Mechanical buckling can pattern the light-diffracting cuticle of *Hibiscus trionum*

**Graphical abstract**

**Highlights**
- Light-diffracting cuticular nano-wrinkles can be mechanically induced
- The direction of the striations aligns with the force direction
- Striation formation requires layers with different mechanical properties

**Authors**
Chiara A. Airoldi, Carlos A. Lugo, Raymond Wightman, Beverley J. Glover, Sarah Robinson

**Correspondence**
bjg26@cam.ac.uk (B.J.G.), sarah.robinson@slcu.cam.ac.uk (S.R.)

**In brief**
Cuticular striations on hibiscus petals form a coordinated pattern that creates iridescence. Airoldi et al. show that the striations can form by mechanical-stress-induced buckling and demonstrate the role of cuticular layers in this process.
Mechanical buckling can pattern the light-diffracting cuticle of *Hibiscus trionum*

Chiara A. Airoldi,1 Carlos A. Lugo,1 Raymond Wightman,2 Beverley J. Glover,1,* and Sarah Robinson2,3,*

1Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK
2Sainsbury Laboratory Cambridge University, Bateman Street, Cambridge, CB2 1LR, UK
3Lead contact
*Correspondence: bjjg26@cam.ac.uk (B.J.G.), sarah.robinson@slcu.cam.ac.uk (S.R.)
https://doi.org/10.1016/j.celrep.2021.109715

SUMMARY

Many species have cuticular striations that play a range of roles, from pollinator attraction to surface wettability. In *Hibiscus trionum*, the striations span multiple cells at the base of the petal to form a pattern that produces a type of iridescence. It is postulated, using theoretical models, that the pattern of striations could result from mechanical instabilities. By combining the application of mechanical stress with high-resolution imaging, we demonstrate that the cuticle buckles to create a striated pattern. Through mechanical modeling and cryo-SEM fractures, we show that the cuticle behaves like a bilayer system with a stiff film on a compliant substrate. The pattern of buckling aligns with the direction of the stress to create a larger-scale pattern. Our findings contribute to the understanding of the formation of tissue-wide patterns in living organisms.

INTRODUCTION

Understanding the formation of patterns on living tissues is of critical importance to the field of developmental biology. A particular challenge is understanding how tissue-wide patterns can be coordinated between cells. Proposed mechanisms include factors that diffuse across the tissues (Hiscock and Megason, 2015; Wolpert, 1988), local cell-to-cell communication to align polarity (Abley et al., 2013; Goodrich and Strutt, 2011), and alignment of proteins and cellular components with mechanical stresses (Bringmann and Bergmann, 2017; Hamant et al., 2008; Nakayama et al., 2012). Here, we focus on a specific type of morphological pattern: the nanoscale cuticular wrinkles (striations) that can occur on the surface of plant epidermal cells. Despite their small size, these striations are functionally very important. In *Hibiscus trionum*, the cuticle at the base of the petal is wrinkled in a pattern that creates a type of iridescence (Moyroud et al., 2017). Petal cells are initially relatively flat and later deform to play a role in development. Many cellular components align with structural color created by semi-parallel nano-ridges (striations) present on the outermost layer of the cell, the cuticle. These structures produce a type of iridescence with a blue-halo effect (Moyroud et al., 2017). Striations while others do not. Here, we combine mechanical perturbations with high-resolution imaging to show that the striations can be mechanically induced, but only in tissues that normally stripe, and that the orientation of the striations depends on the direction of mechanical stress. We conclude that surface striations are induced by mechanical instabilities in tissues with appropriate material properties. Using equations commonly used to describe large deformations of soft materials, we propose a model to study the patterning we observe in our experiments. Our mechanical model and SEM imaging confirmed that the hibiscus petal behaves as a bilayer composed of a film (i.e., the cuticle proper) and a soft substrate (i.e., the cuticular layer).

RESULTS

Surface striations can be mechanically induced on the surface of tissues that naturally stripe

*Hibiscus trionum* petal epidermal cells in the proximal region of the petal appear purple. In addition to pigments, they have structural color created by semi-parallel nano-ridges (striations) present on the outermost layer of the cell, the cuticle. These structures produce a type of iridescence with a blue-halo effect (Moyroud et al., 2017). Petal cells are initially relatively flat and...
topped with a smooth cuticle (Figures 1A, 1C, 1D, 1G, 1H, 1K, and 1L). Striations start to form in the cuticle on the adaxial side of the proximal (purple) region of the petal during early stages of bud development (when the buds reach a size of 12 mm) and are fully established by the time the flower is fully developed (Figures 1B, 1E, and 1F) (Vignolini et al., 2015). The distal (white) part of the petal does not develop striations, but the cells become conical shaped during maturation (Figures 1I and 1J). The abaxial side of the proximal (purple) region of the petal also remains smooth, and the cells remain relatively flat during development (Figures 1M and 1N).

In order to determine whether surface striations can be mechanically induced, we applied in-plane mechanical stress to petals at the early stage when they are 9–10 mm in length, which is prior to striation formation and will be referred to as the pre-striation stage. Mechanical stress was applied using a micro-extensometer coupled to a Keyence microscope with a 500x objective (see Method details) to enable real-time observations of the cuticle (Figure S1A). Strips of petals were cut from the proximal (purple) region of pre-striation stage buds and attached to the extensometer. It was confirmed that the petals did not have fully formed striations and had only a few shallow striations.
at the cell edges (Figures 2A, 2C, S1G, S1K, and 1D). To test whether striations can be mechanically induced, a mechanical force (15–37 mN) was applied in the longitudinal direction, parallel to the direction of natural striation formation. This resulted in an in-plane deformation of 6%–22% (Figures S1B–S1E). Upon the application of the perturbation, we observed striation formation (Figures 2B and S1H; Video S1).

To determine if the orientation of the striations is pre-patterned, we tested whether the same buckling can be obtained by stretching the tissue perpendicularly to the usual orientation of striation formation. Strips of the petal from the proximal (purple) region were placed into the device in the transverse orientation (Figures 2C and S1K). Prior to the experiment, there were no fully developed striations but a few striations at the cell junctions in the longitudinal direction of the petal. Upon application of mechanical stress (23–42 mN, strain of 16%–50%), (Figures S1B–S1E) in the transverse direction, striations formed in the direction parallel to the force application (i.e., perpendicular to the usual orientation of striation formation) (Figures 2D and S1L; Video S2). We can induce the formation of striations in the perpendicular direction just by changing the direction of applied mechanical tension to the petal strip. This suggests that the striations are not pre-patterned by some organ level polarity factor but are produced as a result of mechanical buckling. The strain required to produce striations was slightly higher in the transverse direction (16%–50%, compared to 6%–22% in the longitudinal direction), indicating that there may be a slight bias in the tissue, but not enough to prevent striations from forming. By comparison, compressive stress can be applied by allowing the sample to lose turgor. This creates a complex pattern of striations that likely reflects the local stress pattern and is a result of the geometry of the cell and its neighboring constraints given by its position within the tissue (Figure S2K).

The amount of time taken for striations to occur when external stress was applied was on the order of minutes from when mechanical stress started to be applied to when striations were visible (Videos S1 and S2). The speed of striation formation is consistent with this process being due to the cuticle buckling rather than new cuticle being deposited. The time taken for striations to appear in these experiments is likely a reflection of the time taken to reach the critical mechanical buckling threshold required for the striations to form. To learn more about the striations that we induced, we examined whether their formation was reversible. Upon removal of the force, the striations remained visible (Figure S2A). However, the sample did not return to its original dimensions (see data and code availability section). After 30–45 min in a relaxed configuration, some samples were able to return to dimensions similar to their starting configuration, suggesting that the tissue is viscoelastic (see data and code availability section). In these samples, several induced striations disappeared or became less pronounced (Figures S2C, S2E, and S2L). Similarly, the irregular striations induced by compressive forces generated by dehydration could be removed by rehydrating the sample (Figure S2L).

**Striations could not be mechanically induced in tissues that do not naturally stripe**

We tested whether we could mechanically induce striations in parts of the petal that do not normally form striations (Figures 1G–1N). Striations usually form only on the adaxial side of the hibiscus petal in the proximal (purple) region. Therefore, we imaged the abaxial side of the petal (Figures 1A, 1B, and 1K–1N) while applying forces and strains similar to those that we found to induce striations on the adaxial side of the petal (Figures S1B–S1E). We did not observe striation formation on the abaxial side of the petal (Figures 2E, 2F, and S1J; Video S3). The distal (white) part of the petal also does not usually form striations (Figures 1A, 1B, and 1G–1J). We imaged the distal (white) part of the petal while forces between 14 and 39 mN were applied (Figure S1B). These forces resulted in a slightly higher strain of 25%–64% (Figure S1E). Striations were not observed to form (Figures 2H and S1N). Unlike the purple region, many of the samples taken from the distal (white) part of the petal broke (4/7) with an average breaking force of 28 mN ± 4 (Figure S1F). Some larger ridges formed in both types of samples, but they were not like naturally forming hibiscus striations (Figures S1P–S1R and S1T–S1V; Video S4). The distal (white) part of the petal may have a cuticle that has different properties to the cuticle in the proximal (purple) region, or the distal (white) part of the petal may simply be too fragile to reach the stress/strain necessary to induce striations. However, the abaxial side of the petal did experience the same strain as the adaxial side of the petal. Thus, the lack of striations on this tissue indicates that there may be a difference in the cuticle that means it requires higher forces to buckle.

**The wavelengths of mechanically induced striations are similar to those of natural striations**

To determine if the striations that we induced mechanically were similar to those observed to form naturally, we quantified their wavelength and found they were not significantly different (1235 nm ± 72, n = 10 petals; longitudinal; 1272 nm ± 72, n = 6 petals, transverse; 1329 nm ± 81 n = 5 petals, wild type [WT]) (Figures 3A and S4). In some of the abaxial and distal (white) samples, we observed larger ridges forming (Figures 3B and S1). They have a different appearance, consistent with them

---

**Figure 2. Mechanically induced striations**

Strips were cut from the proximal (purple) region (A–HF) or the distal (white) region (G and H) of pre-striation stage hibiscus petals and mounted in the longitudinal (A, B, E, F, G, and H) or transverse orientation relative to their orientation in the petal (C and D). (A–D) Proximal adaxial region before the application of force (A and C), there are a few striations visible at the edges of cells. After the application of force, many striations appear across the cells in the direction of stress application (B and D). (E–H) The abaxial side of the petal before (E) and after application of stress (F) does not strike. The distal (white) adaxial part of the petal also does not strike (G) before and (H) after application of stress. The arrow (F) indicates the direction of force application, with WT the orientation of the striations in the wild-type petal, and with I the induced direction of striations. Where there are no striations, no arrow is shown.

Scale bar: 10 μm. See also Figures S1–S3.
being induced by the collapse of the cells rather than from buckling of the cuticle. The wavelength of the larger ridges is significantly different to that of WT or induced striations (Figures 3C, S4A, and S4B) and is typically much longer (2514 nm ± 469, n = 3 petals, abaxial longitudinal; 2271 nm ± 337, n = 6 petals, abaxial transverse; 2039 nm ± 212, n = 4 petals, for the distal [white] region). The amount of strain required to induce striations was up to 20% in the case of the longitudinal striations, which are likely most representative of natural striation formation (Figure S1E). To determine if this strain is realistic of something cells might experience, we measured the increase in the length of cells in the proximal (purple) region of the petal during the period when striations form, from the 10-mm stage to 14-mm buds. Cell length, on average, increases from 30 μm in the 10-mm bud to 39 μm in the 14-mm bud, an increase of around 30% (Figure 3D). Cell length doubles to 80 μm before the bud reaches 30 mm, when striations are fully formed. While cell divisions may occur, this number gives a lower estimate of the growth of these cells. We, therefore, concluded that a strain of 20% could be physiologically relevant, even though we note that the timescales are different, and there is no deposition of newly synthesized cuticle during our experiments.
Figure 4. The involvement of layers in striation formation
(A–F) Cuticle model and formation of surface instabilities.
(A–D) Top views of the initial undeformed configuration $\Omega_0$ (left), the configuration at $\varepsilon^*$ (middle), and a configuration deformed above $\varepsilon^*$ (right). $R$ is the ratio of the linear elastic modulus of the film ($E_f$) and the substrate ($E_s$). (A) $R = 1$, (B) $R = 3$, (C) $R = 10$, and (D) $R = 20$. Stiffer films require less strain to trigger surface undulations. For the case of $R = 20$, the critical strain parameter is approximately 22%, whereas at small values of $R$, the sample must undergo a larger deformation to trigger the instability. The color represents the out-of-plane displacement, as shown in the color bar in (F) where the left purple is zero displacement, and the yellow is the maximum displacement of around five times the film thickness.
(E and F) Secondary instability (period-doubling) as a result of a continuous deformation above the primary threshold. In this case, $R = 20$ and a slightly thinner film compared to (D) to accommodate more wrinkles. (E) Top views of the initial undeformed configuration $\Omega_0$ and the configuration at different stages of stretching. (F) Transverse section of the same simulation as in (E).
(G–K) Cryo-SEM fractures of hibiscus petals.
(G) The adaxial proximal part of the mature petal with a fully striated cuticle.
(H) Proximal abaxial part of the same petal with smooth cuticle.
(I and J) Petals of 10-mm buds after stretching them with the extensometer. (I) Proximal adaxial part of the petal of a 10-mm bud after force application. (J) Proximal abaxial epidermis after force application. In (I), we observe striations similar to (G). In (J), we observe no striations developing like in (H); in addition, we observe some curvature in the deeper cuticular layers.

(legend continued on next page)
Mechanics of striation formation on hibiscus cuticle after an externally induced compression

Mechanical transition from a flat configuration into a periodic surface pattern is a common mechanical instability observed in systems composed of thin films resting on soft substrates (Audoly and Boudaoud, 2008; Genzer and Groenewold, 2006; Groenewold, 2001). This structure has been used to describe patterns of flower cuticles, specifically their orientation as a function of cells and petal geometry, which is proposed as the source of stress (Huang et al., 2017) using a linear material model. Such a description is adequate to describe the onset of the primary instability, but it is not adequate for large deformations. Therefore, we consider a neo-Hookean material that is better suited to describe large deformations, such as the ones observed in plant cuticles, and investigate its response to externally imposed deformations such as those imposed in our experiments. The key parameter for the formation of the pattern is the elastic mismatch between the film and the substrate the R-value, as thoroughly detailed in Cao and Hutchinson (2012), Cerda and Mahadevan (2003), and Nikravesh et al. (2020) and briefly in the Method details. When the strain (relative deformation) is below the critical strain for a given R-value, elastic compression remains more energetically favorable than buckling. The larger the elastic modulus of the film with respect to the substrate (R), the lower the strain threshold required for the pattern to be triggered. If the moduli are equal (R = 1), as for a single layer, the system can be stretched by a large percentage, and a stable surface buckled pattern will not appear (Figure 4A). By comparing values of R = 3, 10, and 20, we can see that the larger the elastic mismatch between the film and the substrate, the less strain is required for the surface to buckle (Figures 4B–4D). Once the periodic pattern has formed, the wavelength of the pattern per unit length remains constant, making the amplitude of the striations the quantity that increases with the externally imposed deformation (Cao and Hutchinson, 2012), until a secondary instability is triggered.

Analysis of the ultrastructure of layers in petals after stress application

Research on buckling in bilayer systems and modeling of our experimental setup suggests the importance of the layers in the Hibiscus trionum cuticle. Cryo-fracture scanning electron microscopy was used to examine the ultrastructure of the cuticle. In the mature petal, striations can be observed on the adaxial side (Figure 4G) but not the abaxial side (Figure 4H). While layers can be seen in the cell wall and cuticle on both sides of the petal, the cuticle proper is more clearly visible on the adaxial side of the petal (Figure 4G, arrow). After force application to the younger buds, striations can be seen on the adaxial side (Figure 4I) but not on the abaxial side (Figure 4J). The layers look comparable to those we see in the WT mature petal. Artificially induced striations look equivalent to the WT striations. In the samples to which force has been applied, we observed a superficial layer (cuticle proper) that is more clearly visible in the adaxial part of the petal (Figure 4I, arrow) than in the abaxial part (Figure 4J, arrows), similar to the situation in the naturally striated tissue (Figure 4G, arrow). The cuticle proper remains attached to the layers below and does not delaminate. In some petals, we also observed period doubling of the striated pattern. Such secondary instabilities are commonly observed in buckled systems after reaching a secondary stress threshold (Figure 4K). In this particular experiment, we might have stretched beyond the threshold. Model solutions obtained by further deformation beyond the threshold exhibit similar patterns (Figures 4E and 4F). The presence of such phenomena supports the assumption we made of the cuticle behaving as a film with a soft substrate (Figures 4L and 4M). Transmission electron microscopy experiments in Vignolini et al. (2015) and Moyroud et al. (2017) show the presence of a film and a substrate, and we can hypothesize that the film is composed of the cuticle proper, and the substrate is composed of a cuticular layer (Dominguez et al., 2011; Yeats and Rose, 2013). Although propidium iodide is known to stain pectin (Rounds et al., 2011; Bidhendi et al., 2020), Hong et al. (2017) demonstrated that it could be used to visualize cuticular striations. Staining of the tissue with the cellulose stain, calcofluor white, revealed that the primary cell wall is distant to the cuticular ridges, consistent with the existence of a cuticular layer (Yeats and Rose, 2013) (Figure 4N). Thus, in our model, the film (Figures 4L and 4M, colored in green) represents the cuticle proper (Figures 4N and 4O, red arrow), and the substrate (Figures 4L and 4M, colored in blue) represents the cuticular layer (Figures 4N and 4O, black arrow). The layers are also distinct in the 10-mm bud (Figures S4C–S4F).

DISCUSSION

Wrinkling and buckling in polymers have been investigated before in several different contexts and scales, from DNA conformations (Lee et al., 2018) to filaments composed by biopolymers (Murrell and Gardel, 2012) and brain development (Budday et al., 2014). Here, we have been able to experimentally demonstrate the formation of iridescence-producing nanoscale wrinkles in hibiscus petals simply by applying a force. Modeling papers have postulated that diffraction grating-like structures could be generated by mechanical forces, but experimental proof has been lacking until now. We have also shown that when experiencing comparable levels of stress and strain, only the proximal (purple) adaxial region of the petal buckled, consistent with the natural distribution of striations.
We have investigated a simple model to represent our experimental observations and to verify that the hibiscus cuticle behaves as an artificial material composed of a film on a soft substrate. Two models for mechanically induced striations in petal surface cells have been proposed previously (Huang et al., 2017; Kourounioti et al., 2012). Kourounioti et al. (2012) proposed that mechanical stresses, such as those generated by cuticle over-production, could produce patterns similar to those observed in natural systems. However, they only considered a single layer model, which does not predict any instability per se, nor is it able to explain that only some parts of the tissue buckle. Huang et al. (2017) consider a linear bilayer material to demonstrate that anisotropic stress and cell shape determine the orientation of growth-induced instabilities in plant cuticles. In our case, we observe that strain-induced stress acting on the tissue, rather than a growth mismatch, is sufficient to induce striation formation. Our model is designed to specifically represent the Hibiscus trionum petal cuticle stretched in our experiments and to corroborate the hypothesis that the cuticle behaves like an elastic bilayer system. This hypothesis is confirmed by our SEM imaging of fractured petals, where we observe a specific bilayer structure present in tissue capable of forming cuticles with striation patterns. Using this SEM fracture approach, we also observed period doubling. Period doubling is commonly observed in theoretical and artificial buckled systems after reaching a secondary stress threshold, confirming that the behavior of Hibiscus trionum cuticle is consistent with what has been previously described for synthetic materials (Cao and Hutchinson, 2012; Li et al., 2012).

Further studies will be necessary to understand the origins of the difference between adaxial and abaxial cuticles in hibiscus petals; a difference in the R-value could be key for the different buckling behavior. The fact that the cuticle proper layer appears more prominent in the cryo-SEM fractures (Figure 4) of the adaxial side corroborates this hypothesis. Future investigation will involve the precise characterization of Young’s modulus of the layers in this paper. We can predict a range of R on the proximal adaxial side of the petal based on the wavelength observed in the naturally forming striations and cuticle proper thickness; more details can be found in the Method details, but further analysis is required to measure the stiffness of these layers. Atomic force microscopy (AFM) might be used to estimate Young’s modulus of the layers in the hibiscus epidermis, but it comes with several challenges, the main one being able to measure the substrate accurately.

Our experiments and modeling show that buckling will occur only for the given ratio of material properties between the cuticle proper and the substrate. Thus, while buckling is a passive process, the production of a material that can buckle to produce the striated pattern may be under tight genetic control and can vary in different tissues of the same organism. In plants, mechanical stresses in cells can result from cellular geometry or can be dominated by tissue-level stress, which results from the shape of the tissues or differential growth between the layers (Trinh et al., 2021; Sampathkumar et al., 2014). In the natural setting, the compressive forces needed to induce the buckling could be generated by the growth of the cuticle and the cells, as previously hypothesized (Huang et al., 2017), or the shape of the petal. In the future, it would be interesting to investigate the origin of the stress (e.g., by identifying mutants with altered growth or geometry). The growth of the cuticle likely contributes to striation formation during normal development, but assessing this contribution goes beyond the scope of this article. The discovery of the importance of mechanical forces in epidermal nanostructure development will be key to characterizing such structures in different species and organs and to explaining the different geometries of a vast array of nanostructures.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Visualizing striations while applying mechanical stress
  - Measurement of wavelength and cell length
  - Cryo-SEM
  - Sectioning and staining
  - Model
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2021.109715.

**ACKNOWLEDGMENTS**

We thank the Open Plant initiative and SLCU microscopy facility for assistance with imaging and the Cambridge Advanced Imaging Centre (CAIC) facilities for cryo-SEM imaging. We are grateful to Matthew Dorling for excellent plant care and to Alessandra Bonfanti and Edwige Moyroud for useful discussions. Cambridge University Botanic Garden provided Hibiscus trionum seeds. S.R. was funded by The Royal Society URF (URF \ R1 \ 180196) and Gatsby Charitable Foundation (GAT3395/CDE). C.A.A. was funded by BBSRC (BB/P001157/1 to B.J.G.). C.A.L. was funded by the Human Frontier Science Programme (RGP0019/2017 to B.J.G.). We are grateful to The SLCU Microscopy Core Facility, supported by the Gatsby Charitable Foundation.

**AUTHOR CONTRIBUTIONS**

Conceptualization, S.R. and B.J.G.; methodology, S.R., C.A.A., and R.W.; investigation, S.R., C.A.A., and C.A.L.; modeling, C.A.L.; data analysis and statistical analysis, C.A.A., C.A.L., and S.R.; writing – original draft, S.R., C.A.A., and C.A.L.; writing – review and editing, S.R., C.A.A., C.A.L., and B.J.G.; supervision, S.R. and B.J.G.; funding acquisition, S.R. and B.J.G.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

Received: March 16, 2021
Revised: June 16, 2021
Accepted: August 24, 2021
Published: September 14, 2021
REFERENCES

Abley, K., De Reuille, P.B., Strutt, D.,Bangham, A., Prusinkiewicz, P., Marée, A.F.M., Grieneisen, V.A., and Coen, E. (2013). An intracellular partitioning-based framework for tissue cell polarity in plants and animals. Development 140, 2061–2074.

Alnaes, M., Blechta, J., Hake, J., Johansson, A., Kehlet, B., Logg, A., Richard- son, C., Ring, J., Rognes, M.E., and Wells, G.N. (2015). The FEniCS Project Version 1.5 (University Library Heidelberg).

Audoly, B., and Boudaoud, A. (2008). Buckling of a stiff film bound to a compliant substrate—Part I. J. Mech. Phys. Solids 56, 2401–2421.

Bidhendi, A.J., Chebil, Y., and Geitmann, A. (2020). Fluorescence visualization of cellulose and pectin in the primary plant cell wall. J. Microsc. 278, 164–181.

Bringmann, M., and Bergmann, D.C. (2017). Tissue-wide Mechanical Forces Influence the Polarity of Stomatal Stem Cells in Arabidopsis. Curr. Biol. 27, 877–883.

Budday, S., Steinmann, P., and Kuhl, E. (2014). The role of mechanics during brain development. J. Mech. Phys. Solids 72, 75–92.

Cao, Y., and Hutchinson, J.W. (2012). Wrinkling Phenomena in Neo-Hookean Film/Substrate Bilayers. J. Appl. Mech. 79, 031019.

Cerda, E., and Mahadevan, L. (2003). Geometry and physics of wrinkling. Phys. Rev. Lett. 90, 074302.

Chen, C., Airoldi, C.A., Lugo, C.A., Bay, R.K., Glover, B.J., and Crosby, A.J. (2020). Flower Inspiration: Broad-Angle Structural Color through Tunable Hierarchical Wrinkles in Thin Film Multilayers. Adv. Funct. Mater. 30, 2006256.

Domínguez, E., Heredia-Guerrero, J.A., and Heredia, A. (2011). The biophysical design of plant cuticles: an overview. New Phytol. 198, 938–949.

Farge, E. (2003). Mechanical induction of Twist in the Drosophila foregut/stomodeum primordium. Curr. Biol. 13, 1365–1377.

Genzer, J., and Groenewold, J. (2006). Soft matter with hard skin: From skin wrinkles to templating and material characterization. Soft Matter 2, 310–323.

Goodrich, L.V., and Strutt, D. (2011). Principles of planar polarity in animal development. Development 138, 1877–1892.

Groenewold, J. (2001). Wrinkling of plates coupled with soft elastic media. Physica A 298, 32–45.

Hamant, O., Heisler, M.G., Jönsson, H., Krupinski, P., Uyttewaal, M., Bokov, P., Conson, F., Sahlin, P., Boudaoud, A., Meyerowitz, E.M., et al. (2006). Developmental patterning by mechanical signals in Arabidopsis. Science 322, 1650–1655.

Heisler, M.G., Hamant, O., Krupinski, P., Uyttewaal, M., Ohno, C., Jönsson, H., Traas, J., and Meyerowitz, E.M. (2010). Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling of auxin transport with convergent floral nanostructures enhances signalling to bees. Nature 550, 469–474.

Hiscock, T.W., and Megason, S.G. (2015). Orientation of Turing-like Patterns in the shoot apical meristem and their shaping by mechanical forces. eLife 4, e07811.

Kurihara, D., Mizuta, Y., Sato, Y., and Higashiyama, T. (2015). ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging. Development 142, 4168–4179.

Landrein, B., Kiss, A., Sassi, M., Chauvet, A., Das, P., Cortizo, M., Laufs, P., Takeda, S., Aida, M., Traas, J., et al. (2015). Mechanical stress contributes to the expression of the STM homeobox gene in Arabidopsis shoot meristems. eLife 4, e07811.

Lee, C., Lee, J.Y., and Kim, D.-N. (2018). Publisher Correction: Polymeric design of DNA origami structures through mechanical control of modular components. Nat. Commun. 9, 626.

Li, B., Cao, Y.-P., Feng, X.-Q., and Gao, H. (2012). Mechanics of morphological instabilities and surface wrinkling in soft materials: a review. Soft Matter 8, 5728.

Moyroud, E., Wenzel, T., Middleton, R., Rudall, P.J., Banks, H., Reed, A., Mellers, G., Killoran, P., Westwood, M.M., Steiner, U., et al. (2017). Disorder in convergent floral nanostructures enhances signalling to bees. Nature 550, 469–474.

Murrell, M.P., and Gardel, M.L. (2012). F-actin buckling coordinates contractility and severing in a biomimetic actomyosin cortex. Proc. Natl. Acad. Sci. USA 109, 20820–20825.

Nakayama, N., Smith, R.S., Mandel, T., Robinson, S., Kimura, S., Boudaoud, A., and Kuhlmeier, C. (2012). Mechanical regulation of auxin-mediated growth. Curr. Biol. 22, 1468–1476.

Nikravesh, S., Ryu, D., and Shen, Y.-L. (2020). Instabilities of Thin Films on a Compliant Substrate: Direct Numerical Simulations from Surface Wrinkling to Global Buckling. Sci. Rep. 10, 5728.

Prüm, B., Seidel, R., Bohn, H.F., and Speck, T. (2012). Plant surfaces with cuticular folds are slippery for beetles. J. R. Soc. Interface 9, 127–135.

Robinson, S., Huljev, M., Barbier de Reuille, P., Braybrook, S.A., Schorderet, M., Reinhart, D., and Kuhlmeier, C. (2017). An Automated Confocal Micro-Extensometer Enables in Vivo Quantification of Mechanical Properties with Cellular Resolution. Plant Cell 29, 2959–2973.

Rounds, C.M., Lubeck, E., Hepler, P.K., and Winship, L.J. (2011). Propidium iodide competes with Ca(2+) to label pectin in pollen tubes and Arabidopsis root hairs. Plant Physiol. 157, 175–187.

Sampathkumar, A., Krupinski, P., Wightman, R., Milani, P., Berquand, A., Boudaoud, A., Hamant, O., Jönsson, H., and Meyerowitz, E.M. (2014). Subcellular and supracellular mechanical stress prescribes cytokinesis behavior in Arabidopsis cotyledon pavement cells. eLife 3, e01967.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.

Terpılıowski, M. (2019). Scikit-posthocs: Pairwise multiple comparison tests in Python. JOSS 4, 1169.

Trinh, D.-C., Alonso-Serra, J., Asaoka, M., Colín, L., Cortes, M., Malivert, A., Takatani, S., Zhao, F., Traas, J., Trehin, C., and Hamant, O. (2021). How mechanical forces shape plant organs. Curr. Biol. 31, R143–R159.

Vignolini, S., Moynour, E., Hingant, T., Banks, H., Rudall, P.J., Steiner, U., and Glover, B.J. (2015). The flower of Hibiscus tronon is both visibly and measurably iridescent. New Phytol. 205, 97–101.

Wang, L.F., and Dai, Z.D. (2016). Effects of the natural microstructures on the wettability of leaf surfaces. Biosurf. Biotribol. 2, 70–74.

Wang, H., Shi, H., and Wang, Y. (2015). The wetting of leaf surfaces and its ecological significances. In Wetting and Wettability, M. Aliofkhazraei, ed. (In-Tech).

Whitney, H.M., Kolle, M., Andrew, P., Chittka, L., Steiner, U., and Glover, B.J. (2009). Floral iridescence, produced by diffractive optics, acts as a cue for an-
STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Biological samples  |        |            |
| Hibiscus trionum    | University of Cambridge Botanic garden | N/A |
| Deposited data      |        |            |
| Model code          | https://github.com/calugo/wrinkles/ | N/A |
| Raw data            | https://doi.org/10.17632/dxc6czd8kd.1 | N/A |
| Experimental models: Organisms/strains | University of Cambridge Botanic garden | N/A |
| Hibiscus Trionum    | University of Cambridge Botanic garden | N/A |
| Software and algorithms |        |            |
| ImageJ              | https://imagej.nih.gov/ij/ | N/A |
| ACME controller software ACMErobotX | https://github.com/ACME-Robinson/InstallPackage | N/A |
| Other               |        |            |
| Keyence WHX-5000 microscope with a 500-X objective | Keyence | N/A |
| Verios 460 scanning electron microscope (FEI/Thermo Fisher) equipped with a Quorum PP3010T cryo-apparatus | FEI/Thermo Fisher | N/A |
| Zeiss EVO HD 15 equipped with the Quorum PP3010T cryo apparatus | Zeiss | N/A |
| Leica stereomicroscope M205FA | Leica | N/A |
| ACME                  | Robinson et al., 2017 | N/A |
| Tough tags           | DiversifiedBiotech | TTSW-1000 |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Sarah Robinson (sarah.robinson@slcu.cam.ac.uk).

Materials availability
No new reagents or plant lines were generated during this study.

Data and code availability
- The data have been deposited at Mendeley Data, V1, https://doi.org/10.17632/dxc6czd8kd.1 and are publicly available as of the date of publication.
- All original code for the modeling has been deposited at https://github.com/calugo/wrinkles/ and is publicly available as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Hibiscus trionum seeds were obtained from Cambridge University Botanic Garden, plants were grown in a greenhouse with a 16 hour day light regime, 60% humidity and a controlled temperature of 21°C.
METHOD DETAILS

Visualizing striations while applying mechanical stress

Mechanical stress was applied to the petals using an adapted automated confocal micro-extensometer developed for measuring forces in *Arabidopsis* seedlings while they are imaged with a confocal microscope (Robinson et al., 2017). As the petals are larger than *Arabidopsis* seedlings the ACME extensometer was modified to use a larger 50 g force sensor (Futek LSB200), and have a larger range of movement (Smaract, SLC-1740 - Linear Piezo Stage). To enable visualization of the striations the extensometer was coupled to a Keyence WHX-5000 microscope with a 500-X objective. High magnification on the Keyence microscope allowed us to observe in real-time the tissue surface. To avoid dehydration the sample was floated on an isotonic solution of 0.1M NaCl. Strips of petal were attached to the modified-ACME device using tough-tags (0.94 × 0.50 inches, distal (white), catalog no. TTSW-1000; DiversifiedBio-tech) and cyanoacrylate super glue. Images were obtained at 2000x magnification before application of stretching force. The sample was subjected to mechanical stress until striations were observed, the sample broke or detached or the limiting force of the machine was reached (approx 80 mN). The force was recorded continuously and noted after striations were clearly visible. Images were taken of the sample at 2000x zoom before and after the experiment. Every effort was made to image the same part of the petal. Where it was possible to identify the same feature in the before and after image, the length between the points was measured in ImageJ (Schindelin et al., 2012) and the strain was computed. Where possible the width of the sample before and after the experiment was also measured. Sample numbers L.Ad n = 9–12, T.Ad n = 6, L.Ab = 5–6, T.Ab.n = 6–8, L.W n = 7, not all traits could be measured in all samples. For the reversibility experiments, the force was returned and held at 0 for 30–45 minutes.

Measurement of wavelength and cell length

The measurements of the wavelength were performed using Keyence images at 500, 2000 and 3000 X magnification. We compared the means of several experiments and measurements of naturally striated samples and carried out a non-parametric Kruskal Wallis test - the p values matrix is shown in Figure S3B. Cell length was imaged with a Leica stereomicroscope M205FA Leica, the pigments in the vacuole of *Hibiscus trionum* epidermal cells allow measurements in WT plants with visible light. ImageJ (Schindelin et al., 2012) was used to measure the wavelength and cell length. All measurements were performed using ImageJ [https://imagej.nih.gov/ij/] (Schindelin et al., 2012). Sample size, n = 340, 231, 402 cells, from 2 flowers for 14mm buds and fully developed petals, 3 flowers for 10mm buds.

Cryo-SEM

Cryo-Scanning Electron Microscopy (Cryo-SEM) of petal surfaces was performed on a Verios 460 scanning electron microscope (FEI/Thermo Fisher) equipped with a Quorum PP3010T cryo-apparatus. Plant samples were applied to shuttle-mounted universal cryo-stubs and flash-frozen in slushed nitrogen. After transfer into the prep-chamber, samples were sublimed at –90°C for 3 minutes, sputter-coated with a thin layer of platinum and transferred to the SEM cryo-stage. Both prep-chamber and cryo-stage were set to –140°C. SE-imaging was performed at 1 keV accelerating voltage and 25 pA probe current using the Everhardt-Thornley detector (ETD) in field-free mode. Images were acquired with drift correction, a pixel resolution of 1536 × 1024 pixels and using a 300 ns dwell time and 32 image integrations. Cryo-fractures were prepared and imaged with a Zeiss EVO HD 15 equipped with the Quorum PP3010T cryo apparatus (Wightman et al., 2017). Petals, arranged vertically in a slotted cryo-stub, were flash-frozen as above and then subsequently fractured with a semi-rotary cold-blade followed by 5 nm sputter coating of Gold-Palladium.

Sectioning and staining

Cuticular ridges were visualized by staining with 0.3% propidium iodide for approximately 5 minutes and cellulose was stained using 0.01% calcofluor white in ClearSee for approximately 5 minutes. ClearSee solutions were prepared by mixing xylitol powder (final 10%(w/v)), sodium deoxycholate (final 15% (w/v)) and urea (final 25% (w/v)) in water (Kurihara et al., 2015). The stain was not washed off but extra water was added to enable imaging after the 10 minutes had passed. Images were collected on a Leica SP8 confocal laser scanning microscope using a 63x oil-immersion objective. Samples were excited with an argon laser (488 nm) for PI and 405 nm for Calcofluor. Data were collected in the calcofluor White (420–480 nm) channel and the PI (600–645 nm) channel. Maximum projections were made using ImageJ [https://imagej.nih.gov/ij/].

Model

Geometry, variables, material and model parameters:
See Figure S4A for volumetric definitions and deformation parameters.

\[
\begin{align*}
V &= \text{volume}, \\
A &= \text{area}, \\
L &= \text{length}, \\
R &= \text{ratio of young modulus of film/substrate}, \\
E &= \text{Young modulus}, \\
f &= \text{film}, s &= \text{substrate},
\end{align*}
\]
Critical strain, wavelength, thickness, scaling exponents, shear modulus, Poisson’s ratio, hyperelastic energy strain density, first and second invariants of the deformation gradient, and the right Cauchy-Green tensors.

The critical threshold depends only on the ratio of the film and substrate elastic properties. As mentioned in the main text, post-buckling analysis reveals that once the primary pattern has been triggered, the effect on the pattern of increasing compression is the increasing of the amplitude as:

$$\epsilon_c = R^{-\alpha}.$$  

Equation (1) alongside the scaling law for wavelength:

$$\lambda_c \sim h R^\beta,$$  

constitute the main results we use in this work. In the above expression $\alpha > 0$ and $\beta > 0$. These two results reveal the role of the elastic properties of the layers in the formation of the surface patterns.

Those two scaling laws are obtained for a semi-infinite half-plane occupied by the substrate. For finite substrates the effect of compression in the final configuration being the possibility of bending generating a hierarchical wrinkled pattern as explored in Chen et al. (2020) and Nikravesh et al. (2020) if the base of the substrate is left free. The thickness of the substrate in our system is considerably above the threshold (10-fold thicker; Nikravesh et al., 2020) that would influence film buckling.

However, the main ingredient for the pattern formation is the large ratio between the film and the substrate elastic properties. As mentioned in the main text, post-buckling analysis (Cao and Hutchinson, 2012) reveals that once the primary pattern has been triggered, the effect on the pattern of increasing compression is the increasing of the amplitude as:

$$A_c \sim h(\epsilon_{xx}/\epsilon_c - 1)^{1/2},$$

With a reduction of the wavelength $\lambda$ given by:

$$\lambda = \lambda_c (1 - (\epsilon_{xx} - \epsilon_c))$$

Until the next instability is triggered. For larger values of $R$, (1) and (2) become accurate, with $\alpha = 2/3$ and $\beta = 1/3$.

Finally, in material characterizations under very controlled conditions, the above equations can be used to estimate $R$ itself, for instance using (2) as an equality in the linear limit, we can infer $R \sim \frac{1}{T}(\lambda_c/h)^3$. Pending a more thorough characterization in this work we get $R \sim \frac{h^3}{\lambda_c^2}$ for $k = \frac{<\lambda_c>}{h}$.

In the WT naturally formed striations $<\lambda_c> = 1321\text{nm}$, and according to this formula values of $h$ in the range of 90 to 210 nm would give us an $R$ between 3 and 20. More detailed analysis of the growth of the cuticle proper layer during petal development and AFM experiments will be necessary to determine the real values involved but such detailed analysis goes beyond the scope of this paper.

Exact results can be obtained for linearly elastic materials, semi-infinite substrates or incompressible composites and large values of $R$, and the specifics can be found elsewhere (Genzer and Groenewold, 2006; Li et al., 2012; Holland et al., 2017). Here we only aim to highlight the role played by the geometry and the material parameters.

We consider a neo-Hookean bi-layer system using as a reference configuration a rectangular volume of dimensions $L_y = 1$, $L_x = 2L_y$, and $L_z = L_y/10$. The thickness of the film used in the numerical study was set to $h = L_z/10$. The constitutive equation for the composite was computed assigning strain energy densities given by:

$$\epsilon_c = \text{critical strain},$$

$$\lambda_c = \text{wavelength of striations at the critical deformation threshold (c).}$$

$$h = \text{cuticle proper thickness},$$

$$\alpha, \beta = \text{Threshold and wavelength scaling exponents.}$$

$$\mu_k, \nu_k = \text{shear modulus and Poisson’s ratio, for each layer (k = film or substrate).}$$

$$\psi_k = \text{hyperelastic energy strain density (k = film or substrate).}$$

$$I_1, J_k = \text{the first and second invariants of the deformation gradient and the right Cauchy-Green tensors.}$$

$$U = \text{the total potential energy.}$$

$$u = \text{displacement field.}$$

$$u_R, u_b, u_T, u_D = \text{Boundary surfaces of the volume at } x = 0(R), x = L_x(L), z = 0(B), y = L_y(T) \text{ and } L_y = 0(D) \text{ respectively.}$$

$$\Delta x, \Delta y = \text{in-plane incremental displacement.}$$

$$k_1, k_2 = \text{proportionality constants for the boundary displacements.}$$

$$k_1 = \Delta x/L_y \text{ and } k_2 = -\Delta y/L_y.$$
\[ \psi_k = \mu_k / 2(l_k - 3) - \mu_k \ln(J_k) + (\lambda_k / 2) \ln^2(J_k) \]

Which describe mechanically isotropic subdomains, the indices stand for film and substrate. Such assumption means that each domain requires only two material parameters \( \mu_k \) and \( \lambda_k \), which can be given in terms of the linear elasticity moduli and the Poisson’s and read as \( \mu_k = E_k / (2(1 + \nu_k)) \) and \( \lambda_k = E_k \nu_k / E_k / ((1 + \nu_k)(1 - 2\nu_k)) \), \( l_k \) and \( J_k \) are the first and third invariants of the deformation gradient and the right Cauchy-Green tensors. The solutions discussed in this paper were obtained using \( \nu_f = \nu_s = 0.48 \).

We solved the boundary value problem by finding the configuration that minimizes the total potential energy: \( U = \sum_k \int \psi_k dV_k \). In other words, the variational problem:

\[ dU(u + \xi v) / d\xi = 0 \]

Where \( u \) represents the displacement and \( v \) is an arbitrary vector field. The boundary conditions for the problem provide the parameter control \( \epsilon \).

Let \( u_R, u_L, u_B, u_T, u_D \), represent the boundary surfaces of the volume at \( x = 0, x = L_x, z = 0, y = L_y \) and \( L_y = 0 \) respectively. We are interested in displacements of the form \( u_R = (\Delta x, k_2 y, 0), u_L = (0, k_2 y, 0), u_T = (k_1 x, -\Delta y, 0), u_D = (k_1 x, 0, 0) \) and \( u_B = (k_1 x, k_2 y, 0) \). The linear components \( k_1 x \) and \( k_2 y \) of the boundary displacements are in place to ensure that the deformation of the volume is well defined. The values of \( k_1 \) and \( k_2 \) are obtained by equating the values at the compressed and stretched edges which gives \( k_1 = \Delta x / L_x \) and \( k_2 = -\Delta y / L_y \), which are none other but the estimators used to compute the strain \( \varepsilon_{xx} = \Delta x / L_x \) and \( \varepsilon_{yy} = \Delta y / L_y \) which together with \( \Delta y = \Delta x L_y / (L_x + \Delta x) \) give us the control parameter:

\[ \epsilon = \Delta x / (L_x + \Delta x) \]

We solved the variational problem (5) directly using the finite element method and the FEniCs framework (Alnæs et al., 2015). For the solutions presented here, we employed meshes of \( N_x \times N_y \times N_z = 40 \times 40 \times 51 \) hexahedral cells, defining two material precompiled subdomains (see the code in the link provided), representing the materials, the film subdomain is composed by the uppermost layer of cell elements, which defines a film of initial undeformed thickness \( h = L_y / 51 \).

The solutions of these hyperelastic material models allow us to describe general finite deformations well beyond linear materials, namely primary and secondary buckled configurations for uniaxial compression (Figures 4E and 4F) or some other well known pattern transitions as in Figure S4B obtained by isotropic compression of a rectangular reference configuration, not only cylindrical buckling.

Model code: https://github.com/calugo/wrinkles/

**QUANTIFICATION AND STATISTICAL ANALYSIS**

The wavelength measurements were compared using a non-parametric Kruskal-Wallis H-test which returns the pair \( (H, p) = (1712.691, p < 1e-3) \). Post hoc comparisons between all the groups using the Dunn’s test was then carried out to determine which set of measurements differ. The anova and post hoc analysis were carried out using functions provided by Scipy statistics library and the scikit-posthoc library. The comparative results of the post hoc analysis are shown in Figure S3B. We have utilized the following libraries: Scipy:https://www.scipy.org/citing.html, Scikit-posthocs; Terpilowski. 2019). We have used a post hoc analysis (Dunn’s test) for all samples in Figure S3B. We have performed such analysis in Phyton and generated the Significance table found in Figure S3B. The number of samples is detailed in the figure legend and methods section associated with the individual experiments.
Supplemental information

Mechanical buckling can pattern
the light-diffracting cuticle of *Hibiscus trionum*

Chiara A. Airoldi, Carlos A. Lugo, Raymond Wightman, Beverley J. Glover, and Sarah Robinson
Figure S1. Mechanical perturbation of Hibiscus trionum flowers. Related to Figure 2 and 3. (A-F) Quantification of the applied mechanical perturbations. (A) The extensometer setup. Mechanical stress was applied using a micro-extensometer coupled to a Keyence microscope. To avoid dehydration, we floated the tissue on an isotonic solution of 0.1M NaCl. The sample is held between the two arms, and one moves P. The arm is attached to a load cell which measures force F. The two are connected in a feedback loop such that target forces can be achieved. (B) Forces were applied to the samples, using the modified ACME device, until striations were visible in the case of samples that striated. In the other samples, equivalent forces were applied. (C) Thin strips of petal were tested, 300-750 μm in width. (D) Using the measured width and the force applied the normalised stress was computed, relative to the average stress applied to the longitudinal adaxial proximal (purple) sections. (E) Sample strain was computed from the images. The distal (white) part of the flower strained more. (F) The breaking force of samples a-h from the distal (white) part of the petal. Where no data point is shown the sample did not break. Ad - adaxial side of the proximal (purple) region, Ab - abaxial side of the proximal (purple) region, W - adaxial side of the distal (white) region, L - longitudinal, T- transverse direction of force applied relative to the petal. (B-E) Each dot represents an independent sample. (G-X) Petal tissue before and after stress application. (G, H) Proximal (purple) adaxial petal before (G) and after longitudinal (H) stress application. (I, J) Abaxial petal before (I) and after (J) longitudinal stress application. (K, L) Proximal (purple) adaxial petal before (K) and after (L) transverse stress application. (M, N) Distal (white) adaxial petal before (M) and after (N) longitudinal stress application. Scale bar (G-N) 100 μm. (O-V) Differences between cuticle striations and larger ridges created by collapsed cells. (O-R) are magnifications of the corresponding image in (S-V). (O, S) Proximal (purple) adaxial petal after stress application with the extensometer. (P, T) Abaxial petal after stress application with the extensometer. (Q, U) Distal (white) adaxial tissue after stress application with the extensometer. (R, V) Abaxial petal mounted in the transverse orientation after stress application with the extensometer. The presence of collapsed cells is less widespread than the cuticle striations through the sample and their appearance is different. (W, X) Cryo-SEM fracture of collapsed cells in the abaxial epidermis after stress application. The large ridges appear to have partially lost their pattern after the removal of the force. Scale bar (O-X) 50 μm.
Figure S2. The reversibility of the induced striations in the proximal adaxial part of the petal. Related to Figure 2. (A) Comparison of striation wavelengths after mechanical stress application and after relaxation of the applied force. We measured the striation wavelength in 116 cells after force application and after removal of such force and observed no statistically significant change in striation wavelength p=0.20699. (B-L) Examples of changes in cuticular patterns. (B-I) Striation dynamics before and after the artificially applied force is removed. We removed the force by setting the extensometer to zero force. (B) Striations formed after stress application (C) Striations disappear after >30min upon removal of the force (in the same cells depicted in B). In these cells, we observe a 27.5% reduction in the number of striations per cell. (D) Striations after stress application (in a different sample) (E) Same cells depicted in D after >30min at 0 force. In these cells, we observe a 21.4% reduction in the number of striations per cell. (F-I) The same set of cells followed during the entire experiment from before force application in the sample depicted in D, E. (F) Before stress application. (G) Petal cells depicted in (F) after stress application (H) and after the stress is removed by returning the sample to zero force. In these cells, we observe a 14.9% reduction in the number of striations per cell compared to G. (I) Same region of the petal after 30min in solution at zero force. (J-L) Cuticle changes by altering the cell turgor. (J) Tissue before drying. (K) After drying for 15 min. (L) After 30 min recovery in DI water. Scale bars: 10 µm. (M) The wavelength measured in dehydrated proximal adaxial samples with a parallel striation pattern. The wavelength is comparable to the WT. Number of measurements for each petal: (A) after force application: sample_e 36, sample_f 30, sample_h 22, sample_b 28 and after zero force sample_e 36, sample_f 30, sample_h 22, sample_b 28. (M) WT_f5 108, dehydration_a 56, dehydration_b 44, dehydration_d 50.
Figure S3. Comparison of the wavelengths of natural striations and mechanically induced striations and larger ridges. Related to Figure 2 and 3. (A) The wavelength of all samples analyzed. (B) Significance table of a post-hoc analysis (Dunn’s test) for all samples. All the abaxial and distal (white) ridges are significantly different from the striations observed in the WT proximal (purple) part of the flower and in the stretches performed in the adaxial proximal (purple) part of the petals. T= stretched in the transverse direction, L=stretched in the longitudinal direction, AD=adaxial, Ab=abaxial, Wh=distal (white) adaxial part of the flower. The letter refers to the sample and the date to the day.
Figure S4. Further model analysis and staining experiments. Related to Figure 4. (A-B) Extending the model to other patterns of stress. (A) Cuticle model and deformation assumptions. We consider the deformations for a small region of in-plane area $L_X L_Y$ and thickness $L_Z$ of the sample with undeformed in-plane dimensions $L_W L_S L_Z$, such that $L_X \ll L_S$ and $L_Y \ll L_W$. $L_Z$ is the cuticle thickness which is the sum of the film (h) and the substrate ($H_S$) thickness. (B) Solutions under an isotropic compressive strain. Top-views of the initial undeformed configuration $\Omega_0$, the configuration at different stages of compression. The colour represents the out of plane displacement in relative length units where $L_X = 2$, $L_Y = 1$, $L_Z = 0.1$, $h = L_Z / 10 = 0.01$. (C-F) Sections of Hibiscus trionum petal stained with propidium iodide (red) and calcofluor White (blue). (C) Mature flower adaxial proximal region, (D) Mature flower abaxial proximal region, (E) 10 mm bud adaxial proximal region, (F) 10 mm bud abaxial proximal region. Scale bar (C-F) is 10 µm. The cuticle proper is easily visualised on the adaxial side but is more variable and stains more faintly on the abaxial side.