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Paper

# Does stomatal patterning in amphistomatous leaves minimize the $CO_2$ diffusion path length within leaves?

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## Abstract

Photosynthesis is co-limited by multiple factors depending on the plant and its environment. These include biochemical rate limitations, internal and external water potentials, temperature, irradiance, and carbon dioxide ($CO_2$). Amphistomatous leaves have stomata on both abaxial and adaxial leaf surfaces. This feature is considered an adaptation to alleviate $CO_2$ diffusion limitations in productive environments where other factors are not limiting as the diffusion path length from stomate to chloroplast is effectively halved. Plants can also reduce $CO_2$ limitations through other aspects of optimal stomatal anatomy: stomatal density, distribution, patterning, and size. A number of studies have demonstrated that stomata are overdispersed on a single leaf surface; however, much less is known about stomatal anatomy in amphistomatous leaves, especially the coordination between leaf surfaces, despite their prevelance in nature and near ubiquity among crop species. Here we use novel spatial statistics based on simulations and photosynthesis modeling to test hypotheses about how amphistomatous plants may optimize $CO_2$ limitations in the model angiosperm *Arabidopsis thaliana* grown in different light environments. We find that 1) stomata are overdispersed, but not ideally dispersed, on both leaf surfaces across all light treatments; 2) abaxial and adaxial leaf surface patterning are independent; and 3) the theoretical improvements to photosynthesis from abaxial-adaxial stomatal coordination are miniscule ($\ll 1\%$) across the range of feasible parameter space. However, we also find that 4) stomatal size is correlated with the mesophyll volume that it supplies with $CO_2$, suggesting that plants may optimize $CO_2$ diffusion limitations through alternative pathways other than ideal, uniform stomatal spacing. We discuss the developmental, physical, and evolutionary constraits which may prohibit plants from reaching the theoretical adaptive peak of uniform stomatal spacing and inter-surface stomatal coordination. These findings contribute to our understanding of variation in the anatomy of amphistomatous leaves.

**Key words:** amphistomy; Arabidopsis thaliana; $CO_2$ diffusion; finite element method; optimality; photosynthesis; stomata

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## Introduction

Stomatal anatomy (e.g. size, density, distribution, and patterning) and movement regulate gas exchange during photosynthesis, namely $CO_2$ assimilation and water loss through transpiration. Since waxy cuticles are mostly impermeable to $CO_2$ and $H_2O$, stomata are the primary entry and exit points through which gas exchange occurs despite making up a small percentage of the leaf area (Lange et al., 1971). Stomata consist of two guard cells which open and close upon changes in turgor pressure or hormonal cues (McAdam and Brodribb, 2016). The stomatal pore leads to an internal space known as the substomatal cavity where gases contact the mesophyll. Once in the mesophyll, $CO_2$ diffuses throughout a network of intercellular air space (IAS) and into mesophyll cells where $CO_2$ assimilation ($A$) occurs within the chloroplasts (Lee and Gates, 1964). Stomatal conductance and transpiration are determined by numerous environmental and anatomical parameters such as vapor pressure deficit (VPD), irradiance, temperature, wind speed, leaf water potential, IAS geometry, mesophyll cell anatomy, and stomatal anatomy.

## Competing interests

The authors declare no competing interests.

## Author contributions statement

JLW and CDM conceived of the project, analyzed data, and wrote the manuscript. GJD provided data. TNB contributed to model development and helped edit the manuscript.

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