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# Spatial Analysis of Cell Patterning to Aid Genetic and Phenotypic Understanding of Grass Stomatal Density: A Case Study in Maize

## Background/Objective

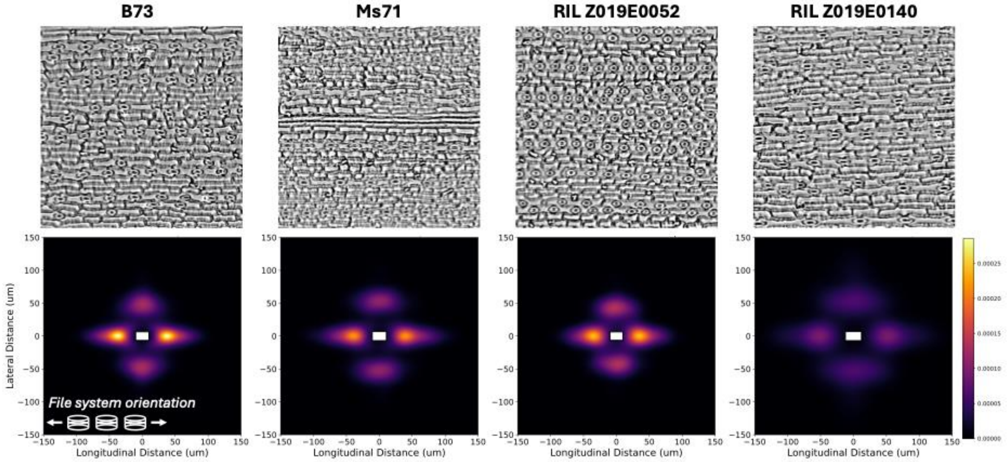
Biological processes involve complex hierarchies where composite traits result from multiple component traits. However, holistically understanding of how sets of component traits interact to underpin genotype-to-phenotype relationships is generally lacking. Stomatal density (SD) is a tractable model system for exploring how high-throughput phenotyping (HTP) data could be exploited by a new spatial analysis approach to better understand a developmentally and functionally important trait. This study investigates how data from a machine learning-enabled HTP method can (1) aid in generating a holistic assessment of a composite-component trait set and (2) help advance knowledge of genotype-to-phenotype relationships and trait networks.

## Approach

Data from 192 recombinant inbred lines of maize [*Zea mays* (L.)] were analyzed by a new stomatal patterning phenotype (SPP) to (1) describe stomatal nearest neighbor relationships as an averaged self-repeating spatial probability distribution; (2) extract key component traits related to cell size, cell packing, and positional probabilities from them; (3) explore interactions between these traits as well as SD via structural equation modeling; and (4) identify stomatal patterning quantitative trait loci (QTL).

## Results

This study exploited the tractability of grass stomatal patterning to demonstrate how spatial analysis of the output from such tools can be mined to extract greater information value. The core set of SPP-derived traits explained 74% of the variation in SD. Analyzing SPP component traits allowed some loci previously identified as generic SD QTL to be recognized as specific to lateral versus longitudinal elements of stomatal patterning.



**Examples of the stomatal patterning variation seen across the RIL population (top) as well as the corresponding patterning phenotypes observed for these respective genotypes (bottom), that allows both spatial occupancy and neighbor frequency to be quantified.**

## Significance/Impacts

This study highlights how novel insights can be gained by decomposing a complex, composite trait (e.g., SD) into a set of component traits that were present in HTP data but not previously exploited. In doing so, it introduces a new spatial analysis method that can be applied universally to quantitatively describe stomatal patterning within and across grass species. It also provides a case study demonstrating how a comprehensive statistical network of component traits extracted from image data can be used to provide novel insights into the genetic basis for variation in a composite trait such as SD.

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