Statistical Properties of Cell Topology and Geometry in a Tissue-Growth Model

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Abstract. Statistical properties of cell topologies in two-dimensional tissues have recently been suggested to be a consequence of cell divisions. Different rules for the positioning of new walls in plants have been proposed, where e.g. Errara's rule state that new walls are added with the shortest possible path dividing the mother cell's volume into two equal parts. Here, we show that for an isotropically growing tissue Errara's rule results in the correct distributions of number of cell neighbors as well as cellular geometries, in contrast to a random division rule. Further we show that wall mechanics constrain the isotropic growth such that the resulting cell shape distributions more closely agree with experimental data extracted from the shoot apex of *Arabidopsis thaliana*.

1 Introduction

Cell division in plants has been studied by plant biologists for over one hundred years (see review in [1]). From simple microscope observations biologists have formulated rules for cell division. During mitosis plant cells are divided into two daughter cells by introducing a dividing cell wall. Hofmeister suggested a rule where new cell walls are formed perpendicular to the main axis of growth, i.e. perpendicular to the main axis of the cell [2]. Sachs noted that new walls form almost perpendicular to old walls [3]. Similarly to Hofmeister's rule, Errara's rule state that the division is along the shortest path dividing the mother cell into two parts of equal volume [4]. More recently, experiments where spherical cells have been compressed into oval shapes agrees with these rules [5,6]. It has also been seen that the arrangement of cytoskeletal structures reveal the placement of new cell walls [1].

Many biological tissues develop in two-dimensional sheets. The epidermal layer in plants is an example, where anticlinal divisions and the lack of cell migration assure the two-dimensional structure of the layer. The epidermal layer can then be described by a network of connected polygons (cells), edges (walls) and vertices, where the connections are updated at divisions only. The predominant existance of three-vertices leads to the average number of cell neighbors (walls)

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to be six following Euler's rule. But the average can be fulfilled by many neighbor distributions, and already in the 1920's F. T. Lewis studied this in growing and proliferating cucumber epithelia [7,8]. He found that although most cells had six neighbors (47%), the distribution was not symmetric, with more five-sided cells (25%) compared to seven-sided (22%). He also noted the non-existance of triangular cells as well as cells with more than nine neighbors. Recently, Gibson *et. al.* found similar asymmetric distributions in epithelial cell layers in several animal tissues including epithelia from *Drosophila melanogaster* wing primordium [9]. Interestingly, they also introduced a probabilistic model where a discrete Markov chain was used to describe topological updates due to cell divisions and the model was able to predict the experimental distribution of the number of cell neighbors.

The approach by Gibson *et al.* focused on the topology of the cells in the tissue, but disregarded details of cellular geometry, growth and proliferation. Here, we use a two-dimensional cell-based tissue growth model to analyze how explicit division rules and wall mechanics lead to different topological as well as cellular shape distributions. We assume isotropic growth, and mainly study tissues with quite homogeneous cell sizes. This resembles the situation in the plant shoot apex, and we compare our models with novel data from the *Arabidopsis thaliana* shoot apical meristem.

Our model allow us to compare Errara's classical division rule (new walls are placed such that the cell is divided into two equally sized daughters along the shortest path) with a random-direction division rule. We also investigate how wall mechanics constraining the purely isotropic growth affect the topology and cell geometry. We compare the resulting tissues with the distributions of the number of neighbors (topology) as well as cell shapes (geometry) from experimental data.

2 Materials and Methods

2.1 Experimental Data

The model results are compared with the experimental data presented in Gibson *et al.* [9], as well as new data from the shoot apex in *Arabidopsis thaliana* (Fig. 1). The shoot data was extracted from a confocal projection using the merryproj software [10]. It is interesting to note that although the statistics is sparse, the overall topological distribution in the shoot data is very similar to the *Drosophila* case as well as the Lewis data [7,8].

2.2 Tissue Model

The model is a two-dimensional model where the spatial degrees of freedom are for vertices, which are connected via edges that represent cell walls. Each cell is described by a polygon, i.e a number of vertices together with corresponding edges. The vertex positions are updated viscously, where we assume that velocities are proportional to the forces acting upon them. The cell walls are treated

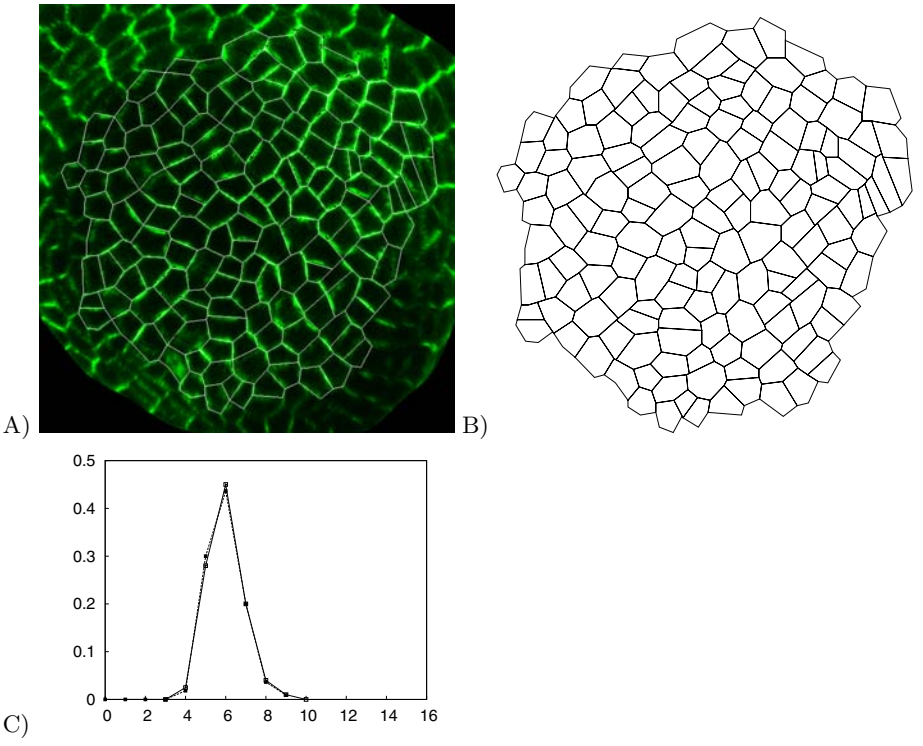

Fig. 1. Data from an image of the meristem of *Arabidopsis* is compared with data from *Drosophila* presented by Gibson *et al.*. A) The original image taken with confocal microscopy. Cell walls are marked manually. B) The template extracted from the image. C) The distribution of the number of neighbors for *Arabidopsis thaliana* marked with filled black squares (■) (110 cells) and *Drosophila melanogaster* marked with empty white squares (□) (2,172 cells) [9].

as mechanical springs. We describe the dynamics with a system of ordinary differential equations originating from an isotropic growth term, wall mechanics, and plastic wall growth. The contribution from wall springs is described by

$$\frac{d\mathbf{v}_i}{dt} = k_w \sum_{j \in \mathcal{C}(i)} \frac{\mathbf{u}_{ij}}{|\mathbf{u}_{ij}|} \left(\frac{|\mathbf{u}_{ij}| - L_{ij}}{L_{ij}} \right) \quad , \tag{1}$$

where $\mathbf{v}_i$ is the position of vertex i, k_w is a material constant setting the strength of the wall springs, L_{ij} is the resting length of the wall spring between vertex i and j, and $\mathbf{u}_{ij} = \mathbf{v}_j - \mathbf{v}_i$. The summation is over vertices connected via edges to vertex i.

Cell walls under tension grow plastically. The change in resting length of a wall spring is

$$\frac{dL_{ij}}{dt} = k_g \Theta \left(\frac{|\mathbf{u}_{ij}| - L_{ij}}{L_{ij}} \right) \quad , \tag{2}$$

where k_g is a constant setting the rate of growth and Θ is the ramp function defined as

$$\Theta(x) = \begin{cases} x \text{ if } x \geq 0 \\ 0 \text{ if } x < 0 \end{cases} . \tag{3}$$

A radial force is used to model isotropic growth of the tissue originating from internal cell pressure. The force on a vertex is described by

$$\frac{d\mathbf{v}_i}{dt} = k_r \mathbf{v}_i \ , \tag{4}$$

where k_r is a constant setting the strength of the radial force.

To decrease computational time, cells on the boundary of the tissue are removed if a cell is outside a given threshold radius, R_t.

Division Rules. If the area of a cell exceeds a threshold value, D_t, the cell is divided into two daughter cells. At division, two new vertices are added at two different walls in the cell, and a new wall connects the vertices. The resting length of the new wall is set to the distance between the two vertices. In addition, the walls at which the new vertices are added are split into two, and the new resting lengths are set proportionally to the split distance such that $L_1^{\text{new}} + L_2^{\text{new}} = L^{\text{old}}$.

The new wall dividing the cell into two daughters is defined by a spatial position and a direction. As an approximation of dividing the cell into two almost equally sized daughters, the new wall is passing through the center of mass of the dividing cell. The direction of the new wall is determined from two different rules. In the first rule, called *random direction*, a random (uniform) direction is chosen. In the second rule, called *shortest path*, the direction of the dividing wall is chosen such that the path through the cell is the shortest possible. This is the model definition of the Errara rule. To avoid four-vertices, walls that are closer to a vertex than a threshold, $w_t L_{ij}$ is moved away from the vertex to the threshold position.

2.3 Simulations

The system of ordinary differential equations is solved numerically with a 5th order Runge-Kutta ODE solver using adaptive stepsize. Data is sampled at ten different time points. As we remove cells at the boundary a new generation of cells is present at each time point. The number of cell neighbors are collected for all cells, excluding cells at the boundary. Five different intial states are used for each model to gather statistics. The initial states are all one single cell represented by a regular polygon with three, five, seven, nine, or eleven vertices. Data from the 50 different time points is averaged to give final distributions for each model.

We use in house developed software allowing for discrete updates between each time step taken by the numerical ODE solver. In these updates we check cells for division and removal. Parameter values used in the simulations are presented in Table 1.

Table 1. Parameter values used during simulations

Parameter	With mechanics	Without mechanics
k_w	0.05	-
k_g	0.01	-
k_r	0.05	0.05
D_t	1	1
R_t	10	10
w_t	0.1	0.1

3 Results

3.1 Comparing Different Division Rules

First we compared the topology distributions from the two different cell division rules. The result is presented in Figs. 2A and C, where the data from the simulations are presented together with the experimental distributions. For both division rules the distributions have their maximum at six cell neighbors, but while the distribution for the shortest path division rule match the experimental data well, the distribution for the random direction division rule is broader compared to the experimental data.

3.2 Removal of Spring Wall Mechanics

To study how removal of wall mechanics affects the distribution of cell neighbors, we kept the radial force that drives the isotropic growth, but removed the cell wall springs.

The result is presented in Figs. 2B and D. What might be found surprising is that the differences between the distributions with and without wall spring mechanics are very small. This is true for both the shortest path division rule and the random direction division rule.

3.3 A Quantative Measurement of Cell Shapes

A striking result from our simulations is that even if the mechanics has no or little effect on the distributions, there is an obvious visual difference of cell shapes between simulations with and without mechanics. Examples of cell shapes from simulations with the two different division rules are presented in Fig. 3. Clearly, the cell shapes emerging using the shortest path division rule is more plant-like, and also the simulations with mechanics look more like cellular tissue in comparison with the non-mechanical simulations.

To quantify differences in cell shape, we measure the ratio between the length of the boundary of a cell squared and the area of the cell. In Fig. 4 this measure is presented for different simulations and compared with the *in vivo* data. First, it can be seen that the shortest path division rule generally has a closer match to the experimental values than the random direction division rule. The random

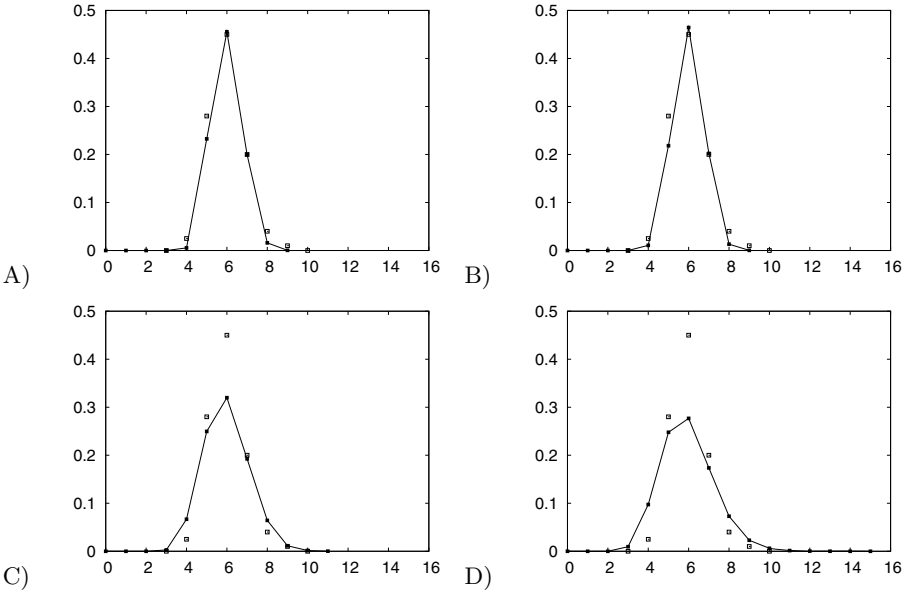

Fig. 2. Distributions of number of neighbors for simulations with different division rules with and without mechanics. Filled black squares (■) marks results from simulations while empty white squares (□) marks experimental data. A) Shortest path division rule with mechanics. B) Shortest path division rule without mechanics. C) Random direction division rule with mechanics. D) Random direction division rule without mechanics.

direction division rule not only differ more in the average value, but it also has a much larger spread among cells. A closer inspection also reveals that simulations with mechanics is closer to the experimental values than simulations without mechanics.

4 Discussion

We have used a simple two-dimensional cell-based tissue growth and proliferation model to investigate the dependence on cell division rules on statistical properties of cell topology and geometry. We used an isotropically growing tissue and showed that one of the classical rules for plant division (Errara's rule), where new plant walls appear at the shortest path that divides the cell in two equally sized dughter cells, indeed do produce a skewed topology distribution seen *in vivo* with an average of six neighbors but with more five than seven neighbor cells (Fig. 2A). On the contrary, the 'control' model with new walls placed in a random direction did not follow this distribution (Fig. 2C).

For each division rule we performed two sets of simulations, one with wall mechanics and one without. While the non-mechanics simulations follow pure isotropic growth, wall mechanics constrains the growth via a wall growth model.

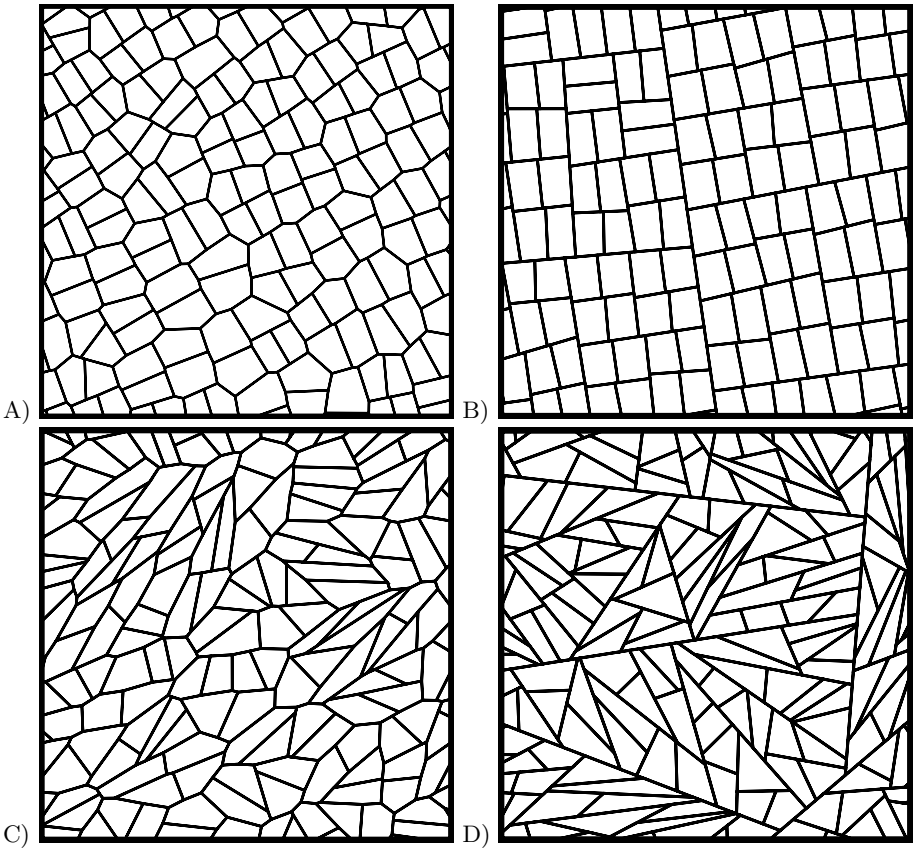

Fig. 3. Examples of simulations with different division rules. Simulations with the shortest path division rule with (A) and without (B) mechanics. Simulations with the random division rule with (C) and without (D) mechanics.

When comparing distributions of number of neighbors there was no difference between the sets with mechanics and the simulations without mechanics, although a visual difference could be seen, where the simulations with mechanics produced more plant-like cells (Fig. 3).

To investigate this further we quantified cell shapes and compared with novel data from the *Arabidopsis* shoot apex. We could again see that Errara's division rule produced a statistical distribution very similar to our measured data, while the random division rule produced far more asymmetric cell shapes. In this case, we could also see a small difference between simulations with or without mechanics, where including wall mechanics generated shapes more similar to the *in vivo* data (Fig 4).

In conclusion, we have showed that statistical properties of cell topology and shape indeed can be used to discern among different model hypotheses for cell proliferation. Interestingly, divisions at random directions do not lead to correct

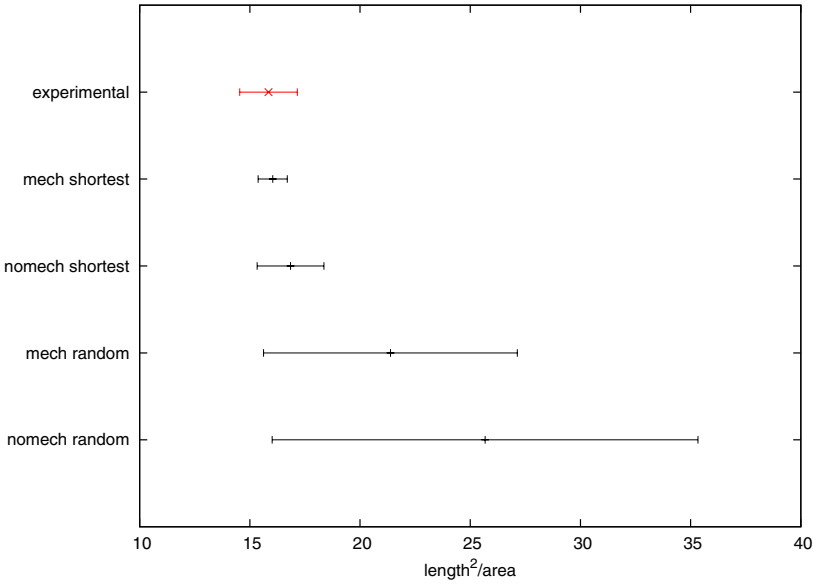

Fig. 4. Mean and standard deviation for the ratio between length of cell boundary squared and cell area. The data point marked with a cross (×) shows the experimental data. Other data points marked with a vertical dash (|) show results from simulations. Simulations have been done with the two different division rules, with and without mechanics.

topology or cell geometries, while Errara's classical cell division rule do agree with the statistical properties from experimental data. Of course, statistical agreement is only a first test which many hypotheses may pass, and ultimately the hypotheses must be compared with statistics of single cell data. Still, we have presented a useful methodology, where explicit and mechanistic hypotheses that combine into a cellular plant growth and proliferation model can be compared on merits based on experimental data.

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