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Mechanics, geometry and genetics of epidermal cell shape regulation: different pieces of the same puzzle

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Pavement cells in the leaf epidermis of many plant species have intricate shapes that fit together much like the pieces of a jigsaw puzzle. They provide an accessible system to understand the development of complex cell shape. Since a protrusion in one cell must fit into the indentation in its neighbor, puzzle cells are also a good system to study how cell shape is coordinated across a plant tissue. Although molecular mechanisms have been proposed for both the patterning and coordination of puzzle cells, evidence is accumulating that mechanical and/or geometric cues may play a more significant role than previously thought.

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Introduction

Cells with an elaborate, jigsaw puzzle-like shape appear in the epidermis of many plant species, including the model plant *Arabidopsis thaliana*. Progressing from simple polygon-shaped meristematic cells, they develop into large cells with many interlocking lobes (convex areas) and indentations (concave areas), that often resemble puzzle pieces (Figure 1). Because of this dramatic change in form during development, puzzle cells have become an attractive system for investigating cell-shape control.

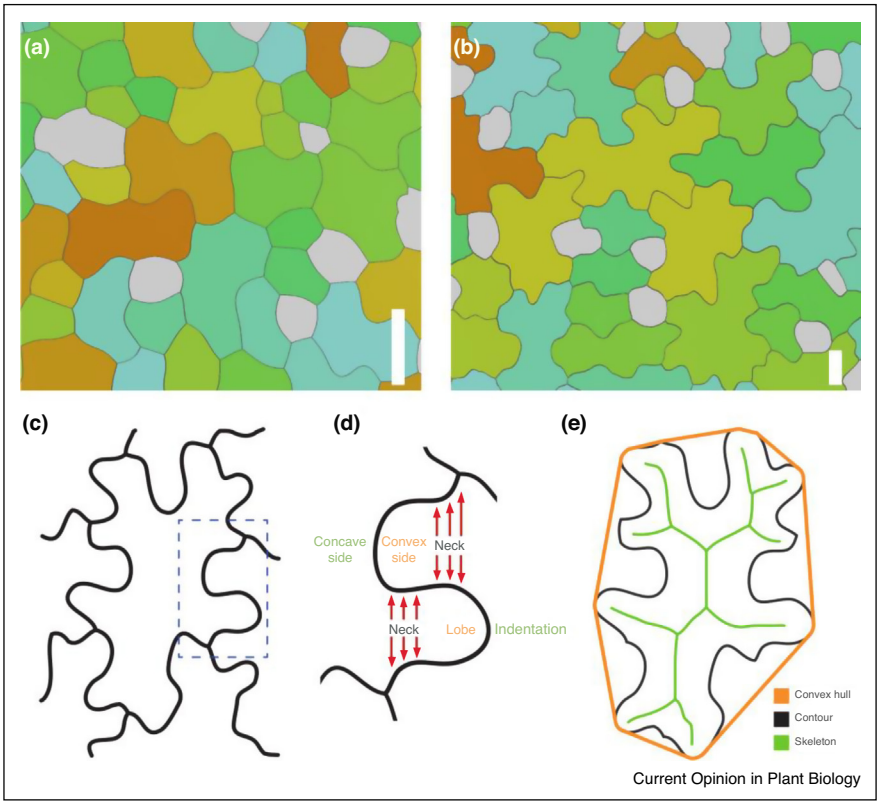
Understanding puzzle cell development has been challenging, as it appears to involve feedbacks and interactions at several scales. These feedbacks include various self-organizing components that act at the sub-cellular scale. Molecular interactions for cell-wall partitioning [1–7], sub-cellular cytoskeleton organization [8••,9,10] and differential cell wall mechanics [11,12••,13,14•] all interact to produce the lobes and indentations. Since a lobe in one puzzle cell must fit into the corresponding indentation in its neighbor, coordination of these processes must occur at the supra-cellular level. Possible candidates to provide this coordination are extra-cellular signaling molecules [15], mechanical or geometric cues [13,16••,17] or a combination of the two.

When striking or unusual cell shapes are observed, it is natural to wonder about their function, as form often follows function in biology (for a recent review see [18]). Several hypotheses have been proposed to explain the interlocking puzzle shape of these cells. It has been proposed that puzzle shapes may be important for the correct spacing of other epidermal cell types such as stomata and trichomes [19] or to help the leaf to remain flat and thereby optimize light capture [20]. Another hypothesis is that the interlocking shapes may increase adhesive strength between cells, increasing the stability of the epidermis [21,22] that is often under considerable tension from internal cells [23]. A related idea is that puzzle cells might help the tissue to undergo large reversible deformations, such as when the tissue is stretched or bent [14•].

Sapala *et al.* (2018) have recently proposed a different function for the puzzle cell shape, that is related to the mechanical stresses the cell walls encounter due to their turgor pressure [16••]. Green plant tissue relies on turgor pressure for its shape. It behaves like a pressurized cellular structure, not unlike an inflatable mattress. When turgor pressure is reduced, the structure collapses and the plant wilts. Sapala *et al.* (2018) propose that the puzzle shape allows the formation of large pressurized cells in the epidermis of plant organs that grow isotropically, as seen in many leaves. If the cells had simple shapes, large cells would bulge out excessively under pressure and burst (imagine an air mattress without the vertical seams) [16••]. Of course, long, thin cells would also work, as in the air mattress, however these would only be possible in plant organs that grow mostly anisotropically, such as roots or stems.

Studies on pavement cell development have been impeded by difficulties in reliably quantifying the growth, shape and mechanics of these cells, and in understanding the nature of patterning within cells and its coordination between adjacent cells. Here we review recent work addressing these shortfalls and highlight how they begin to substantially change our understanding of pavement cell function and development, which may be directly related.

Figure 1



Pavement cell shape. (a,b) Cell shape in the epidermis of an *Arabidopsis thaliana* cotyledon. **(a)** Small cells (2 days after germination) have relatively isodiametric shapes, while **(b)** larger cells (6 days after germination,) display very complex, jigsaw puzzle-like shapes. The emergence of the puzzle shape occurs early in organ development. Scale bars, 20 μm. Cells belonging to stomatal lineage (grey) do not become puzzle-shaped. **(c)** Cells with a jigsaw-puzzle like shape that interlock with neighboring cells are called puzzle cells. The inset (dashed box), is shown in **(d)** and demonstrates the basic terminology used to describe puzzle cell morphology. **(e)** 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).

Quantifying puzzle cell shape

Puzzle-cells have complex, recognizable shapes that nevertheless are highly variable. This has made it challenging to reliably quantify cell shape changes during development or identify cell-shape differences between various mutants. Shape measures provide a means to determine specific geometric aspects of cell shape. The simplest one is circularity, which indicates how close a cell shape is to a circle (see Box 1A for definitions). The perimeter or area of a cell can be compared to its convex-hull (Figure 1e) to give a measure of the convexity of a cell representing the amount of indentations or concave regions the cell has. Conversely, one can take the ratio of the largest empty circle (LEC) that fits inside a cell and compare it to the cell area, giving another simple measure of how the cell deviates from a circular shape. These simple to compute measures are useful in coarsely evaluating differences in cell-shape, focusing on the general degree of lobeyness. Although most are not directly related to the mechanism of pavement cell formation, the LEC by itself (without the area ratio) provides a proxy for stress in the cell [16••]. It follows that measures related to the mechanism controlling pavement-cell morphogenesis may be especially useful in characterizing the phenotypes of various puzzle cell shape mutants.

For more advanced quantification, measures characterizing the number and geometry of lobes and indentations are required. These measures are often directly relevant to proposed mechanisms, for example the number of lobes at a given cell size could indicate the periodicity of an intra-cellular partitioning mechanism. Manual measurement of these features indicate they are indeed biologically relevant [2,7,24], however it does not offer a reliable means of objective quantification. To address this problem several methods for puzzle cell quantification have been proposed. They can be roughly divided into two categories: those that focus on the cell contour and those that use skeletons to approximate the overall form of the cell (Box 1B, Figure 1e).