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## Geometric plasticity at leaves from *Ctenanthe oppenheimiana* probed by measure of distances between stomata

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# Geometric plasticity at leaves from *Ctenanthe oppenheimiana* probed by measure of distances between stomata

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**Abstract.** We measure the stomatal plasticity at *Ctenanthe oppenheimiana* leaves based on distance between stomata pairs in microscopic images. The distance measurements revealed that the plasticity induced by environmental adaptations are measurable using the distance between neighbor stomata. Plants exposed to extreme light irradiation times from 24 to 4 hours per day, presented discernible relations between stomatal distances. The average distances between stomata pairs revealed a powerful tool to predict changes of plasticity of stomata according with different light acclimation conditions at *Ctenanthe oppenheimiana* plants.

## 1. Introduction

Stomata is a cell that act as valve localized at plant leaves and stems performing the exchange of gas with the atmosphere [1]. They control the relation between the carbon gain and water loss to adjust photosynthetic needs with environmental and atmospheric conditions [2]. The plant epidermis is highly plastic during environmental adaptation, thus the number of stomata per leaf area can be highly variable and adapted according to the environmental conditions [3]. In this case the quantity and quality of light are also critical for stomatal plasticity on the leaf epidermis.

Therefore, the distribution of stomata in the epidermis can be a very valuable quantitative parameter in the analysis of plasticity as it provides many details of distance and angle between stomata that can change at plant adaptation to certain environmental conditions.

In this study, we attempt to develop computational metrics based on distance between stomata pairs located in the microscopic images from the live *Ctenanthe oppenheimiana* plants, exposed to different conditions of light acclimation.



## 2. Methods

### 2.1. Optic Microscopy and plant acclimation

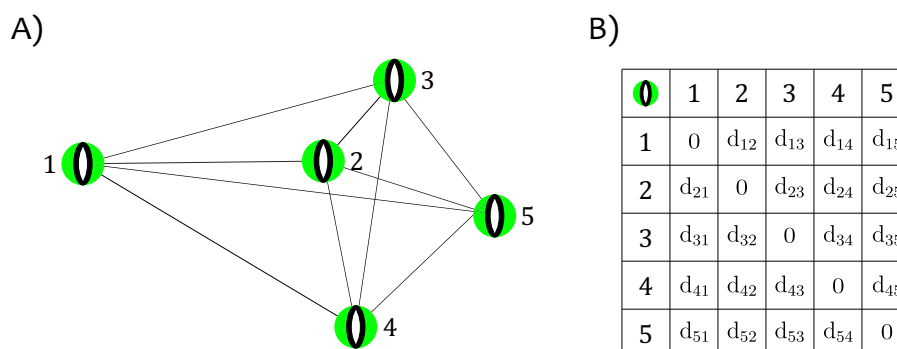
We performed optic microscopy with *Ctenanthe oppenheimiana* leaves from plants artificially adapted at controlled growth chambers. The microscope used was Axio-Lab A1, Zeiss, adapted with cameras axiocam ERc5s. The magnification used was  $100\times$ . The plants used at microscopy were adapted with continuous 55% relative humidity, photon flux from  $100\mu\text{mol}/\text{m}^2 \times \text{s}$ , temperature  $25^\circ\text{C}$  and light photo periods from 24 and 4 hours per day during a period of 60 days.

### 2.2. Measure of distances between stomata

The microscopic images were processed using Matlab image processing toolbox. The stomates were identified manually in the images, their centroids ( $s_i$ ) were identified through an algorithm which found the central coordinates of each one of the manually marked dots, the cartesian centroid is represented by ( $s_{ij} = [s_i, s_j]$ ). Thus, the Euclidean distance between a pair of stomates  $s_i$  and  $s_j$  is calculated as follows:

$$d(s_i, s_j) = \sqrt{(s_i - s_j)^2 + (s_i - s_j)^2}. \quad (1)$$

Images with magnifications of  $100\times$  were used. A distance matrix between each pair of centroid stomata at image is calculated as illustrated in Fig. 1.

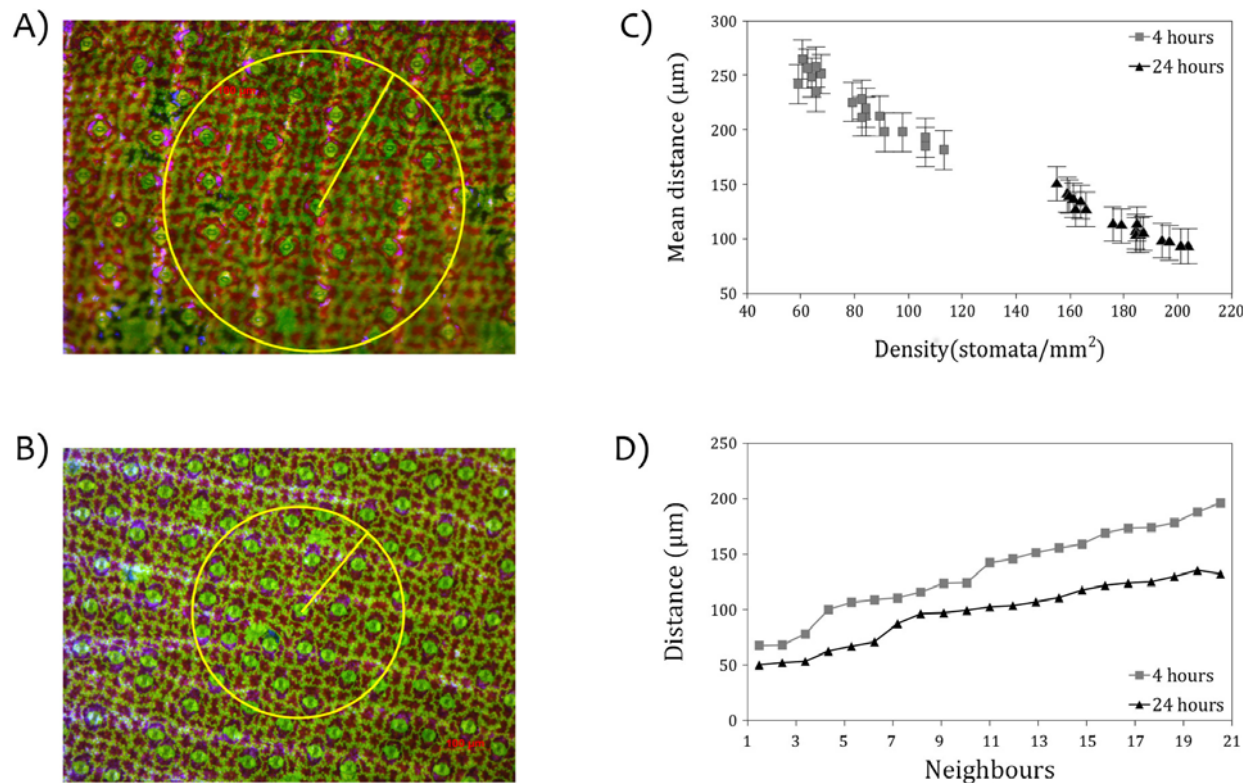


**Figure 1.** A) Illustration of five stomates (green) connected with each other by lines. B) Distance matrix of each stomata pair. The matrix indexes represent each stomata, and  $d(i, j)$  is the distance between each pair of stomata found in A).

## 3. Results

The first evidence of the stomatal plasticity, observed in the leaves of the light acclimatized plants, was the great alteration of the stomatal density and the distance between the stomata of abaxial leaf epidermis according with the photo period. It was observed that the distance between stomata is inversely proportional to the stomatal density in the leaves. The stomatal density varied between the groups of plants studied at different light photo-periods of 4 and 24 hours per day under constant photon flux. Figure 2 depicts how the mean distance between the 20 nearest neighbor stomata varies in function of the stomatal density. Stomata from 3 images per leaf from 18 different plants submitted to photo periods of 4 and 24 hours per day were considered to distance measurements.

The graph above (Fig. 2C) shows that there is a range at the dispersion that has no points. It occurs in densities varying between 115 and 155 stomata/ $\text{mm}^2$ , which indicates that the



**Figure 2.** A) image of a plant exposed to photo period 4 hours per day. The generic stomata chosen is at center of the yellow a circumference with radius of the  $200\mu m$  sweeping an area where 20 nearest stomata are located close to the central stomata B) Plant exposed to photo period of the 24 hours per day. A generic stomata is chosen over the surface and delimited by a circumference of  $264\mu m$  radius that cover the 20 nearest neighbors of this central stomata. The stomatic density observed at image A) is  $82\text{ stomata}/\text{mm}^2$ , and at B, equal to  $150\text{ stomata}/\text{mm}^2$ . C) Correlation between stomatal density and the mean distance between the 20 nearest neighbor stomata of each one of the stomata of the images from epidermis of the 18 plants acclimated to photo periods of the 24 (squares) and 4 hours per day (triangles), respectively. The error bars indicate the variation obtained in the average distance measurement from 3 images per plant. D) Graph showing the distance, in ascending order, of the central stomata of the circumference to each of the 20 nearest neighboring stomata for the plant image samples in A) and B).

intermediate acclimation conditions such as photo periods of 8, 12 and 16 hours per day can fill this interval.

#### 4. Conclusions

We showed that the stomata distribution over the leaf surfaces (*Ctenanthe oppenheimiana*) varies according with the light acclimatization conditions. The changes at the stomatic plasticity over the leaves can be performed by measures of distances between stomata. Therefore, the precise measures of the stomatic distances, specifically at the (*Ctenanthe oppenheimiana*), can be useful as a sensor to environmental changes that plants can be exposed in general.

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