Solution: Exercise 8

We start with the observation that epidermal cells in leaves make jigsaw-puzzle-like shapes (Fig. 1, 2). Then we make a hypothesis that these shapes are a way for cells to accommodate mechanical stress while having a large surface area.

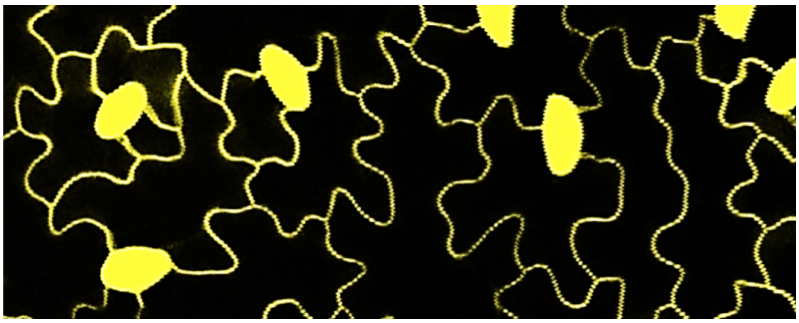


Figure 1: Outlines of jigsaw-puzzle-like shaped epidermal cells in the leaf of *Arabidopsis thaliana*.

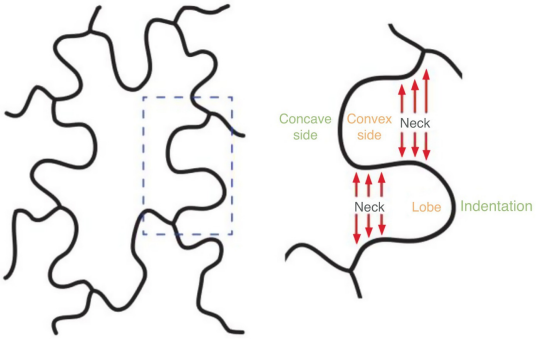


Figure 2: Cells with a jigsaw-puzzle like shape that interlock with neighboring cells are called puzzle cells. The inset (left image, dashed box), is shown on the right and demonstrates the basic terminology used to describe puzzle cell morphology. Adapted from [1].

1. **What is mechanical stress? Provide the simplest definition possible.**
   Mechanical stress is the force exerted on a body divided by the area of the cross-section of this body.

2. **How do we measure mechanical stress:**

   i) **computationally:**
   Finite Element Method (FEM) simulations, which are commonly used in continuum mechanics. For a review of the usage of FEM in plant biology, see [2].

   ii) **experimentally:**
   There is no experimental method to directly measure stress in plant tissue, which is why we need to rely on computational methods.

3. **Starting from a small cubic cell, how do you expect maximal mechanical stress in a single cell to behave if you:**

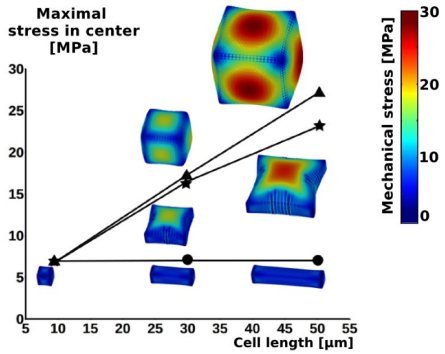
   i) **elongate the cell (expand it in one direction),**

ii) **expand the cell in 2 directions,**

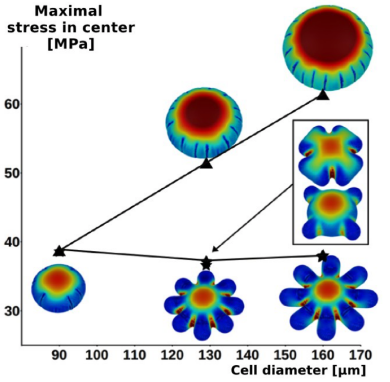
iii) **expand the cell in 3 directions,**

iv) **add lobes to the cell?**

Maximal stress in the cell wall increases when large, isotropic surfaces are created. Elongating the cells or adding lobes does not contribute to the maximal stress values as long as it does not increase the largest surface (Fig. 3).



(a) Graph points show stress in cells expanded in one dimension (circles), two dimensions (stars) or three dimensions (triangles). Enlargement in two or more dimensions substantially increases stress in the center of the cell walls.



(b) In isolated circular cells, pressure-induced stress increases with diameter (triangles), similarly to (a). Adding lobes, regardless of their length, width or number (inset) does not influence maximal stress in the cell wall in the center (stars).

Figure 3: Cellular stress patterns in finite element method (FEM) simulations. Cell walls have uniform, isotropic material properties (Young's modulus = 300 MPa) and are inflated to the same turgor pressure (5 bar). Adapted from [3].

4. **How would you evaluate the hypothesis that puzzle shapes are a way of avoiding high mechanical stress in large cells?**
By performing FEM simulations of stress on cell shapes coming from real tissue (extracted from microscopy data) and on simplified polygons created based on junctions between the real-life shapes (Fig. 4). This way we have two options for cell shape for similar cell area.

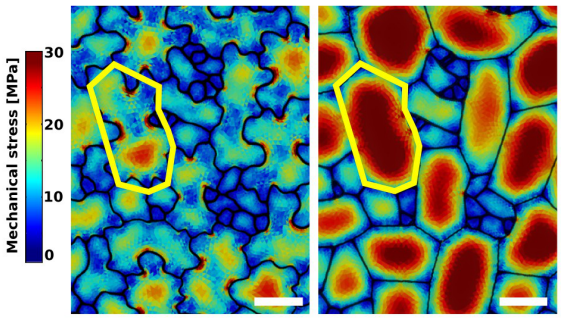


Figure 4: Relationship between cell shape and stress. Left: Principal stresses generated by turgor in vivo were simulated in a FEM model on a template extracted from confocal data. Right: a simplified tissue template using the junctions of the cells shown in the left pannel. The yellow outline marks a corresponding cell in both panels. Total area and number of cells is the same, however, the maximal stress is much lower in the puzzle-shaped cells compared to the more isodiametrically-shaped cells. Scale bars, 50 μm. Adapted from [3].

5. **FEM simulations are computationally costly. What quick proxy would you choose for mechanical stress?**
One good option is to use the radius of the largest circle that can fit inside the cell contour (also called largest empty circle or LEC, Fig. 5), since stress is the highest in the isotropic, wide parts of the cell (compare Fig. 3,4).

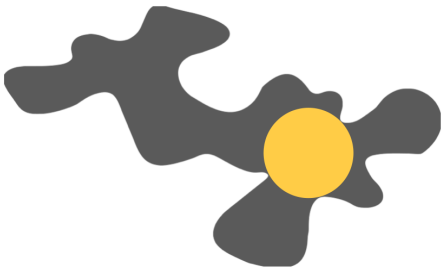


Figure 5: Yellow: the largest circle that can be fit into a cell of interest (gray). The size of this circle, called Largest Empty Circle (LEC), is a convenient proxy for mechanical stress as it is conceptually simple and easily implementable in cell size quantification software such as MorphoGraphX [4].

6. **What measure of cell shape would you use to quantify the 'puzzleness' of cells? Think about how these different measures would perform in distinguishing cells of different shapes.**

   a) Simple (cell perimeter, cell area)
   b) Circularity (4πArea/Perimeter)
   c) Convex hull-based (area or perimeter of the cell/area or perimeter of the convex hull, Fig. 6). This measure will from now on be called **lobeyness** [3].
   d) Skeletonization (calculating the 'skeleton' of each cell to know the number of lobes, Fig. 6)
   e) Elliptical Fourier Analysis (deconstructing the cell shape into waves)

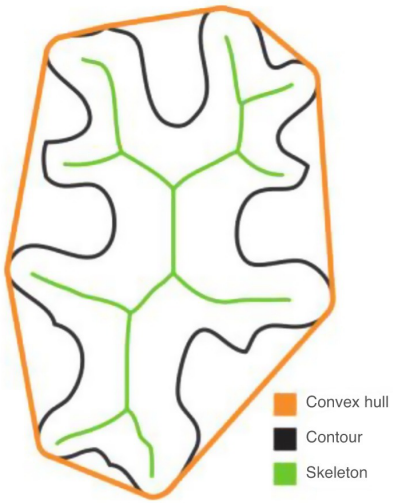


Figure 6: Measures of puzzle cell shape are typically computed from the cell contour (black), its convex hull (orange), or a skeleton approximating the overall form of the cell (green). Adapted from [1].

7. **Now that you are equipped with a good shape measure, how would you check the plausibility of the hypothesis that puzzle shapes are observed in large cells?**
If you cannot create transgenics, collect leaves from different plant species (for example, leaves of different trees that grow on your campus) and compare shape and size of epidermal cells. Here (Fig. 7), this analysis was performed for 24 species of bushes and trees and saw that indeed, small cells were much less likely to create puzzle shapes than large cells.

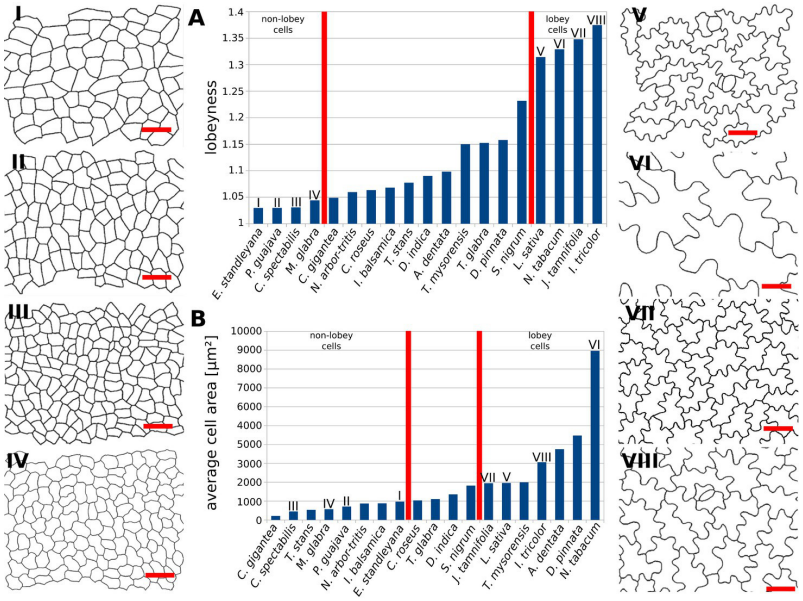


Figure 7: (A) Average cell lobeyness (for definition, see Question 6c). (B) Average cell area. (I-VIII) Pictures of leaf epidermal cells of species corresponding to numbering in (A) and (B), numbered by the order of appearance in (A). Scale bars, 50 μm. Adapted from [3].

8. **How would you check if there is a relationship between cell shape and organ shape?**
Find a mutant/transgenic line in which leaves have an altered shape (an easy phenotype to notice) and measure epidermal cell shape. Below (Fig. 8): *A. thaliana* transgenic line

overexpressing LONGIFOLIA1 (*p35S:LNG1*) in a range of phenotypes from wildtype-like (A,B) to elongated leaves and epidermal cells (C,D).

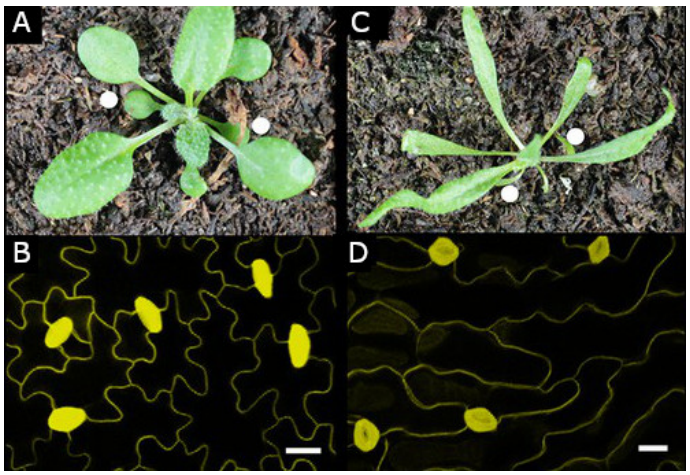


Figure 8: *p35S::LNG1* transgenic plants with wild type-like phenotype (A,B) and a strong phenotype with dramatically elongated cotyledons and leaves (C,D). B,D: confocal images of epidermal cells in cotyledons (marked by white dots in panels A and C, respectively). Scale bars: 20 μm. Adapted from [3].

9. **Can you think of other ways in which cells could potentially minimize stress on the cell wall or try to withstand it?**

- Divide
- Thicken cell wall
- Strengthen cell wall by altering its chemical composition

References

[1] A. Sapala, A. Runions, and R. S. Smith, "Mechanics, geometry and genetics of epidermal cell shape regulation: different pieces of the same puzzle," *Current Opinion in Plant Biology*, vol. 47, pp. 1–8, Feb. 2019. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S1369526618300311

[2] A. J. Bidhendi and A. Geitmann, "Finite Element Modeling of Shape Changes in Plant Cells," *Plant Physiology*, vol. 176, no. 1, pp. 41–56, Jan. 2018. [Online]. Available: http://www.plantphysiol.org/content/176/1/41

[3] A. Sapala, A. Runions, A.-L. Routier-Kierzkowska, M. Das Gupta, L. Hong, H. Hofhuis, S. Verger, G. Mosca, C.-B. Li, A. Hay, O. Hamant, A. H. Roeder, M. Tsiantis, P. Prusinkiewicz, and R. S. Smith, "Why plants make puzzle cells, and how their shape emerges," *eLife*, vol. 7, p. e32794, Feb. 2018. [Online]. Available: https://doi.org/10.7554/eLife.32794

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