

Reply to 'Early shaping of a leaf'

Feng et al. reply — Computational modelling has often been used to fill the gap between our understanding of the biophysical properties of cells and our observations of the organ shapes resulting from morphogenesis. In our recent study published in *Nature Plants*¹, we used computational modelling to decipher how the observed biomechanical differences in various leaf primordia domains can explain the initial asymmetry in leaves¹. Coen and Kennaway suggest that our modelling results are seemingly counter-intuitive². Here, we would like to further elaborate on our model assumptions, and to emphasize the importance of epidermal-growth-control for understanding biological shape.

Formulated as early as 1882, by Julius Sachs, the epidermal-growth-control hypothesis derives from the observation that if the epidermal layer of an organ is separated, the epidermal layer shrinks while the inner tissue layers expand³. In other words, the epidermal cells in plant organs experience and resist tensile stress due to the expansion of turgid inner cell layers. Notably, the interaction between the epidermal layer and inner layers is reminiscent of the way that cell walls limit protoplast expansion. In some of their modelling analysis, Coen and Kennaway found that soft regions tend to form bulges². They suggest that the reduction in cell growth rate should be proportionate to wall stiffness, which requires the assumption that plant cells grow by tensile stress coming from turgor pressure that stretches the elastic cell wall. Based on this assumption and the observed patterns of wall stiffness, they found that the leaf cross-section shape, which our model reasonably matches, is counter-intuitive. In such simulations, leaves do not have epidermal constraints on growth. When the restriction from the epidermal layer is removed, our model similarly predicted that soft cells grow faster than stiffer cells (Fig. 1a,b), although we used an energy-minimizing modelling framework. Because the lack of epidermal restriction results in a free boundary problem, it is not surprising that no remarkable tissue shape could be achieved.

Using identical parameters but adding a fixed epidermal layer, we were able to obtain opposite results, that is, stiff cells have a faster growth rate (Fig. 1c). We also allowed the epidermal layer to expand from the tensile force exerted by the inner cells. From a physical viewpoint, increased expansion of

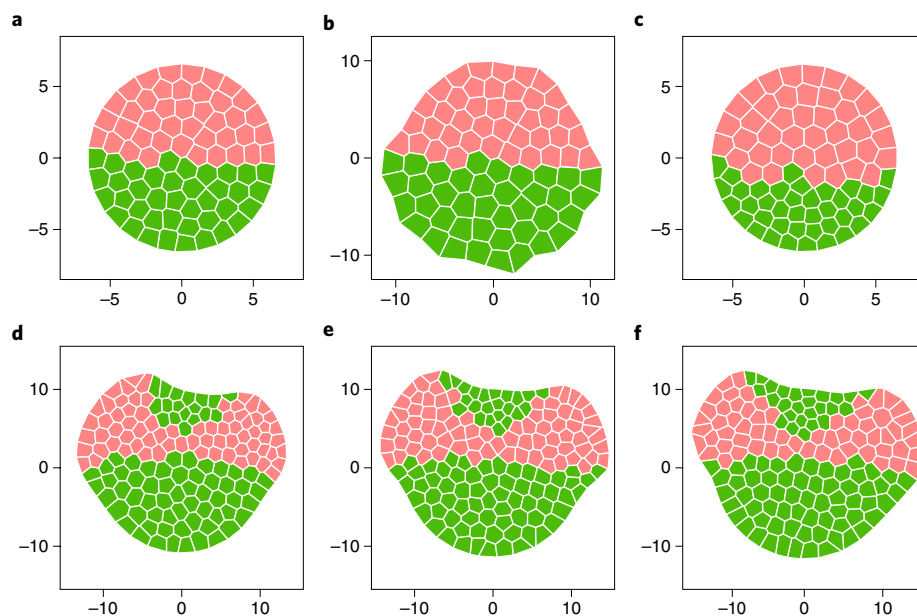


Fig. 1 | Simulation results under different constraints. **a**, Initial state. **b**, Final state with no epidermal constraint and no cell division. **c**, Final state within a fully constrained space without cell division. **d–f**, Simulation results for different initial growth potential ratios in a partially constrained space. **d**, $Fr < Fg$; **e**, $Fr = Fg$; **f**, $Fr > Fg$, where Fr and Fg describe the gradient of energy of pink (stiff) and green (soft) cells, respectively. F is defined as growth potential, $F = dE/dA$, where E is the cell potential energy, and A is the cell area. In each panel, x and y axes indicate dimension length and are in the same arbitrary unit.

the stiff inner cells in a semi-confined space occurs at the expense of reduced expansion of the soft cells, and the system would allow this to happen if expansion of the stiff cells could reduce the system's potential energy more efficiently. In fact, the larger the ratio of $\lambda_{stiff}/\lambda_{soft}$, the higher the growth rate reached by the stiff cells; where λ is the parameter that describes the resistance to deviation from the target area. To some extent, λ reflects cell wall stiffness. Other recent theoretical studies also highlighted the importance of epidermal restriction on plant organ shape^{4,5}.

The Coen and Kennaway model assumes that the specified reduction in cell growth rate is in proportion to wall stiffness, which they called 'growth by turgor'². This implies a further assumption that cell growth is driven by elastic walls yielding to uniform turgor pressure. Although consistent with some short-term observations, this could be an oversimplified assumption. The polymer-like cell wall structure exhibits elastic, plastic, viscoelastic and viscoplastic behaviours⁶. For example, plasticity — that is, irreversible wall deformation in response to a transient

force — is widely seen⁶. In their model, elastic behaviour is nicely described in a quantitative manner. However, the modelling framework does not take plastic and viscoelastic behaviours into account.

In our Monte Carlo-based energy-minimization modelling algorithm, one does not explicitly specify the relationship between cell growth rate and cell wall elastic, plastic and viscoelastic behaviours. Cell growth is described by generalized potential energy, which indirectly reflects wall behaviours. To explain experimental observations, we assumed that stiff inner cells exert larger forces on their neighbouring epidermal cells. This can be achieved by assuming that stiff cells reach plastic deformation earlier, or assuming that stiff cells have a higher turgor pressure. In fact, the presence of different turgor pressures in different cells in hypocotyls has recently been proposed⁵.

Although the energy minimizing approach has the advantage of simplifying calculations, it does not allow precise manipulation of all cell growth parameters. For example, wall stiffness values can be set

as constants, but cell growth rates are not directly specified. Cell growth rate is affected not only by wall stiffness, but also by current cell size (that is, current area), which changes over time. To test the robustness of our model to parameters, we evaluated whether the shape evolution is sensitive to the ratio of growth potentials between the two cell groups under a specific constraint. We assigned different target areas but kept other parameters constant to obtain three different situations (Fig. 1d–f). Although the relative growth potential of the two cell types changed significantly, the shape evolution showed relatively minor changes, suggesting that shape evolution is insensitive to a range of growth potential values in a partially confined space. Thus, a range of cell growth rates can produce similar shapes when cell wall stiffness is fixed. From these simulations, it is also evident that we do not need an assumption that ‘both the stiff and soft tissues have the same specified growth rate’, as suggested by Coen and Kennaway².

Technically, our model is solved by the Monte Carlo method with intrinsic randomness. When cell division occurs, we re-divided the grid with the centroid of cells to ensure that the simulated region is a Voronoi diagram. At the same time, to match experimental observations, we adjusted the cellularity of certain regions so that their properties are fixed. With these

simulation strategies, cells at the boundaries between stiff and soft regions may switch their identities during growth. Nevertheless, we do not foresee an effect on shape evolution by this approach.

From the mechanical point of view, the epidermal-growth-control hypothesis suggests that the mechanical roles of the epidermal and inner cells in shaping leaf development are different. The epidermal cells provide mechanical constraint, while the inner cells translate the mechanical features into local growth and provide a driving force against the epidermal layer. Consistent with this division of labour, we used a hybrid numerical framework to separately describe these two cell types.

Computational modelling must use simplifying assumptions. Depending on the biological question of interest, we may need to use realistic assumptions to replace some simplifying ones. The three possibilities named by Coen and Kennaway are all reasonable considerations. In addition to possible non-uniform turgor, assuming isotropic cell growth is clearly an oversimplification. Measuring inner cell wall stiffness would also clarify the role of pectin de-methyl-esterification in wall mechanics. Furthermore, oversimplification of wall behaviour as purely elastic may not properly recapitulate the evolution of organ shape. Further experiments on these issues are expected to provide more precise

biophysical parameters, which would allow more elaborative models to better describe leaf-shape change. □

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Competing interests

The authors declare no competing interests.

Additional information

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