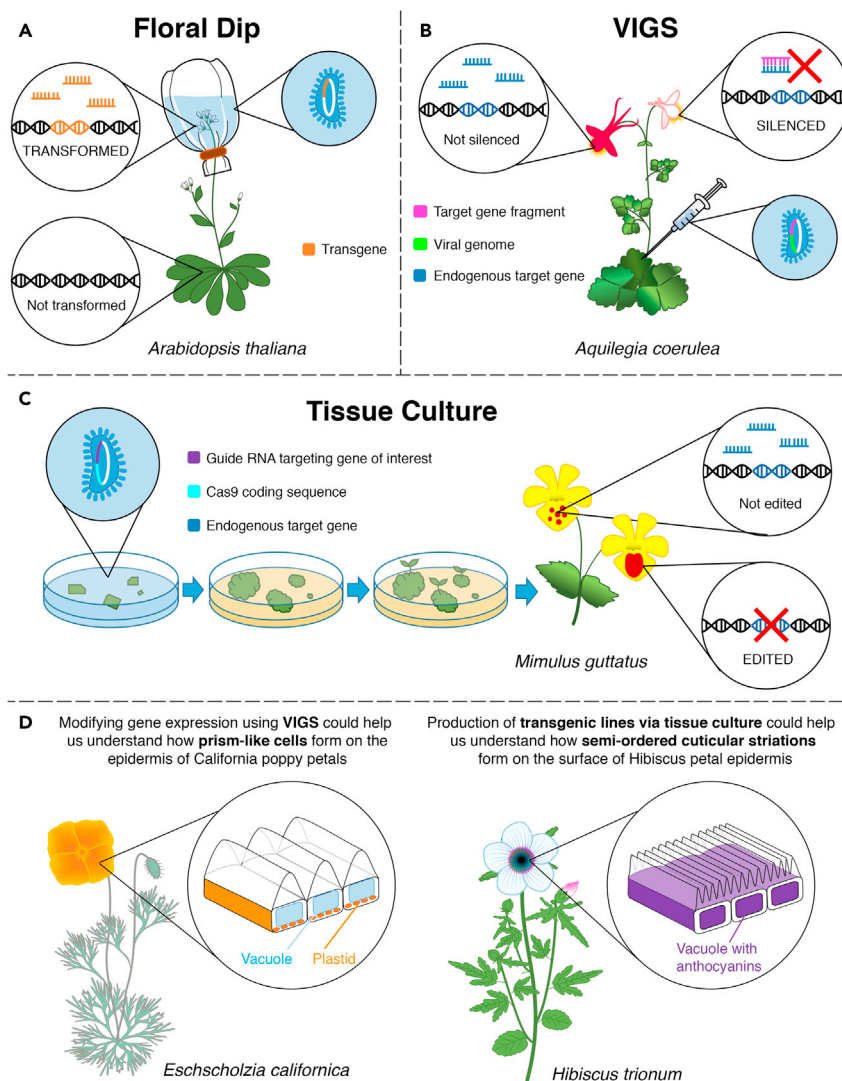


Figure 2. Experimental methods to modify gene activity in plants and investigate the mechanisms controlling the development of tridimensional features on plant surfaces

(A) The floral dipping method is commonly used to transform *Arabidopsis*, but it is often not suitable for other model plants or emerging model systems.

(B) Virus-induced gene silencing (VIGS) is an efficient tool to affect gene expression transiently, and it has been used successfully to silence a gene required for spur development in *Aquilegia coerulea* (Ballerini et al., 2020).

(C) Tissue culture is used to generate *Mimulus guttatus* transgenic lines used to study the development of spotted pigmentation patterns on the ventral petal (Ding et al., 2020).

(D) VIGS and/or tissue culture protocols are now available for emerging model systems well suited to investigate the mechanisms controlling the development of epidermal structures with interesting optical properties such as prism-like cell shape in California poppy (*Eschscholzia californica*) and cuticular ridges at the base of Venice mallow (*Hibiscus trionum*) petals.

protecting against herbivores, pathogens, or damaging UV rays (Aragón et al., 2017; Arya et al., 2021; Schulte et al., 2019; Vermeij, 2015).

The sculpting of the epidermis helps petal to function as multisensory billboards. In addition to increasing color depth, conical cells also provide grips for visiting insects, thus contributing both visual and tactile cues for pollinators before and after landing (Whitney et al., 2011). The trichomes (hairs) that surround the flower of *Espositoa frutescens*, a cactus from the Andes mountains, are more absorbent to ultrasonic frequencies than the rest of the flower, creating an acoustic contrast that can be used by pollinating

bats to locate flowers efficiently (Simon et al., 2019). Structural patterns still matter after pollinator departure, as not only must pollen grains reach a recipient flower but they also need to adhere to the pistil, the female reproductive structure. The top of the pistil, or stigma, comprises hundreds of stigmatic cells (Figure 1E), each corresponding to a single epidermal cell. Distinct stigmatic cell geometries are often paired with specific pollen types, maximizing pollen capture efficiency (Basso-Alves et al., 2011). Pollen adhesion to different plant parts can even be fine-tuned with surgical precision: for pollination to be successful pollen departure from the flower donating the pollen must be facilitated, whereas pollen grains need to be retained efficiently by the receiving flower. The stigma of false dandelions (*Hypochaeris radicata*) is covered with flexible conical papillae that clump together when wet. Atomic force microscopy (AFM, Table 2) measurements showed that these act as a gripping system where pollen adhesion strongly increases over time so that pollen grains that reach the pistil of accepting flowers are locked on the stigma and cannot leave (Ito and Gorb, 2019). In flowers acting as pollen donors, the pollen produced by the male organs first fall onto the style (portion of the pistil directly below the stigma) before relocating on the bodies of visiting insects. Crucially, the style surface is radically different from the pistil top: papillae are absent, and pollen grain adhesion does not increase when the style is wet, allowing easy transfer to pollinators (Ito and Gorb, 2019).

Glossy plants devoid of epicuticular crystals are often more prone to infestation by pest than waxy ones—this is because cuticular waxes can interfere with the ability of predatory insects to attach to the surface and conduct their predatory activities. Wax crystals and other cuticular structures are effective shields against a broad range of harmful visitors: surface topography can prevent both insect legs and eggs adherence, interfere with predator motion by acting as obstacles or making the surface slippery, and even modify the optical appearance of leaves, influencing herbivores choice (Gorb and Gorb, 2017; Lewandowska et al., 2020). Microscopic elements, and in particular wax crystals with specific thickness and tridimensional organization, also interfere with the attachment and successful colonization by fungal spores and microorganisms susceptible to cause plant diseases (Aragón et al., 2017). Cell shape can influence plant-herbivores interaction too: sharp and stiff trichomes, sometimes reinforced by biomineralization, can cause mechanical damage and prevent insects from chewing, moving, or depositing their eggs on the epidermis. Similarly, the silica needles that adorn the exterior of many grasses deter herbivores using a dual mechanism: first by causing mechanical damage, second by facilitating the transfer of bacteria and pathogenic fungi to predators (Lev-Yadun and Halpern, 2019). Interestingly, physical deterrents against herbivores and pathogens are sometimes effective defense against other plants too, preventing the attachment of dodders, a genus of parasitic plants (Runyon et al., 2010).

The distribution of those tridimensional elements matters when it comes to protecting plant surfaces. For instance, trichome geometry, orientation, and position could play a role in keeping predators at bay (Salemo et al., 2018; Vermeij, 2015). An elegant modeling study recently showed that mechanosensitive trichomes that produce defensive compounds in response to vibrations specifically respond to acoustic waves produced by chewing caterpillars (Liu et al., 2017). Interestingly, those trichomes tend to accumulate near leaf veins. The physical proximity between so-called “acoustic antennae” and a long-distance transport system raises the exciting possibility that higher-order patterning of plant surface could effectively couple local perception of a predator with the activation of systemic defense mechanisms.

The texture of the epidermis affects how easily pollutants and dust can be removed from plant surfaces (Burkhardt, 2010; Lu et al., 2018). Adhering particles block efficient light capture, and any residual water layer on leaves surfaces is also likely to reduce photosynthetic gas exchanges. Thus, self-cleaning properties and hydrophobicity can significantly impact on a plant photosynthetic capacity. The texture of plant surfaces also directly participates in the accumulation of attractive or repellent compounds on the plant exterior: such molecules can be directly produced by epidermal cells (e.g., glandular trichomes of stinging nettle) or be a component of the cuticular microstructures themselves (e.g., crystals containing substances acting as allomones or toxic molecules). Alternatively, compounds produced elsewhere, by a visiting animal or nearby plants, can be captured by plant surfaces, as certain textures adsorb or absorb the vapors of organic compounds present in the air layer covering the epidermis (Himanen et al., 2010; Mofikoya et al., 2018). These trapped compounds are perceptible by animals and neighboring plants. They can contribute to camouflage, making the plant less detectable to herbivores. Plants can also “borrow” predator-repellent compounds from their neighbors and display them on their surface (Himanen et al., 2010). Captured compounds can even participate in plant-plant communication by triggering the expression of defense genes in the receiving plant (Peng et al., 2011). However, whether it is the surface topography itself or its chemistry

Table 2. Multidisciplinary techniques and their possible contributions to investigate structural patterning in plants.

	Example of techniques	Type of information obtained	Example of contribution to structural patterning understanding	Advantages (+) and Shortcomings (-)
MICROSCOPY	Scanning Electron Microscopy	Precise details of an organ/tissue/cell surface. Cuticle/cell wall thickness and organisation using cryofractures	Characterisation of surface ornamentations (e.g. cuticular ridges, epicuticular wax crystals, etc...) and cell wall characteristics	(+) High resolution, rapid and applicable to any species. (+) No need for staining nor fixation (Environmental SEM or CryoSEM). (-) Sample freezing, critical point drying or sputter coating could damage surface features when fragile and induce cellular collapse.
	Transmission Electron Microscopy	Cell ultrastructure	Identification of cuticle motifs	(+) High resolution. (-) Sample fixation is time-consuming and can induce artefact (i.e., change in cuticle or cell wall apparent thickness).
	Confocal microscopy	Spatial and temporal information related to gene expression, protein localisation, cell geometry and growth parameters	Extract cell geometry parameters, Live imaging or time-lapse to track cellular and molecular changes over time during pattern formation and follow cell lineages	(+) Allow imaging of cell behaviour, protein trafficking and gene expression in live tissue. (-) Often relies on transgenic approach which restrict its used to transformable species. (-) When used staining might induced artefacts. (-) Autofluorescence frequent in plant tissues can interfere with imaging and restrict the range of tissues that can be imaged properly.
MECHANICS	Atomic Force Microscopy (AFM)	Measure mechanical properties of tissues, cell wall or cuticle, Image surface topography, Infer turgor pressure	Measuring ability of tissues or organic material to deform and develop striation, follow wax crystals formation on plant surfaces during development or during repair post-wounding	(+) Provide direct access to the elastic modulus of biomaterials with minimal sample preparation. (+) High spatial resolution. (-) Apparent stiffness measured sensitive to turgor pressure and to the cell geometry and dimension, especially cell curvature and aspect ratio. (-) Lack of protocols capable of generating reproducible results and absolute values for mechanical parameters. (-) Only assesses mechanical properties at the surface of the sample and the deforming force is applied perpendicular to the stresses relevant for cellular growth.
	Automated confocal micro-extensometer (ACME)	Measure changes in mechanical properties at the cellular level over time	Characterization of the role of cell mechanics in pattern formation	(+) Measurement of mechanical properties in living tissues, in plane of growth. (+) Can be coupled with confocal microscope allowing simultaneous imaging. (-) Tissue/organ must be accessible. (-) Measurements difficult on thin and fragile tissue. (-) If the sample has been harvested from the plant, desiccation will occur and induce artefacts.
CHEMISTRY	Fourier transformed infra-red spectroscopy (FTIR)	Identification of main functional groups, chemical bonds, and arrangement of the biochemical compounds (e.g. cell wall and cuticle composition analysis) in biomaterial	Characterisation of cuticle composition. Distribution across tissue (e.g. quantity of cutin in the petal cell wall-cuticle continuum of Arabidopsis)	(+) Allow rapid and direct imaging of any plant surface, without fixation or labelling of samples. (+) Provide detailed spatial information.
	RAMAN	Identification of chemical compounds and their distribution across a tissue	Mapping of plant constituents such as cuticle compounds or pigments at the tissular or cellular resolution	(-) Mostly provide qualitative information (presence/absence of a compound) rather than quantitative. (-) Exact identification of compounds detected often time-consuming and difficult (gives molecular rather than structural formula) – requires further analysis (i.e., NMR, Mass spectrometry, etc...).
	Liquid extraction surface analysis (LESA) mass spectrometry (MS)	Analysis of chemical compounds (e.g. cuticle composition)	Characterization of the chemical heterogeneity across a tissue and its contribution to pattern formation	
OPTICS	Optical goniometer	Measure of the light scattered by a structure as a function of the observation angle	Characterization of the role of disorder in structural colour production by patterns tissues	(+) Allow direct and rapid measurements over large surface areas. (-) High illumination intensities and prolonged exposition can damage the tissue and generate artefacts.

Each technique presents advantages and drawbacks that need to be considered when deciding on the most suitable experimental setup. Combining multiple methods is one approach commonly adopted to mitigate technical shortcomings and reach reliable conclusions.

(e.g., leaves lipid content correlate positively with their ability to capture hydrophobic terpenes) that contributes most significantly to volatile capture often remains to be established.

UNDERSTANDING PATTERN FORMATION ON PLANT SURFACES USING THE TOOLS OF MODERN EXPERIMENTAL BIOLOGY

The plant epidermis is a living tissue, generated by groups of undifferentiated cells that acquire characteristic shapes and textures as development progresses—a process known as cell differentiation. By

manipulating this process *in planta* and capturing its dynamics with enough spatiotemporal resolution, the mechanisms regulating the formation and distribution of surface features can be dissected. We provide below a brief overview of current approaches used to modify gene expression in living plants for readers, especially nonbiologists, who might not be familiar with those techniques. We also highlight how those methods could specifically help us understand the mechanisms sculpting the epidermis of plants by focusing on two specific examples: prism cells and cuticular ridges.

Manipulating gene expression to understand structural pattern formation

Higher order structural patterns on a plant epidermis often involve contrasting cell types. Different cell types emerge because cells can deploy the information encapsulated in their genome each in their own unique ways: particular genes can be switched on (expressed) or switched off (repressed) with extreme spatiotemporal accuracy so that even neighboring cells can eventually acquire distinct biochemical capabilities and morphologies. A key experimental approach to investigate the mechanisms regulating the structural patterning of plant surfaces is the targeted manipulation of gene expression. This often relies on the production of transgenic plants, which carry the machinery (transgenes) necessary to change the expression level of a gene of interest (i.e., overexpression or downregulation), modify the timing of its expression (e.g., constitutive expression), or trigger its expression in cells where it is normally silent (e.g., misexpression or ectopic expression). Most methods to introduce transgene into plants do not offer any control on where the transgene is incorporated in the genome—as the local genomic landscape influences gene expression, the phenotype intensity often varies between transgenic lines. Genome editing methods offer a powerful alternative. The CRISPR/Cas9 technology is particularly versatile and now widely used in a range of species (Sukegawa et al., 2021). It enables the targeting of any site in the genome with surgical precision—routine genome editing in plants introduces point mutation in genes of interest so that they fail to produce functional proteins (i.e., gene knock-out), but more sophisticated approaches such as those targeting the DNA motifs that control when and where a gene is normally expressed (Rodríguez-Leal et al., 2017) or those capable of precisely replacing or introducing a DNA sequence emerge in plants (Sukegawa et al., 2021).

The most widely used approach to introduce a transgene in the classic model *Arabidopsis* utilizes the natural ability of a soil bacterium, *Agrobacterium*, to transfer part of its DNA (transfer DNA) to infected plant cells, a process known as transformation. Most of the transfer DNA sequence can be replaced with any sequence of interest, providing a convenient vector to deliver transgene into plant cells (Chilton et al., 1977). Introducing foreign DNA in a plant cell is only the first step: to generate a transgenic individual, a full organism must be obtained from this modified plant cell using *in planta* or *in vitro* methods. *In planta* methods require the co-culture of the targeted plant tissue (usually reproductive organs) with a transformed *Agrobacterium*. These are based on the assumption that either egg cells or sperms will accept the transgene and will give rise to a transformed embryo after fertilization (Chee and Slightom, 1995). Although *in planta* transformation methods are straightforward in *Arabidopsis*, attempts to apply these methods to other species often fail (Bent, 2000). *In vitro* methods require tissue culture: fragments of plant tissues such as roots, embryonic stems, or pieces of leaves are co-cultivated with transformed *Agrobacterium* and grown in presence of hormones promoting cell proliferation and then differentiation into a full transgenic plant (regeneration). Specific hormone cocktails supporting regeneration must be empirically deduced for each species and limiting factors include the recalcitrance of some species to respond to culture conditions (Benson, 2000) and the regeneration time, as several months are often necessary to obtain plantlets. This is particularly problematic when studying structural patterning, as plant surfaces with interesting properties are often found in nonmodel organisms with long life cycles. However, those methods can be effective in establishing novel model systems, well suited to investigate the formation of pigmentation motifs (Ding et al., 2020; Figure 2C) or structural patterns such as cuticular striations that can form diffraction gratings and provide visual cues for pollinating insects (Figure 2D). Indeed, theoretical approaches (see next section) suggest that cell growth and cuticle production are central to the emergence of such semi-ordered striations (Antoniou Kourouniotti et al., 2013; Huang et al., 2017). The development of transformation protocols for a species that produces such diffraction gratings on its petals (e.g., *Hibiscus trionum*, Figure 2D) would provide a mean to manipulate *in vivo* the direction and extent of cell growth and the synthesis of cuticular components. This represents an exciting venue to test experimentally the predictions of theoretical models.

A comprehensive understanding of the processes used by plants to sculpt their surface requires the modification of gene expression in a wider range of species. Although this remains an ongoing challenge, solutions are emerging. In 2019, Gordon-Kamm and colleagues proposed a way to get around species

recalcitrance by exploiting morphogenic genes involved in controlling plant growth and development, to facilitate the regeneration step (Gordon-Kamm et al., 2019). Virus-induced gene silencing (VIGS) is also a promising approach. VIGS enables targeted gene downregulation by exploiting viral gene silencing, an innate plant defense system (Lange et al., 2013). The effect is transient and only affect parts of a plant, but this method has been very successful in uncovering the genetic basis of spur development in Columbine (*Aquilegia*) (Ballerini et al., 2020) (Figure 2B). It has also been validated in California poppy (Becker and Lange, 2010) and constitutes an effective approach to investigate the formation of the prism-like cells that render the petal epidermis of California poppy particularly glossy (Wilts et al., 2018) (Figure 2D).

High-resolution imaging to capture the dynamic of structural pattern formation

Until recently, our knowledge of plant structural patterns was limited to the observation of fully developed mature tissues, using optical or scanning electron microscopes (Table 2), the latter being particularly useful to image cuticular features (Figure 1). However, pattern establishment requires the specification of distinct cell identities in young developing tissues that are actively growing and dividing. Such a dynamic process is challenging to study. To understand how genes activity alters cellular growth, division, and differentiation in a controlled manner to produce a robust structural pattern, the methods described above must be coupled with approaches capable of capturing cell behavior with high spatiotemporal resolution. New powerful bioimaging techniques (Prunet and Duncan, 2020; Rambaud-Lavigne and Hay, 2020) provide valuable tools to boost advances in the field.

Arabidopsis cotyledons and sepals are good systems to study surface patterning being easy to access and displaying a substantial range of cell sizes, shapes, and identities. Confocal time-lapse imaging in a living tissue is an important tool to capture dynamic processes such as cell growth and division or protein production, movement, and accumulation at cellular and subcellular scales (Table 2). In the context of pattern formation, being able to capture images regularly over time is of particular interest to characterize the emergence of specific cellular shapes and features and to track the behavior of molecular players that may regulate or bring about those changes. Confocal time-lapse imaging has been instrumental to describe the emergence of cotyledon jigsaw puzzle cells (Sapala et al., 2018), sepal trichomes (Hervieux et al., 2017), giant cells (Meyer et al., 2017), and cuticular ridges (Hong et al., 2017). These chronological image series represent a large amount of data that need to be rigorously analyzed to quantify changes in cellular behavior as patterns emerge. Image processing softwares are now available to extract cellular contours and surfaces, perform 2D or 3D reconstruction, and identify individual cells (segmentation) from confocal z-stacks (Barbier de Reuille et al., 2015; Erguvan et al., 2019; Fernandez et al., 2010). For instance, MorphoGraphX (MgX) can automatically track cellular lineage and quantify growth rate and direction as well as cell division rate and orientation. These parameters must be taken into account to understand both when and how tridimensional features form (Coen et al., 2004): trichome precursors were shown to grow twice as fast as their neighboring cells before bulging (Hervieux et al., 2017), revealing the existence of cell growth heterogeneity across a patterning tissue and early divergence in the behavior of cells destined to become trichomes. By quantifying cell lobeyness change overtime in cotyledons cells, Sapala and colleagues showed that their characteristic puzzle shape starts to appear long before the cotyledon reaches its final shape, when tissue growth is still isotropic (Sapala et al., 2018). These results rely on accurate cell segmentation and often involve manual correction of segmentation errors. Software benefiting from machine learning developments such as PlantSeg (Wolny et al., 2020) or pipelines combining convolutional neural networks with watershed-based segmentation techniques (Eschweiler et al., 2019) can bypass those issues and perform superior cell segmentation guided by neural network predictions.

The establishment of different cell types and sizes can be determined by early divergence in cell proliferation activities across the epidermis. Spatiotemporal patterns of growth and cell division can be extracted from time-lapse series (Hong et al., 2017), but it is not always possible to follow a given tissue over time. Indeed, live tracking individual cells can be challenging, especially when growing tissues, for instance petals, are hidden under other plant parts. In this case, clonal analysis can be performed after labeling sets of epidermal cells at fixed time points, to extract information such as the main direction of growth and its rate from the clone patterns obtained (Sauret-Güeto et al., 2013). Dividing cells can also be marked using transgenic lines expressing the labile chimeric mitotic cyclin *CycB1;1:uidA* reporter (Huang and Irish, 2015) or stained with ethynyl-2'-deoxyuridine (EdU) that incorporates into newly synthesized DNA (Yang et al., 2019). These techniques were instrumental to show that cell division predominantly occurs in the distal part of developing *Arabidopsis* petal where cells will become conical (Huang and Irish, 2015; Yang

et al., 2019). Failure to divide also contributes to surface patterning: endoreduplication occurs when DNA replication is not followed by cell division. This phenomenon, mapped using techniques such as DAPI staining and flow cytometry, often takes place in cells destined to become the largest cells of an epidermis, such as those at the base of cabbage petals (Kudo and Kimura, 2002) or the giant cells dispersed across the Arabidopsis sepal surface (Meyer et al., 2017).

Controlled cell growth can influence the texture of plant surface. Conical cells start as rather flat cells on the epidermis of young petal. Detailed live-confocal examination revealed that outgrowth emergence coincides with the formation of an ordered array of previously randomly arranged microtubules in conical cell precursors. Preventing cells from remodeling their cytoskeleton is sufficient to impair correct cell expansion and thus directly impacts on the topography of the mature petal (Ren et al., 2017). Cell growth also affects the formation of tridimensional cuticular elements. Hong and colleagues showed that the appearance of cuticular ridges on the surface of Arabidopsis sepals coincides with the slowdown of growth and the end of cell division (Hong et al., 2017). Discrepancy between the growth of the cuticle and the underlying cells could even be a general mechanism, allowing epidermal cells to sculpt their surface (Antoniou Kourounioti et al., 2013; Huang et al., 2017). Indeed, when present on anisotropic cells, cuticular striations often align with the main direction of cell growth, forming for instance parallel ridges on flat tabular cells at the base of *Hibiscus trionum* petals (Antoniou Kourounioti et al., 2013; Moyroud et al., 2017) or adopting a “star” pattern on rose conical cells, by radiating from the cone tip to the cell base (Figure 4C SEM). However, control of cell growth does not solely explain the formation of a specific texture. The chemical composition and organization of the cuticle also matters: a comparative study of cuticle ultrastructure in various genetic backgrounds established a clear correlation between the amount of 10,16-dihydroxyhexadecanoic acid, a typical monomer of cutin, and the presence/absence of cuticular striations as mutants that only produce very low amount of this specific cutin monomer fail to form the stellate cuticular ridges normally found on the conical cells of Arabidopsis petals (Mazurek et al., 2017). Interestingly, the expression pattern of *CUTIN SYNTHASE 2 (CUS2)*, a gene involved in cutin biosynthesis, matches the zone of slower growth rate in sepals, where cuticular ridges form (Hong et al., 2017), and transgenic lines analysis showed that *CUS2* is crucial for ridge maintenance during sepal growth, but the precise mechanism is still unknown.

What make neighboring cells start behaving differently? One of the current challenges is to link growth patterns to genetic activities as processes instructing cells what features to develop, where, and when to remain poorly understood. As in animals, molecular cues are needed to coordinate these processes (Vargesson, 2020; Wolpert, 2016). “Morphogen-like” factors such as phytohormones, transcription factors, micro-RNAs, or small peptides have been suggested to act as diffusible signals, delivering spatial information to epidermal cells and specifying fate (reviewed in Gundu et al., 2020; Klesen et al., 2020; Rogers and Schier, 2011). By combining high-resolution imaging with transgenic approaches to introduce fluorescent sensors and reporter constructs in plants, the expression of multiple genes and the activity and outputs of hormonal pathways can be followed simultaneously in developing tissues (e.g., Galvan-Ampudia et al., 2020). For example, imaging Arabidopsis expressing a marker of auxin signaling implicates this pivotal plant hormone in the elaboration of at least two contrasting cell shapes, influencing both conical cell expansion in petals and lobe formation in jigsaw leaf cells (Dang et al., 2020; Grones et al., 2020). The genetic networks that specify and execute cell shape programs are far from being completely understood but interestingly transcription factors regulating cell geometry can also participate in cuticle production (Oshima and Mitsuda, 2013), providing epidermal cells with a simple way to coordinate structural patterning at the micro- and nanoscale. Being able to quantify gene expression with cellular resolution is starting to illuminate our understanding of pattern establishment. By carefully monitoring the levels of an HD-Zip transcription factor, *MERISTEM LAYER1 (ATML1)*, in individual epidermal cells of the sepals and simultaneously capturing the size and shape of nuclei and cells, Meyer and colleagues demonstrated that *ATML1* expression varies among and within cells over time and that high levels of *ATML1* during the G2 stage of the cell cycle are associated with giant cell formation (Meyer et al., 2017). Thus, cell-autonomous fluctuation-dependent mechanisms can account for the distribution of different cell sizes on plant surfaces.

COMBINING PHYSICS, CHEMISTRY, AND MATHEMATICS TO UNDERSTAND PATTERN FORMATION AND PROPERTIES

The plant epidermis is a complex dynamical system in which gene regulation, intercellular signaling, and morphogenesis all interact. Thus, identifying genetic players behind pattern formation is necessary but not sufficient to understand the overall mechanisms leading to a peculiar topography. In this section, we

use selected examples to illustrate how computational mathematics, physics, and chemistry are joining forces with experimental biology to shed light on the processes that carve the surfaces of plants.

Role of mechanics in cell-shape acquisition

Turgor pressure pushes cell wall, and thus both cell wall mechanical properties (as extensibility and stiffness) and turgor pressure play a key role in growth (Cosgrove, 2016; Sampathkumar, 2020; Trinh et al., 2021). Their roles in the morphogenetic process have been reviewed in details in Altartouri and Geitmann (2015), Chebli and Geitmann (2017), Eng and Sampathkumar (2018), and Sampathkumar (2020).

Spatial variations in cell wall properties influence morphogenesis, contributing directly to the formation of structural patterns. The wall of growing plant cells is a complex assembly of cellulose microfibrils embedded in a polysaccharide matrix, made of pectins and hemicelluloses. Cell wall composition, ultrastructure, and thickness (100 nm to 1 μ m) vary locally and influence the wall ability to expand and adopt certain shapes. Enzymes and other proteins are also present in small quantity and can play a key role in remodeling of the wall during cell growth. Indentation methods such as AFM (Majda et al., 2017) or cellular force microscopy (Routier-Kierzkowska et al., 2012) can measure the mechanical properties of the cell wall in surface (Bidhendi and Geitmann, 2019a, 2019b). Brillouin microscopy, a noninvasive method well suited to assess the mechanical properties of live tissues (Elsayad et al., 2016), has also recently been used to draw a relative stiffness map through the thickness of the wall (Altartouri et al., 2019). These techniques have been essential to discover that the acquisition of specific cell shapes relies on the mechanical properties and constraints imposed during growth. Brillouin microscopy on Arabidopsis cotyledons showed for instance that stiffness is not uniform along the periclinal wall of developing jigsaw-puzzle cells. Such stiffness differential between lobe and neck regions of emerging protrusions could play a central part in promoting the expansion of cell wall undulations at later stages (Altartouri et al., 2019). The tool arsenal to probe the role of mechanics in cell morphogenesis is ever expanding (Table 2). To understand how a tissue reacts to mechanical stress such as deformation, and to measure its mechanical properties while growing, an automated confocal micro-extensometer (Table 2) capable of applying a controlled mechanical stress by stretching the living sample is now available (Robinson et al., 2017).

Cell wall anisotropy is linked to the orientation of the cellulose microfibrils (Baskin, 2005). The relationship between cellulose deposition and the mechanical properties of growing cell walls is a very active field of study that has yielded significant discoveries in the last few years. Cortical microtubules usually orient along the maximum stress direction (recently reviewed in Hamant et al., 2019) and guide the trajectories of the cellulose synthase (Paredes et al., 2006) and cellulose microfibrils deposition. This can reinforce the direction of maximal stiffness in the wall and promote growth in the perpendicular direction (Sampathkumar et al., 2014; Zhao et al., 2020). However, growth does not always occur perpendicularly to cellulose microfibrils orientation: probing the structure of the cell wall at the nanoscale revealed that cellulose microfibrils are organized in stacked layers, giving the cell wall a polylamellate architecture. Interestingly, cellulose microfibrils in onion epidermal cell wall are arranged along the same direction within a layer, but this orientation shifts between adjacent layers so that across the entire wall the orientation of cellulosic fibers is almost isotropic (Cosgrove, 2018a, 2018b; Kafle et al., 2017).

Cellulose deposition is guided by the orientation of microtubules (reviewed in Li et al., 2015; Paredes et al., 2006). Thus, to understand cell shape patterning, the dynamic remodeling of the cytoskeleton must be captured, and tools have been developed to quantify microtubule anisotropy at the cellular (Boudaoud et al., 2014) and subcellular (Tsugawa et al., 2016) levels. Molecular actors that sculpt plant surfaces by controlling the organization of the cytoskeleton have also started to be identified. Small GTPases from the Rho family (ROPs) and their partners are responsible for the organization of both microtubules arrays and microfilaments networks that promote lobe formation in puzzle cells (Fu et al., 2005, 2009). Interestingly, the formation of the circumferential microtubules arrays that control conical cell expansion also involves ROPs and is jointly regulated by two pathways: one involving KATANIN1, a microtubule-severing enzyme (Ren et al., 2017) and one implicating ANGUSTIFOLIA, a repressor that lowers the reactive oxygen species levels that would otherwise inhibit microtubules ordering (Dang et al., 2018).

We are starting to better understand how epidermal cells control their shape, but the mechanisms prompting the emergence of distinct cytoskeletal behavior in different cells to create the overall pattern remain elusive.

Interestingly, differences in turgor pressure have recently been observed between neighboring cells, using AFM (Long et al., 2020). This is yet to be tested in epidermal cells, but spatial variations in turgor pressure could act as a cue to pattern plant surfaces. At a higher scale, mechanics could also act as a supracellular instructive signal, regulating the overall patterning of an organ surface. Indeed, mechanical forces control the direction of cell growth and cell division patterns but also provide some positional information that can inform cell fate specification (Mirabet et al., 2011).

Modeling approaches to probe the mechanisms sculpting plant surfaces

A myriad of molecular and mechanical inputs is likely to coordinate cell behavior and produce structural patterns. Computational modeling is a useful approach to decipher the respective contribution of each parameter, by using Finite Element Method (FEM), a numerical approach used to solve 2D (Hernandez-Lagana et al., 2021; Kierzkowski et al., 2019) or 3D problems (Bassel et al., 2014; Belteton et al., 2021) in continuum mechanics (reviewed in the context of shape changes in Bidhendi and Geitmann, 2018). Most importantly, mathematical models and simulations permit the exploration of several hypotheses *in silico* and make predictions by incorporating growth properties, division, mechanical signals, gene activity, and signaling aspects. These predictions can then be tested experimentally, and the results obtained can be used to refine models. This iterative approach is key to challenge competing models, discriminate between hypotheses, and help design and select subsequent experiments most likely to be informative.

FEM simulations were used to investigate the role of mechanics in puzzle-shape formation and trichome bulging (Belteton et al., 2021; Hervieux et al., 2017; Sapala et al., 2018), and computational modeling was instrumental to show how differential growth between neighboring cells generate mechanical stresses and conflicts, leading to a diversity of cell shape through local deformation (Rebocho et al., 2017). Theoretical approaches were also deployed to understand the formation of cuticular ridges. A model invoking mechanical buckling could generate different cuticular patterns by modulating two parameters: the rate of cuticle production and the extent of anisotropic cell growth (Antoniou Kourouniotti et al., 2013). This model is in broad agreement with experimental data associated with ridges formation on the sepal surface (Hong et al., 2017), but it remains to be tested rigorously in petals (Figure 2E).

Modeling has been widely used to get insight into lobe formation in pavement cells. The model first proposed by Majda and colleagues, in which structural heterogeneity in anticlinal walls can initiate wavy cell contours in presence of tension (Majda et al., 2017), has recently been challenged (Bidhendi and Geitmann, 2019a, 2019b). By reproducing the FE simulations, Bidhendi and Geitmann suggested that anticlinal wall stretching only produce weak bend in the wall, rather than a significant wavy curvature (Bidhendi and Geitmann, 2019a, 2019b). Models explaining pavement cell morphogenesis are still debated (Altartouri et al., 2019; Belteton et al., 2021; Bidhendi et al., 2019), but their re-evaluation and associated discussions are central to challenge assumptions and illuminate the processes at play.

Pairing modeling with accurate quantifications can help relate patterns of gene activity to cell behavior, a task known to be extremely difficult (Rebocho et al., 2017). For instance, the computational model built by Meyer and colleagues supports their experimental observation that a high level of ATML1 during the G2 phase of the cell cycle is essential for giant cell fate specification (Meyer et al., 2017). It also allowed the authors to define a fluctuation-driven process, involving a weak positive feedback loop, as a patterning mechanism capable of sculpting the plant epidermis (Meyer et al., 2017). Software able to integrate image processing and simulation modeling, directly using templates from confocal images, play a key part in linking the regulation of gene expression to cell morphogenesis. Means to include mechanics simulation (e.g., MorphoMechanics) in MgX will be extremely useful and are currently in development.

The chemistry of plant surfaces impacts on structural pattern formation

Plant cuticles are complex biomaterials, commonly composed of two major components: a polymer of cutin and a cocktail of intra- and epicuticular waxes. Other constituents such as cutan, polysaccharides such as pectin or cellulose, and phenolic compounds are also frequently present, and these can act as nanofillers and impact on the mechanical behavior of the cutin polymer (for a recent review see Khanal and Knoche, 2017). In short, cutin behaves as a viscoelastic polymer but its extensibility can be greatly modified via impregnation with other components: for example, waxes and cutan are known to increase cuticle stiffness (Khanal et al., 2013; Takahashi et al., 2012). Chromatography, mass spectroscopy, nuclear magnetic resonance (NMR) spectroscopy or RAMAN, and IR imaging have all been applied to plant material (Table 2), but most studies have focused on analyzing

cutin and wax chemistry on a limited number of species, where cuticle can be isolated or easily extracted (Fernández et al., 2016; Heredia-Guerrero et al., 2014). To determine whether chemical heterogeneity plays a part in the cuticle ability to form different nanotextures on different tissue parts and to investigate possible links between changes in cuticle chemistry and emergence of specific surface features during development, access to spatially and temporally resolved information is required.

Direct surface analysis methods (Table 2, Giorio et al., 2019, 2015; Hemalatha and Pradeep, 2013) can now be used to detect the presence of chemicals *in situ* and generate spatial maps of plant surfaces metabolites. Recent analysis showed that distinct compounds can associate with different cuticular textures: methods based on LESA-MS can easily detect epicuticular waxes and cutin monomers on the striated surface of *H. trionum* and *H. richardsonii* petals basal regions but not on the distal smooth portions (Giorio et al., 2015, 2019). Other studies have linked chemical composition with distinct cuticle architectures (Fernández et al., 2016), showing that different amounts of 10,16-dihydroxy hexadecanoic acid, a monomer of cutin, lead to the cuticle adopting different organizations. Remarkably, only specific configurations appear compatible with nanoridge formation (Mazurek et al., 2017).

The cuticle is not an invariable material, but its formation follows a dynamic process, changing in structure, composition, and properties as the epidermis grows: Bourgault and colleagues recently showed that the composition of maize leaves cuticle follows a base-to-tip gradient as development progresses (Bourgault et al., 2020). Thus, not only the nature of the chemicals present but also the order and timing of their production and delivery to the plant surface are likely to deeply affect the final topography. Self-organization could also be instrumental: self-organization properties are thought to be involved in wax crystal growth (Fernández et al., 2016) especially during healing responses after wounding. Huth and colleagues mechanically removed the epicuticular waxes found on the surface of Eucalyptus leaves and used AFM to show those structures are capable to regenerate their original morphology—interestingly the process work regardless of leaf age (Huth et al., 2018). Environmental factors associated with global warming, such as temperature increases, and frequent droughts can significantly modify the composition and properties of the cuticle (reviewed in Heredia-Guerrero et al., 2018; Suseela and Tharayil, 2018). Those modifications are thought to improve plants protection and their ability to buffer the impact of climate change, and it will be important for future studies to investigate how these affect the topography of the cuticle.

The chemistry of the cell wall can impact on the formation of both cuticular and cellular patterns. First, the inner side of the cuticle is often enriched in polysaccharides from the cell wall, which govern the cuticle elastic behavior, and thus are likely to impact on its ability to generate distinct textures (Guzmán et al., 2014; Khanal and Knoche, 2017). Second, the cell wall is chemically modified during growth, affecting its stiffness and its ability to deform and expand to create different cell shapes. Cell wall loosening enzymes, such as EXPANSIN1, are important to maintain the sharp tip of conical cells found on Petunia petal (Zenoni et al., 2004). As with cuticle analysis, a variety of approaches from solid state NMR (Wang et al., 2015) to diverse imaging techniques such as AFM and Field Emission SEM (Zhang et al., 2017; Zheng et al., 2017) (Table 2; Figure 3) have been employed to investigate the behavior of cell wall constituents. Cellulose appears the stiffest component of the wall, whereas hemicelluloses and pectin network are softer (Mirabet et al., 2011). However, the mechanical properties that result from the interactions between those elements are still far from understood (Cosgrove, 2018a, 2018b). Haas and colleagues have used 3D-STORM super-resolution microscopy and cryoSEM to study leaf pavement cell wall (Haas et al., 2020). Pectin nanofilaments were seen oriented perpendicularly to the cell outer surface in the anticlinal walls of the pavement cells, but this organization was not found in the periclinal walls (Haas et al., 2020). This contrasts with previous studies that consider a physical connexion between both walls as major for cells morphogenesis (Bidhendi and Geitmann, 2019a, 2019b; Chebli et al., 2021; Cosgrove and Anderson, 2020; Zhang and Zhang, 2020). Haas and colleagues propose instead that de-esterification of pectin homogalacturonan nanofilaments could, by swelling, contribute to cell growth and shape formation independently from turgor pressure (Haas et al., 2020). This theory is actively debated and will require more experimental and theoretical investigations (Bidhendi and Geitmann, 2019a, 2019b; Chebli et al., 2021; Cosgrove and Anderson, 2020; Zhang and Zhang, 2020). Nevertheless, it implies that there is still a lot to learn about the physical properties of the cell wall and how those relate to the production of structural patterns at the microscale.

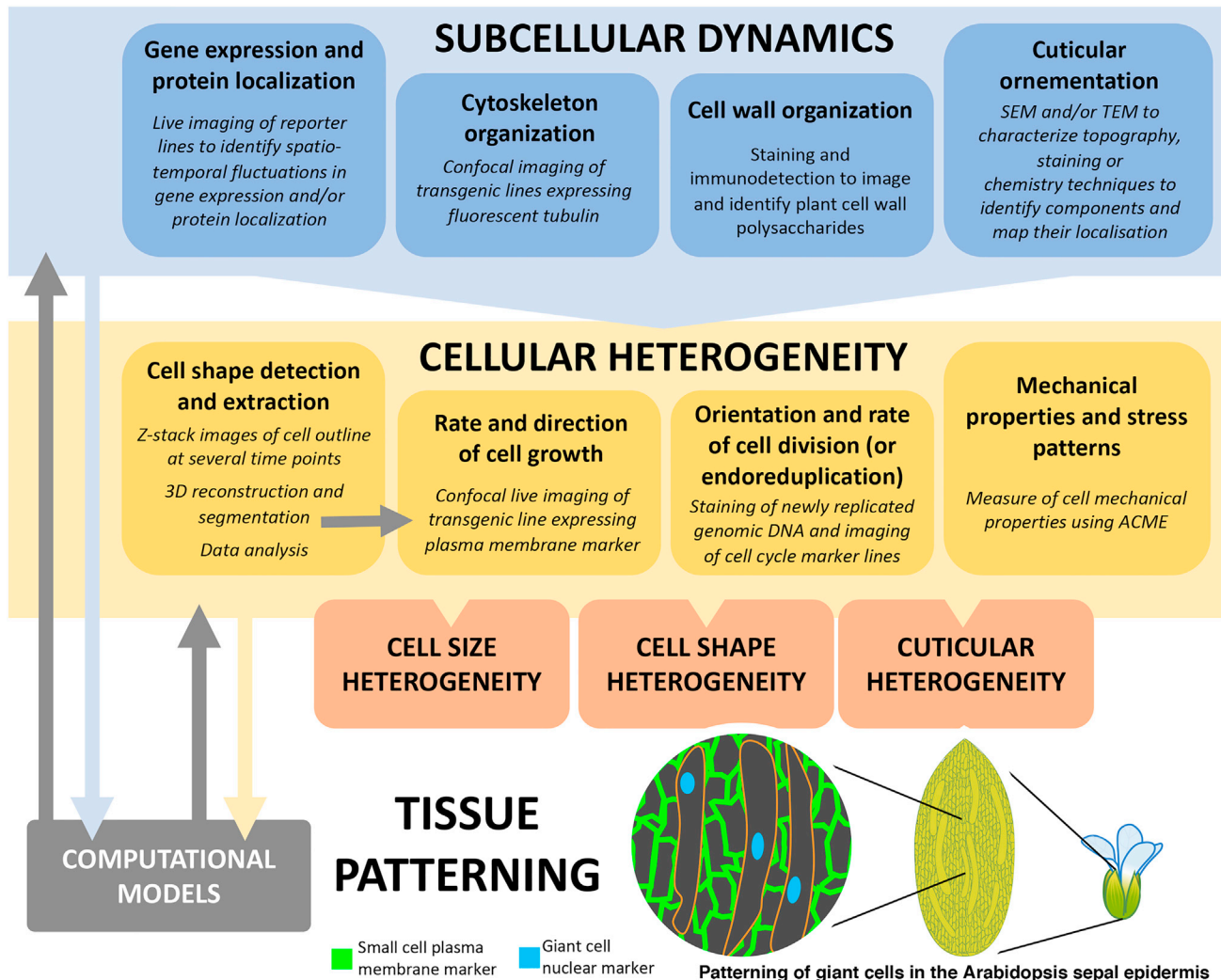


Figure 3. Typical workflow combining quantitative analysis of multiscale aspects, experiments, and theoretical modeling, to characterize pattern formation on plant surfaces.

EXPLOITING NATURE'S CREATIVITY TO CREATE "SMART" MATERIALS

Biomimetics ambition is to manufacture structures that imitate ones found in Nature, using synthetic components to produce useful materials. Given the remarkable range of qualities structural patterns confer to the plant surfaces, it is not surprising those often serve as starting point to create new materials with transparent, self-cleaning, self-healing, or light-harvesting properties (Figure 4). Plant biomimetics is the focus of several excellent reviews (Barthlott et al., 2017; Kumar et al., 2019; Speck and Speck, 2021). Here, we chose to highlight recent examples to illustrate specific key points and promising new venues.

First, the manufacturing areas to which biomimetics can contribute are as diverse as the plant structures that inspire them: as the structures that sculpt petal epidermis assist the collection and focusing of sunlight, they have often been used to design and improve photovoltaic systems. Based on the micropatterning of the rose petal epidermis, researchers have created a scattering bio-film that improves light capture (Li et al., 2017), whereas the texture of pansy petals (*Viola* sp) has led to the development of solar cell coating (Schmager et al., 2017). The high aspect ratio of *viola* conical cells gives efficient light harvesting properties and a low reflection loss and replicating this structure using a transparent coating encapsulating a silicon-based solar cell improved by 6% the power conversion efficiency (Schmager et al., 2017). Plant structural patterns also impact construction and architectural design with the development of a new generation of building material and innovative facades to optimize shading systems and reduce heat costs (Durai Prabhakaran et al., 2019).

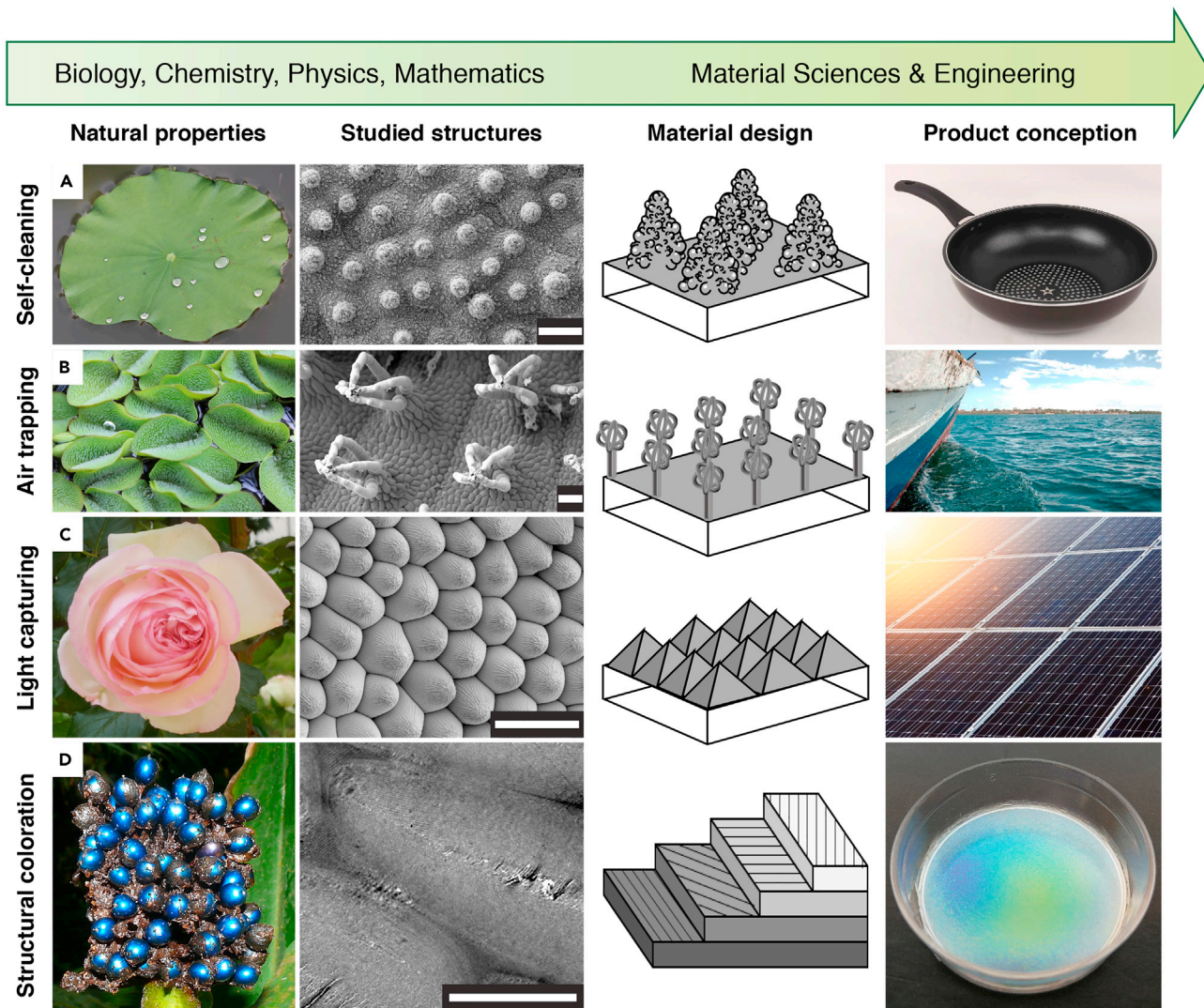


Figure 4. Multidisciplinary approaches are based on plant science research power biomimetics and lead to innovations

(A) The self-cleaning properties of Lotus (*Nelumbo nucifera*) leaves rely on the presence of papillae-like cells covered with hydrophobic waxes. This hierarchical structure has inspired Material Sciences and Engineering for the conception of nonsticky pans.

(B) The ability of *Salvinia* fern leaves to trap air and repulse water rest upon the elaborate multicellular plier-shaped hairs found on the epidermis. These microscopic egg-beater features have been mimicked to engineer drag-reducing coating surfaces for the maritime shipping field.

(C) The conical cells that make the rose petal epidermis are efficient light harvesters and have inspired the design of improved solar cell coatings.

(D) The helicoidal stacking of cellulose fibers within the cell wall of *Pollia condensata* fruit creates an intense blue structural color. This ability to create visible color using transparent and renewable material such as cellulose nanocrystals property is a promising venue to produce sustainable and nontoxic colored materials. Scale bars in the SEM images in A, 20 μm ; B, 100 μm ; C, 50 μm ; and D, 10 μm . Picture credit for Natural properties column: (A) Wilhelm Barthlott & Christoph Neinhuis, (B) Iseempa, and (D) Juliano Costa via Wikimedia Commons. Picture credit for Product conception column: (B) Baxito and (D) Yapadaryko via Wikimedia Commons. Picture credit for Studied structure column: (A & B) Raymond Wightman. SEM image used in Studied structure (D) is based on the TEM image used in Figure 2C (Vignolini et al., 2012).

Second, biomimetic approaches have recently managed to recreate the dynamic behavior of biological material. Not only the final architecture is reproduced, but the mechanisms leading to its production itself are mimicked. Indeed, some plant leaves have the ability to self-heal by secreting a new wax layer once damaged (Huth et al., 2018). These regeneration properties have been chemically simulated by incorporating the chemical *n*-nonadecane wax into a PDMS molding of a lotus leaf, creating a surface able to self-repair in about 20 min, making it the quickest self-healing material without external stimuli (Wang et al., 2020).

Third, there is more than one way to achieve a chosen effect. As different species have evolved specific properties independently from each other, some structures might be easier to replicate than others or be better suited to different applications. Hence, exploring natural diversity is a way to exploit nature ingenuity to our advantage. The epidermis of the floating fern *Salvinia molesta* exhibits a superhydrophobic behavior, reminiscent of the self-cleaning effect first reported for lotus leaves (*Nelumbo nucifera*) (Figure 4). However, although lotus leaves stay clean and dry, thanks to the hierarchical roughness created by papillae-like cells covered with hydrophobic waxes, the fern surface is covered with multicellular pincer-shaped hairs. Those microscopic eggbeaters are mostly hydrophobic except for their tip that lacks the hydrophobic coating of wax crystals. This results in water being pinned at the top of the hairs, creating a layer of trapped air retained on the leaf surface and supporting respiration and photosynthesis when submerged (Barthlott et al., 2010). This discovery led to the development of drag-reducing coating surfaces. If applied to shipping, it could decrease the friction between hull and water up to 30% and reduce fuel consumption and CO₂ emission (Busch et al., 2019).

Replicating structural patterns found on the plant surface is not only useful to produce devices with interesting properties but also advances our understanding of the biological structures themselves, as the exact dimensions and geometry of the replicas can be varied at will and the impact on the properties recorded. Comparing the shape (hemisphere, pyramids, etc.) and characteristics (irregularity, height, position, and tilt angle) of epidermal cells found on the surface of various leaves and petals revealed that patterns comprising hemispherical features exhibit the best antireflection properties (Huang et al., 2016; Li et al., 2018; Schmager et al., 2017). The complex hierarchical pattern of multicellular hairs found on *Salvinia* was also successfully replicated in 3D using laser lithography, permitting the production of materials with similar hydrophobic and air retention properties (Tricinci et al., 2015). It also showed that the amount of air retained on the surface directly depends on the number of filaments that compose each “head” of the eggbeater so that the greater the number of filaments, the greater the amount of air trapped. The “air mattress” on the surface of *Salvinia* can sometimes collapse, but interestingly the fern is able to reconstitute this air layer—by combining theoretical modeling and 3D printing techniques, Xiang and colleagues showed that this recovery behavior stems from microgrooves generated by adjacent cells at the leaf surface (Xiang et al., 2020). This microstructure feature, overlooked in the past, could influence future strategies to optimize underwater structures and make them resistant to extreme environments.

Finally, even if the biomimetic field is inspired by nature’s ingenuity, fabricated devices are not necessarily made of sustainable materials. In the context of environmental awareness, several researchers are now aiming to develop a new generation of biomimetic materials using organic, renewal and biodegradable elements as building blocks. Cellulose is the most abundant organic polymer on earth and represents a fantastic sustainable raw material: helicoidally stacked fibrils of cellulose in *Polia* fruits skin give them their shiny metallic blue appearance: each cell gives a specific color as the thickness of cellulosic layers varies from cell to cell, creating a pixelated effect (Vignolini et al., 2012). This organization relies on the self-assembly properties of the cellulose nanocrystals. Those can be exploited using evaporation processes and fine-tuned to selectively produce a range of different colors (Frka-Petesic et al., 2020), and this led to the creation of sustainable cellulose-based colored films as alternatives to potentially harmful colorants routinely used in textiles, cosmetics, or food industries (Parker et al., 2018).

Conclusions and outlooks

Plants are fantastic architects that can sculpt a remarkable diversity of microscopic features on their surfaces. Those hidden patterns provide the outer layer of plants with striking properties that participate in all aspects of plant interactions with the environment but also with neighboring plants, visiting animals, or hostile pathogens. However, we are only just starting to “scratch the surface” of plant structural patterns.

First, it is clear that the function of those structures greatly depends on their hierarchical organization and their spatial distribution, but the mechanisms that decide what structure form where and thus the overall patterning of the epidermis are generally not well understood. Although we can now identify correlations between the presence of specific compounds and the formation of specific structures, establishing causality remains difficult. In particular, the precise mechanisms accounting for how peculiar cocktails of chemicals could lead to a specific pattern at the plant surface, as well as the mechanisms specifying boundaries between two differentially textured regions across a tissue still need to be unraveled.

Acquiring spatiotemporal quantitative information related to emerging surface features is necessary to understand how, where, and when they form. Such data are essential to decipher the mechanisms that control cell shape and cuticle patterning. It is also useful to infer the likely function(s) of the structural patterns produced as the properties of tridimensional features often depends on their organization. Newly developed analytic tools, together with live-imaging approaches and techniques borrowed from other disciplines, have started to relate different cell behavior and identities to different gene activities and decipher patterning mechanisms. However, recent findings all rely on a high-quality imaging and accurate quantifications, but imaging a complete organ in real time with sufficient resolution is still limited to easily accessible structures (e.g., root tips, leaves or sepals). There is a strong demand for developing novel microscopy techniques supporting the imaging of deep tissues and hidden organs without compromising the physiological state of the living material during the experiment. A recent study used light sheet fluorescence microscopy to investigate developmental processes occurring beneath several cell layers in floral buds, including whole-organ growth (Valuchova et al., 2020). Even if limitations persist, such a technical tour de force represents a promising avenue.

Finally, our understanding of the cellular mechanisms controlling structural patterning in plants largely comes from a handful of classic model systems. Features of interest are generally found in species not previously studied at the bench, and a broad understanding of structural pattern establishment will rely in part on the development of novel model systems (Figures 2D and 2E). Organ size is also a limitation: many flowers are much larger than those of *Arabidopsis* rendering both confocal imaging (Prunet and Duncan, 2020) and data processing complex, as segmentation of a large numbers of cells is difficult. Overcoming such technical difficulties is not a simple task but new experimental and quantitative methods need to be developed so that developmental aspects can be studied in species exhibiting patterns with interesting properties and functions. This will also enable us to understand how conserved the mechanisms sculpting plant surface are: are the molecular entities that pattern plant epidermis as diverse as the structures they produce, or can similar mechanisms be re-deployed in different organs to create contrasting architectures?

We believe that to fully understand the formation of structural pattern, it will be crucial to pursue a multi-disciplinary research strategy (Figure 3), which combines molecular genetics in novel model systems, multiplexed imaging, and computational modeling with techniques and expertise from chemistry, physics, and computer science disciplines.

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AUTHOR CONTRIBUTIONS

E.M., L.R., and S.G. wrote the manuscript and prepared the figures and tables. S.G. produced the graphical abstract. All authors approved the revised version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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