To grow or not to grow: How are developmental decisions made in leaves?
Keywords: leaf morphology, auxin distribution, petiole, tomato

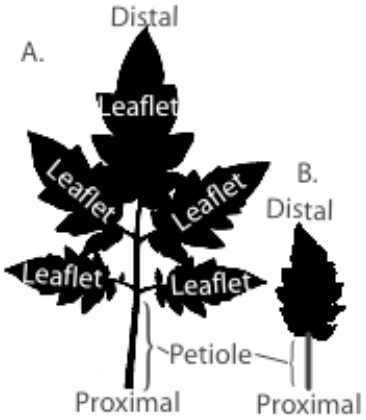

Figure 1- **A.** Complex leaf. **B.** Simple leaf.

**Introduction:** The angiosperm leaf is a flattened structure with conserved function in light capture, but has incredible morphological diversity in shape. How such leaf shape diversity is achieved is still unknown. Leaves are broadly split into two categories, simple and complex (Fig 1), with the complex form showing a multitude of leaflets on the leaf. One major factor that may influence leaf complexity is the distribution of the phytohormone auxin. Auxin maxima not only predict leaf initiation[1-5], but also leaflet initiation and blade outgrowth (expanded flattened portion on a leaf or leaflet) on leaf primordia[6]. Exogenous microapplication of IAA, a synthetic auxin, on developing leaf primordia causes ectopic leaflet initiation and blade growth[6]. Most complex leaves have a punctuated growth pattern with regions of blade outgrowth interrupted by regions of no outgrowth. The proximal region of most leaves is the petiole where blade outgrowth is completely suppressed. I propose to address the following question: **What regulates blade outgrowth in the compound leaf and in the proximal end, the petiole, during leaf development and how does this influence leaf complexity?**

While it is common to think that where blade/leaflets exist specifies leaf complexity, it is just as important to understand the regions where blade and leaflets do not occur. With the exception of the CUC genes[7], which determine boundaries between leaflets but do not appear to have a role in suppressing outgrowth in the petiole, the regulatory mechanisms directing these regions of no outgrowth is greatly unknown. It is these regions lacking outgrowth on a complex leaf that greatly define leaf shape through blade and leaflet dissection. My project aims to elucidate how these regions act in establishing the diversity of leaf complexity through two approaches: molecular characterization and exogenous auxin application.

**Study System:**. *Solanum lycopersicon* (tomato) and its wild relatives exhibit a great variety in leaf shape ranging from simple to complex with great variation in petiole length and are an emerging developmental system to study leaf morphogenesis. Most are sexually compatible and capable of genetic manipulation through transformation and Virus Induced Gene Silencing (VIGS)[8]. Additionally, the tomato leaf primordium is easily accessed by dissection and the early drafts of the completed genome of tomato are available, allowing access to a wealth of genomic tools.

**Hypothesis 1: The cells in the developing petiole are not competent to respond to leaflet and blade initiation factors i.e. auxin.** To test this hypothesis, small dots of IAA will be applied to the base of developing leaf primordia. If ectopic leaflet/leaf blade cannot be initiated in regions of elongated petiole tomato varieties, such as the Pruden's Purple variety, we can conclude that these regions are unresponsive to auxin maxima, providing important insight into why petioles do not initiate blade or leaflet growth. This work will be expanded to closely related species *Solanum aviculare* and *S. lacinatum*, species which have complex leaves and blade growth along proximal regions of the leaf (petiole) and also *S. tuberosum* and *S. americanum*, which have defined petioles. This data will provide important insight into why petioles do not initiate outgrowths and allow me to determine if the role of IAA in leaflet and blade outgrowth is evolutionary conserved, since thus far IAA applications have only been characterized in tomato.

**Hypothesis 2: Auxin gradients are not maintained in the petiole during petiole establishment.** My preliminary work in the *trifoliate* tomato mutant shows that the proximal portion of leaf primordium (corresponding to the presumptive petiole) has low levels of the auxin reporter PIN1:GFP. Additionally, I applied auxin to the petiole region, but was unable to get ectopic leaflets to form. I will cross or transform auxin reporters PIN1:GFP and DR5:GUS into tomato varieties which have varying degrees of petiole length, to assess differences in auxin distribution in the proximal regions of leaf primordia. Stronger accumulation of auxin reporters in the distal regions of leaf primordia compared to proximal would support the idea that auxin accumulation is suppressed in petioles. In addition, varieties with elongated petioles should have decreased proximal signal compared to the proximal signal in varieties with shorter petioles.

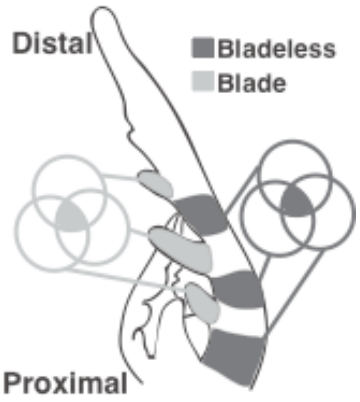


Figure 2 Transcriptomics of developing leaf. Non-overlapping genes between the dark gray and light gray triangles will be analyzed

**Hypothesis 3: The proximal regions in leaf development should have differentially expressed genes compared to the distal regions, where leaflet and blade outgrowth occurs.** The recently sequenced genome of tomato allows us to use high throughput sequencing to infer differential expression. RNAs from specific tissue are converted to cDNA and sequenced to get a full transcriptional identity of specific tissue. These sequences are aligned to the known genome and the number of reads corresponds to the level of gene expression. I will use Laser Capture Microdissection, RNA extractions, and sequencing to compare gene expression profiles of tissue from the distal bladed and bladeless regions and the proximal regions in all the varieties. I will look to see how much expression overlap exists in **a)** the genes that are similarly expressed in the petiole compared to the distal bladeless regions (dark gray) and **b)** blade regions (light gray) compared to bladeless regions, revealing candidate genes for petiole tissue specification (Fig. 2). These experiments will be performed at different developmental stages to achieve time context of gene expression. I will pick five selected genes for transformation or VIGS to monitor the consequences of gene downregulation in various species. This will allow me to understand how developmental timing, gene expression, and hormonal regulation generate leaf complexity.

**Personal interest, experience, and broader impact:** The origin and continual motivation behind my research has been understanding the evolution of plant morphology and I have spent the last three years ensuring that I am well equipped to succeed in my research objectives. My current lab is focused on the evolutionary development of plants using tomato as a model developmental system, with a history of working with non-model species. I have been actively learning bioinformatic skills through classes offered, group learning, and involvement in programming projects. Plant form has inspired people for thousands or years and I know first hand that work exploring the beauty of nature is a motivator for learning more about science. The results from this research will not only contribute to advancement in scientific understanding of leaf form, but will be used as a backdrop in my continual efforts to bringing science to the audience with the goal to excite young scientists and especially non-scientists about Biology.

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