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Research Statement

Introduction

My research focuses on using genomic and transcriptomic approaches to study natural variation and the evolution of developmental novelty among closely related species and cultivars of plants. What are the developmental mechanisms through which natural and artificial selection alter the morphology of organs? To answer these questions, I use quantitative genetic approaches and focus on the incipient differentiation of leaves from the indeterminate stem cell population of the vegetative meristem. As a model, I study members of the tomato complex (*Solanum* sect. *Lycopersicon*), including wild species adapted to varying desert and riparian habitats as well as domesticated varieties. Despite profound morphological differences in leaf morphology and shoot apical meristem architecture, wild tomato species and domesticated varieties are inter-fertile, allowing for rigorous genetic approaches to be applied in this group to study their evolution.

I propose using quantitative genetics to compare the transcriptional networks of the meristem and incipient leaf across different species of tomato. By determining the genetic basis of the transcriptional changes that occur during the differentiation of an organ, and how these changes vary across evolutionary distance, a comprehensive understanding of the underlying mechanisms governing morphological change can be determined.

Past Research

Most of my research in the past has focused on leaf development. At Cold Spring Harbor Laboratory, my dissertation research with Dr. Marja Timmermans looked at the role of small RNAs, both microRNAs (miRNAs) and *trans*-acting short interfering small RNAs (ta-siRNAs), in establishing the adaxial-abaxial (top-bottom) axis of leaves. I discovered that ta-siRNAs accumulate outside their domain of biogenesis as a gradient, leading to the hypothesis that

these small RNAs can act as morphogens that pattern leaves. Using laser-capture microdissection, we also isolated the meristem, young leaf primordia, and the epidermis to create an “atlas” of miRNA precursor expression in the shoot apical meristem, revealing complex transcriptional control of miRNA families during development.

Current Research

My current research as a post-doctoral fellow in the lab of Dr. Neelima Sinha, working closely with Dr. Julin Maloof, uses tomato as a model to study the genetic basis of natural variation and environmental response. Central to this work is the use of 76 introgression lines (ILs), each harboring an introgressed segment of the wild tomato *Solanum pennellii* genome in an otherwise domesticated tomato background. Together, the introgressed segments of the 76 lines span the entire genome, and finding a phenotypic difference between an IL and domesticated tomato indicates that the responsible genes controlling the trait lie in the introgressed region.

The transcriptomes of the vegetative apices of ILs are analyzed using next-generation sequencing techniques, under simulated sun and foliar shade. Because each IL contains a single introgressed segment, differentially-expressed genes can be classified as *cis*- (within the introgression) or *trans*-regulated (genes outside the introgression). Such a comprehensive dataset allows not only genetic x environmental effects to be discerned, but the outlines of a transcriptional network, as defined by evolutionary changes between two species.

My contribution to the project has been to statistically analyze gene expression and its relationship to phenotype. QTL analysis of gene expression (“eQTL”) in the ILs reveals master regulators of gene expression regulating the genome-wide expression of disease-resistance and heat shock response transcriptional programs. Moreover, using data from an inter-species atlas of tissue-specific expression between *Solanum lycopersicum* (domesticated tomato) and its wild desert relative *S. pennellii*, genes regulated by particular eQTL show tissue-specific expression. This data is complemented by laser-capture microdissection studies analyzing genes enriched for expression in leaf primordia and the meristem. The intersection between eQTL arising from natural variation and cohorts of genes with similar

tissue-specific expression profiles suggests that discrete transcriptional modules regulating tissue-specific expression are modulated over evolutionary time.

Proposed Research

For my future research, I propose to study the differences between closely related species in the shifts of transcriptional regulation that occur as cells lose the indeterminate state associated with the meristem and differentiate into leaf primordia. My proposal unites rigorous quantitative genetics using the *S. pennellii* ILs I studied as a post-doc with detailed, cell type-specific developmental techniques I have always practiced. The Specific Aims below detail 1) comparing cell type-specific gene expression profiling between different tomato species, 2) meristem and leaf primordium-specific eQTL analyses using the *S. pennellii* ILs, and 3) an examination of non-cell autonomous effects of eQTLs using periclinal chimeras.

Specific Aim 1: What tissue-specific differences in gene expression underlie the varying shoot apical meristem sizes and architectures amongst members of the tomato clade?

Not only are there disparate differences in leaf morphology amongst wild tomato species, but the architecture and size of the shoot apical meristem, from which leaves originate, varies between species (**Fig. 1**). For example, the meristem of domesticated tomato is enlarged relative to that of its desert relative *S. pennellii*, whereas the primordia of domesticated tomato are smaller than those of *S. pennellii* at equivalent stages (**Fig. 1E-F**).

A. Characterize shoot apical meristem architectures amongst wild tomato species

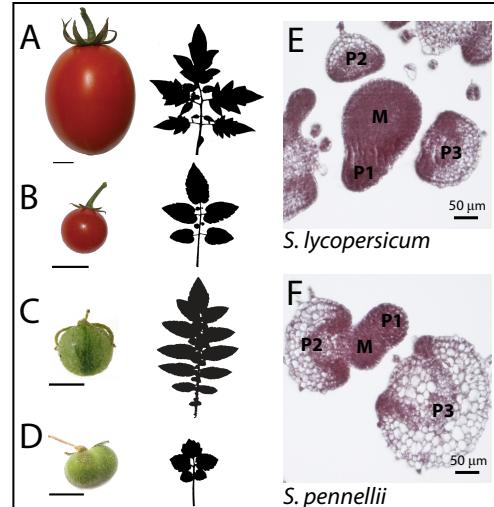


Figure 1: Natural variation between domesticated tomato and wild relatives. **A-D)** Examples of fruit and leaves from A) *Solanum lycopersicum*, B) *S. pimpinellifolium*, C) *S. habrochaites*, and D) *S. pennellii*. Note the differing colors and sizes of the fruit, and differences in leaf complexity and morphology. **E-F)** Differences in leaf morphology originate in the shoot apical meristem. Note the larger meristem (M) and smaller leaf primordia (P1-P3) in domesticated tomato (E) compared to its wild relative *S. pennellii* (F), suggesting prolonged indeterminacy.

- a. Detailed histology suggests that the shoot apical meristem of domesticated tomato is intrinsically more indeterminate than *S. pennellii*, given the larger meristem and delayed primordium development (**Fig. 1E-F**). What do the shoot apical meristems of other tomato species look like and how does this correlate with their different morphologies and degrees of leaf complexity?
- b. Shoot apical meristems of *S. lycopersicum*, *S. pimpinellifolium*, *S. harachaite*s, *S. pennellii*, and *S. peruvianum* will be quantitatively analyzed using micro-CT scanning techniques we are currently developing and/or confocal imaging.

B. Transform wild tomato species with meristem- and incipient leaf-specific reporters for *in vivo* isolation of specific cell types for expression profiling.

- a. *In situ* hybridization will be performed on gene candidates I have identified from domesticated tomato using laser capture microdissection that exhibit meristem- and primordium-specific expression. Transcripts with robust expression in all species with tissue-type specificity will be considered further.
- b. Wild tomato species will be transformed with reporters to isolate cell type-specific nuclei using the INTACT method (Deal, R. and Henikoff, S., *Nat. Protoc.*, 2011).
 - i. Transgene reporters expressing in specific cell types will allow affinity purification of biotinylated nuclei which will be isolated and analyzed for gene expression differences between tissues, species, and tissue x species interaction.
- c. Further uses of transformed lines:
 - i. Studying differences between species in the epigenetic marks marking differentiated primordia from the meristem
 - ii. Studying differences in species in their heteroblastic progression (differences in the morphology of leaves arising from different nodes) by sampling the shoot apical meristem at different times.

Specific Aim 2: What is the genetic basis for differences in the transcriptional programs between the meristem and differentiated primordium amongst closely related species?

Using the *S. pennellii* introgression lines (“ILs”), I have completed an eQTL analysis of the genetic basis of gene expression in vegetative apices of tomato. The analysis revealed master *trans* regulators of transcriptional programs regulating disease resistance and the heat shock response. Interestingly, master regulatory loci were enriched for genes expressed in a tissue-specific fashion, suggesting that evolution modulates the spatial expression of transcriptional cohorts of genes. What are the genetic regulators of the differences in gene expression between the meristem and leaf primordia amongst species?

- A. Separate eQTL analyses will be undertaken on the 1) P1/P2 leaf primordium (that is, the youngest dissectible primordium) and 2) resultant dissected shoot apex enriched for meristematic cells of the vegetative apex. This analysis will be conducted in the *S. pennellii* ILs using the previous high-throughput RNAseq methods developed in the Sinha/Maloof labs.**
 - a.** Dissection of young differentiated primordia from the meristem tissue is possible in the *S. pennellii* ILs owing to the enlarged meristem in tomato.
 - b.** Cell-sorting, or even INTACT transgene reporters would require the creation of another introgression line population and would not be comparable to previous results collected in this stable genetic resource. Further, the transgene is prone to silencing. Laser-capture microdissection for so many samples is also impractical.
- B. eQTL analyses of the meristem, primordium, and the entire vegetative apex (already completed) will be compared.**
 - a.** Candidate master regulators of expression, or eQTL which colocalize with the extensive data on leaf morphology I have collected in the ILs, will be studied further
 - b.** *In situ* hybridization of candidates will reveal the developmental context of gene expression, and ectopic expression and/or silencing of the gene using appropriate tissue-specific promoters will reveal candidate function.

Specific Aim 3: What are the non-cell autonomous effects of eQTL between the epidermis and underlying layers of the shoot apical meristem?

Bilateral communication between the meristem and leaf primordia is well-established. Even if tissue-specific, eQTL analyses fail to separate cell-autonomous effects on gene expression from non-cell autonomous, an important consideration in determining developmental mechanism. *Solanum* species are easily grafted and can generate periclinal chimeras, which possess cell layers from different species (for example, a *S. pennellii* L1/epidermal layer and domesticated underlying layers).

- A. ILs harboring master regulator eQTL or eQTL of other developmental interest will be used to create periclinal chimeras with transgenic domesticated tomato tissue to be isolated using the INTACT method.**
 - a. A domesticated tomato line with an L1/epidermis-specific phenotype marker (e.g., a glabrous line, or chlorotic phenotype marking the L2/3) will be transformed with a ubiquitously expressed INTACT transgene
 - i. Phenotypes differentiating the L1/epidermis from underlying layers will facilitate successful periclinal chimera generation
- B. Reciprocal periclinal chimeras, either harboring the IL of interest in the epidermis or underlying layers and the domesticated/INTACT genotype in the other layer, will be compared to periclinal chimeras lacking the IL genotype**
 - a. By isolating cell types expressing the INTACT reporter, the non-cell autonomous effects of the IL genotype (non-INTACT) can be determined
 - b. INTACT-expressing cells in periclinal chimeras with and without the influence of the IL genotype will be compared using RNAseq methods.

Summary

The tomato clade (*Solanum* sect. *Lycopersicon*) provides an unprecedented opportunity to study the genetic basis of evolutionary changes in development. The inter-fertility of tomato with its wild relatives has been exploited to create numerous genetic resources. Quantitative genetics and developmental studies provide complementary perspectives in understanding natural variation, but are rarely combined. The proposed work seeks to understand a fundamental biological process, the differentiation of a leaf primordium from the stem cell niche of the meristem, by combining eQTL and cell-type specific isolation techniques. By combining new genetic and developmental tools, rigorous genetic evaluation of the differences in the initiation of an incipient organ between species can be discerned.