# Aspen Stomata Density and Triploidy
## Developed in Compucell3D

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**Abstract**

This research represents two 'efforts'. First, a model was produced of stomatal development to address the question of decreased stomata density in tripoidal western aspen. Second, research was done to provide a foundation for a future model that will address the seeming contradiction between triploid stomata density and drought tolerance.

*Keywords:* CPM , Stomata, Polyploidy, Triploidy, CompuCell3D, Western Aspen

## Introduction

With drought severity and frequency projected to increase in the future [Backhaus 2014] and with an estimated 35% of plants being polyploidal( Wood 2009 ), it is vital that we understand plant adaptation to drought stress, if polyploids are inately more drought tolerent, and how stomata function and develop under drought conditions. Brianne Palmer's research into stomatal density and karyotype of western aspen provides a glimpse into how these factors relate and raises interesting questions. One question is, is the decrease in stomatal density observed in triploid western aspen due to triploid cell size? The average stomatal density at 800x600 px for diploids was 28.74 and 23.19 for triploids. The average length for diploid stomata was 27.339 micrometers and 31.82 micrometers for triploids.

The reason this question is important to understanding drought tolerance in plants is the decrease in stomata density in triploidal plants would appear to have a negative effect on plant drought tolerance. It was observed by Carpenter and Smith (1975) that Appalachian hardwoods growing on dry sites had smaller and denser stomata. This is the opposite of triploid western

aspen which were observed to have larger and less dense stomata (Palmer 2017). It may be the case that western aspen are less drought tolerant then their neighboring diploid western aspen( Dixon and DeWald 2015). However, the highest proportion of western aspen tirploidy occurs in drier areas (Mock et al. 2012). Hepsworth et al (2015) observed artificially decreasing stomatal density enhanced drought tolerance, and Zhang et al (2015) found that polyploidy correlated with drought tolerance in two apple cultivars. There appears to be contradictory evidence to what stomata morphology with what kerotype produces the most drought tolerant plant and opens up possibility for further research.

## 0.1 Stomata Development

While this model does not focus on stomata function, and therefore, cannot shed light on ideal plant morphology for drought tolerance, it does address the mechanism behind the differences in stomata density between diploid and triploid western aspen.To achieve this end, stomata development is modeled using the "three steps for cell-type differentiation" (TSCD) outlined by Keiko et al (2007) and Bergman and Lau (2012).

TSCD functions as follows:

- Stomata lineage is started by pluripotent Meristemoid mother cells(MMC) differentiating themselves from Protodermal cells by producing SPEECH-LESS (SPCH) transcription factor.SPCH drives MMC formation and the asymmetric division of these cells. This process of determining SPCH production is possibly stochastic, but that is not certain.
- MMCs undergo asymmetric division, which creates smaller meristemoids and larger stomatal lineage ground cells (SLGC) .
- Meristemoids then differentiate into Guard mother cells (GMC). This process is driven by the transcription factor MUTE, which terminates the 'stem cell likeness' or pluripotency of merstemoids. This locks the GMC into becoming a stomatal cell.
- The GMC then devides symmetrically into Guard cells (GC). At this step the stomata is formed and has it's 'mouth' like shape. The transcription factor FAMA drives this division.
- Protodermal cells and SLGCs become pavement cells, the waterproof cells that form between stomata.

Under certain conditions SLGCs can produce SPCH like MMCs and asymmetrically split off another meristemoid, in a process called 'spacing'. Additionally, meristemoids can divide to produce new SLGCs in what is

called amplifying. If two meristemoids are produced next to one another some conflict resolution happens and one cell continues on to form a stomata while the other changes to a SLGC. How this conflict is resolved is not clear to me.

It is important to note that stomata arrange themselves in different patterns in different species. These patterns can range from spirals to the "at least one cell separation" in Arabidopsis. Since I was unable to find the stomata pattern in aspen and Bergman and Lau's paper focuses on Arabidopsis stomatal development, I used the Arabidopsis stomata development as the foundation for the algorithm.

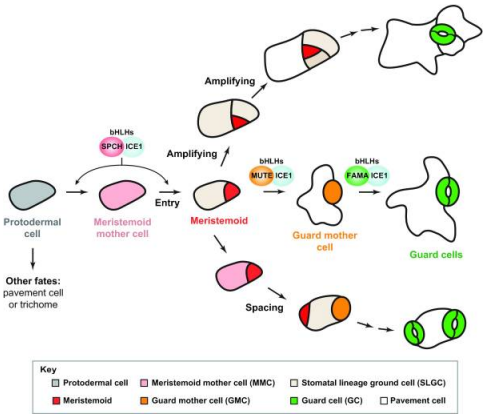


Figure 1: Cell fate transitions and divisions during Arabidopsis stomatal development. (Bergman and Lau 2012)

## 0.2 Polyploidy and Cell Size

The last key piece of information is the effect of polyploidy on cell size. In Tsukaya (2013) it is concluded that ploidy level increases cell volume, however the reason is unknown. Tsukaya goes on to state that '...plant cells can increase their volume without increasing ploidy level. Thus, cell size, cell function and ploidy level are not directly associated, and genetic regulation plays a role in ploidy level-dependent cell size.' It has also been noted that a doubling of the genome does not result in a doubling of volume occupied by chromatin, it only causes a 1.6 increase in nuclear envelope surface area(Comai 2005).Comai does state, however, that "Increasing the genomic content of an organism usually increases cell volume." This conclusion is supported by Marinho et al (2013) which mentions that polyploidal organisms my increase their cell size.

## 0.3 Western Aspen Stomata and Polyploidy

Brianne Palmer's research suggests a link between stomata density and triploidy in western aspen. The overlap between diploid and triploid stomata lengths combined with a trend toward longer stomata lenghts in triploids provides evidence that something is increasing triploid stomata length at the community level, but not absolutely.Meaning the cell sizes of stomata in

both diploid and triploid individuals are not being controlled by one factor, but at a population level there is a trend. This seems consistent with how polyploidy increases cell volume, while not absolute, it does generally result in increase cell volume.

The effect of polyploidy on stomata density can be thought of like drawing dots on a balloon then inflating the balloon. As the volume of the balloon increases the distance between the two points also increases. In this case, as the volume of pavement cells increases relative to the increase in stomata volume the distance between stomata must increase. Since the bounding box used to measure stomata density (800X600 px) does not scale with relative cell size, the stomata density inside this box will decrease as the length between stomata increases.

## Method

This model uses two algorithms. The first, is a 2D CPM of the TSCD which produces a 'ground' model of diploid stomata development using arbitrary cell area. The volumes for triploid stomata developemnt are increased by inferring area from the average increase in triploid stomata length. Second, two images produced by this model, one for diploid and one for triploid, are then run through a MatLab function which calculates the average euclidean distance between all GCs. From here we can find the increase in distance between, and decrease in density of stomata in triploids relative to diploids.

### CPM Stomata Development

The CPM functions as follows:

1. The model is initialized with MMCs stochastically distributed among protoderm cells.
2. MMCs asymmetrically split into meristemoids and SLGCs.
3. 'Conflicts' where two meristemoids are next to each other are resolved by the cell 'checking' if its neighbor is a meristemoid and if it is it switches cell type to SLGC.
4. Spacing and amplification are accomplished by protoderm and SLGCs checking their neighbors to see if there is a meristemoid. If not, the cell either 'enters' the process as a MMC, in the case of protoderms, or asymmetrically divides, in the case of SLGCs.
5. Once cell fate is determined (i.e. after 250 mcs or even distribution of meristemoids) protoderm and SLGCs change type to pavement cells.

(Pavement cells have low contact energy to achieve the 'puzzle piece' shape seen in nature.) Meristemoids change cell type to GMCs.

6. GMCs symmetrically divide and change type to GCs.
7. The cell area is then increased for the tripoild. Since stomata form a generally circular shape and the actual average stomata width was not known the relative increase in area was determined using the equation for area of a circle and their radii found by dividing the length in half. $l_t = 31.820, l_d = 27.339, r_t = 15.910, r_d = 13.670. A_t / A_d = 795.225/587.023 = 1.355$ The arbitrary values used for diploid were then multiplied by 1.355. This uniform increase in cell 'volume' may not be true to nature, but seems like a best guess.

Figure 2 is a side by side comparison of plant epidermis with stomata (small circular cells) and pavement cells (jigsaw puzzle piece shaped cells). In hindsight abstracting out the symmetrical division of GMCs into GCs would have produced a more true to image effect, since, one GMC tended to retain 'roundness' better than two GCs. Increasing contact energy did not 'squeeze' the two GCs closer together as I had thought. This may have had a negative effect on the results, but I thought it was necessary to include the final GMC division in this model. Also, understanding the division of GMCs will provide useful in future models.

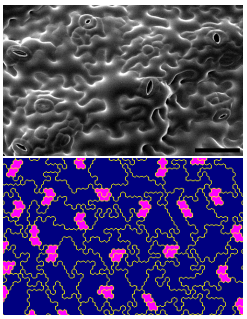

Figure 2: Comparison of leaf epidermis (Stomata and Pavement cells) and CMP output of the same. Pink cells are stomata guard cells(GCs) and blue are pavement cells. http://dev.biologists.org/content/131/21/5215

### MatLab Distance Calculations

To calculate the average euclidean distance two images, one of the CPM tirpolid and diploid, were fed into a MatLab function that works as follows:

1. The image is eroded to remove the cell boundary line produced by the CPM.
2. The image was then converted to binary black and white.
3. Centroids for each stomata GC pair were found.
4. The euclidean distance from each centroid to each other centroid was found using the pdist() function.

5. The average of these distances was found using the mean() function.

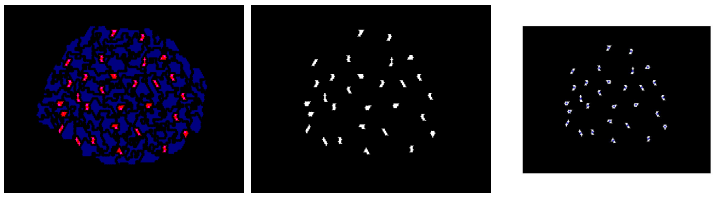

Figure 3: 1) Errosion on image. 2) Binary thresholding. 3) Centroids

## Results

The average stomata distance for the triploid CPM was found to be 183.357 px and the 172.062 px for the diploid model. This means there was a 6.656% increase in distance between triploid and diplod stomata(the centroid between GCs). Since an increase in distence between stomata is effectively a proportional decrease in th size of the bounding box, the triploid bounding box is functionally 87.310% the size of the diploid bounding box. From this we can infer that the CPM triploid density is 28.74 (the observed stomatal density)* .8731 = 25.093 triploid stomata/new bounding box.

The observed average density of stomata was 23.19 in tripoilds. While the inferred CPM triploid density is greater than the observed triploid stomata density it does fall with in the confidence interval (20.3 - 26.1) provided by Palmer. Furthermore, it shows that stomatal density is negatively affected by an increase in cell area, due to triploidization.However, it does suggest that increased cell size due to triploidization is not an absolute factor in increased cell size and decreased stomata density. This is consistent with how polyploidization seems to affect cell volume.

Another, potential, reason for the higher than expected CPM triploid stomata density may be due to GC shape in the model. As mentioned above, dividing the GMCs to GCs did not produce the desired 'rounded' shape of GCs around stomata. This in turn, may have offset the centroids in the MatLab function.

## Discusion

One important observation is that the size of triploidal aspen leaves, at least anecdotally, do not appear to be larger than those of diploids. So, in effect, triploid leaves do have a lower density of stomata compared to

diploids. This would appear to be contradictory to my results, which suggest density is relative to the bounding box used to measure cells. If the cells proportionally increase then the bounding box should also proportionally increase to keep stomata density constant. However, this only means the leaf is a defacto bounding box.

The question remains why are western aspen so prevalent in drier areas of the west if larger, less dense stomata have a negative effect on drought tolerance? One potential answer is, larger stomata may enable more efficient photosynthesis (Parkhurst 1994). Another, interesting possibility, is that autotetraploid cassave down regulates production of Oxidoreductase NAD(P) (Feifei et al 2014). Oxidoreductase NAD(P) is an electron carrier between photosynthesis and the Calvin cycle. A reduction in electron transport would result in a reduction in ATP synthesis, but also a reduction in H20 loss. It appears that desert plants have adopted a similar strategy. In some desert plants the photosynthetic tissues can be highly reflective which resulted in upwards of a 53% reduction in electron transport rate (Stemke 2011).

To test best drought tolerance strategy, I propose a 'plant leaf' model with the following characteristics:

1. Stomata development which is influenced by keryotype, as well as, expression of SPCH, MUTE, and FAMA regulated by the environment.
2. Stomatal gas exchange. Meaning functioning stomata, which uptake CO2 and expel O2 and H2O, which in turn 'feed' the Calvin cycle and nutrient transport.
3. Photosynthesis which is directly controlled by proportional light absorptance. Meaning the reflectivity of photosynthetic cells can be adjusted.
4. A functioning Calvin cycle where NAD(P) and ATP biding proteins can be adjusted to reflect observed differences in keryotypes.
5. The model must be 3D, because as Parkhurst (1994) mentions, "Intercellular diffusion is fundamentally a three-dimensional process..."

While there still exists gaps in my knowledge as to how these processes exactly relate, a model that can effectively demonstrate these characteristics would be valuable to understanding plant adaptation to drought stress. Additionally, once ideal drought tolerance characteristics are known from the model these characteristics may be used to determine plant species best fit for survival in drought prone regions. This could be useful from finding plants best suited to erosion control in areas prone to severe weather events (long droughts followed by floods) to engineering drought hardy crops.

## Acknowlegements

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## References

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