Online Proceedings

10

Plant Genetics, Ecology and Evolution

Session Moderator: Veronica Di Stilio, Biology

JHN 026

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Visualizing and Quantifying Peptide Behavior in *Arabidopsis* Stomatal Differentiation and Epidermal Patterning

Emily K. (Emily) Lo, Senior, Biology (Molecular, Cellular & Developmental)

Levinson Emerging Scholar, Mary Gates Scholar

Mentor: Keiko Torii, Biology

Stomata, valve-like pores encircled by guard cell pairs on the plant epidermal surface, serve as the primary gateway for gas exchange with the environment as well as water movement through the vasculature. During organ morphogenesis in *Arabidopsis thaliana*, the differentiation process from an unspecialized protodermal cell to a fully mature pair of guard cells, in addition to the coordination of proper stomatal spacing and density in the epidermis, is regulated by a complex signaling pathway that involves a series of interactions between positional signaling peptides and transmembrane receptor kinases. The secreted signaling peptides EPIDERMAL PATTERNING FACTOR (EPF) 2, EPF1, and STOMAGEN (EPFL9) play major roles in this differentiation pathway. Previous studies have extensively investigated the functions of EPF2, EPF1, and STOMAGEN; however, the effective range of these peptides after secretion has yet to be determined. Here, we use a Cre-lox Gal4 recombination system to induce site-specific mosaic overexpression of our target peptides in the *Arabidopsis* epidermal layer, in which induced recombinant cells are positively indicated by fluorescence. Preliminary results have confirmed the efficacy of the Cre-lox system as well as consistency of stomatal patterning phenotypes with the overexpression of each peptide. Using this system, we will visualize and quantify the distance over which EPF2, EPF1, and STOMAGEN can influence stomatal patterning in *Arabidopsis*. By better characterizing the peptide activities that regulate stomatal patterning in plants, this project can lead to novel insight on the relationship between stomatal development and plant productivity, which has potential applications for agricultural innovation.

The Effect of Light Quality on Flowering Gene Expression

Ella Rand (Ellie) Taagen, Senior, Biology (Molecular, Cellular & Developmental)

Mentor: Akane Kubota, biology

Plants use information about daylight to align their flowering time with seasonal changes, increasing reproductive success. Long-day conditions promote flowering in model organism *Arabidopsis thaliana*. The gene *FLOWERING LOCUS T (FT)*, which encodes florigen and promotes flowering, has been understood to show evening peak expression in long-day specific manner. The molecular mechanism responsible for *FT* regulation has been widely studied using 16-hour fluorescent white light, 8-hour dark, and constant 22C as simplified long-day conditions. To expand our understandings on flowering regulation in nature, where multiple environmental signals synergistically regulate flowering, our lab analyzed *FT* expression in plants grown outdoors during Seattle's summer solstice (15h59m daylight, 21.2C high, average 1971 to 2000). In contrast to what has been reported under simplified long-day conditions, a morning and evening peak of *FT* expression was observed. Since 2014 we have succeeded in reconstructing natural double *FT* expression in growth chambers with 16-hour fluorescent light supplemented by far-red LED and temperature oscillation. My project addressed the effect of far-red light, as measured by the red to far-red light ratio (R:FR), on morning *FT* expression. *Arabidopsis* seedlings were grown for two weeks in chambers under constant temperature and 16-hour fluorescent light supplemented with variable strength of far-red light conditions, mimicking shade (0.25 to 0.75:1), natural sunlight (1:1) and fluorescent light (2:1). *FT* expression was analyzed using real-time quantitative reverse transcription PCR. As a result, higher FR ratio increased *FT* expression in wild-type. We are currently analyzing phytochrome mutants, lacking functional red and far-red light photoreceptors, to investigate how they are involved in *FT* regulation. *FT* function is widely conserved in angiosperms and identifying environmental inputs and the

associated genes for flowering regulation in *Arabidopsis* under natural conditions can contribute to researching effects of climate change on flowering time in agriculturally relevant species.

The Importance of Circadian Timing in Mediating Plant-Pollinator Interactions

Le Ann Pham Nguyen, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Takato Imaizumi, Biology
Mentor: Myles Fenske, Biology

Many plant-pollinator interactions are thought to depend on coordination between the timing of plant scent emission and pollinator activity. Certain plants of the genus *Petunia* exhibit rhythmic scent emission based on the circadian clock, but little is known about the role of the clock in pollinator behavior. The pollinator of *Petunia axillaris*, the hawkmoth *Manduca sexta*, is thought to be nocturnal based on mostly anecdotal evidence. To understand the temporal relationship between *Manduca sexta* and *P. axillaris*, it is necessary to first determine the precise timing of hawkmoth activity. We hypothesized that moths would display circadian timing in their flight behavior, complementing the timing of scent emission from *P. axillaris*. To test this, we observed the flight behavior of male hawkmoths in response to 12-hour light/12-hour dark conditions, continuous dark, or continuous light. The moths were entrained to the 12-hour light/12-hour dark cycle during their pupation and their first two days post-cocoon emergence, and they were introduced to a wind tunnel on the third day for the experimental conditions. The number of moths flying within a ten-minute period was recorded continuously for one day or every four hours for three days. Hawkmoths exhibited flight activity only during the subjective night phase of continuous dark conditions. This rhythm was maintained over multiple days of continuous darkness, demonstrating that it is not dependent on light input. We also examine how desynchronizing the clocks between plant and pollinator affects pollination success, and lastly if it's possible to induce a pollinator shift by shifting the timing of scent release.

Exploring the Genetic Basis for Diverse Floral Symmetry among Rhododendrons

Haley Alyssandra (Haley) Deal, Senior, Environmental Science & Resource Management
Mentor: Benjamin Hall, Biology
Mentor: Valerie Soza, Biology

Flowering plants exhibit diverse forms of floral symmetry that represent many independent changes throughout their evolutionary history. The selective and genetic basis to these changes has provided scientists with a complex puzzle that Darwin once referred to as an "abominable mystery." Recent genetic studies began to uncover the genetic causes for this mystery, revealing the presence of certain genes that show correlations with this evolutionary trend. One such gene, the *CYCLOIDEA* (*CYC*) gene, has been identified as a major contributor to changes of floral symmetry. We have found this gene in the sequenced genome of *Rhododendron williamsianum* where it has experienced multiple duplications. The goal of my research is to identify the main duplications in one of the *CYC* copies (*CYC2*) in the Rhododendron genus. I accomplished this by using polymerase chain reactions to isolate any *CYC2* copies present in two species from each of the subgenera of *Rhododendron*: *Hymenanthes*, *Azaleastrum*, *Therorhodion*, and *Rhododendron*. I then used cloning and sequencing to obtain the sequences of the *CYC2* copies from each of the sampled species. I will conduct a phylogenetic analysis of these sequences in order to reconstruct a gene tree that will display a summary of the main *CYC2* duplications that have occurred in the genus and when they emerged. Preliminary results from four species sampled thus far show that two independent duplications in *CYC2* have arisen in both *Hymenanthes* and *Rhododendron*. My expanded analysis, sampling species from additional subgenera, will provide a more thorough history of this gene and its duplications throughout the genus. This will allow for more robust testing for correlation between symmetry and gene duplication in the future.

Comparative Leaf Growth Strategies in Response to Water Stress and Shade: Uncovering an Ecophysiological Role of Leaf Mass per Area (LMA) in *Populus tremuloides*

Alec Stephen (Alec) Baird, Senior, Biology (Plant)
Mary Gates Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Elizabeth Van Volkenburgh, Biology
Mentor: Janneke Hille Ris Lambers, Biology

Developmental phenotypic plasticity can be a vital means to buffer the sudden and persistent effects of anthropogenic climate change, through facilitation of species persistence and possible facilitation of species range shifts. Integrating our understanding of plastic physiological growth mechanisms with functional traits may prove useful for interpretation of the ecological impacts of climate change. We investigated the influence of water-stress and light limitation on leaf mass per area (LMA), leaf anatomy, and leaf gas exchange of juvenile *Populus tremuloides* trees. Leaves that developed while water-stressed had significantly elevated LMA, due to increased leaf density from significantly higher spongy mesophyll surface area per leaf area ($A_s mes$/A) and reduced stomatal conductance (g_s) but only marginally reduced photosynthesis (Pmax). In shade, leaves had significantly reduced LMA, due to reduced thickness, density, and combined $A_m es$/A, but maintained the same $A_s mes$/A, and reduced palisade mesophyll surface area per leaf area ($A_p mes$/A),

which was coupled with a reduction of g_s and P_max. Furthermore, water-stressed leaves had elevated intrinsic water use efficiency (WUE_i: P_max/g_s) while the shaded leaves, despite reduced g_s, maintained similar WUE_i. Our results suggest that, under water-stress and full sunlight, greater A_smes/A conferred maintenance of photosynthetic rate possibly through reduction of mesophyll resistance (r_m), at the cost of reducing expansive leaf area (and increasing density) as leaves were more dense but were 20 % the area of control leaves. In shade, suppressing A_pmes/A while maintaining A_smes/A may be a plastic strategy to increase laminar light capture, as leaves had reduced density and thickness but were still 50 % the area of control leaves . Together, in *P. tremuloides* saplings, these stresses induced developmental plasticity in LMA with differing plastic underlying anatomy and function; we discuss future implications specifically in the context of developmental plasticity, growth trade-offs, and the ecological impacts of climate change.

Investigating Nitrogen Fixation in Growth-Promoting Bacteria: Transposon Mutagenesis of Endophytes

Pierre Michel (Pierre) Joubert, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Sharon Doty, Environmental&Forest Sciences

Growth promoting endophytes have been isolated from wild poplar and willow plants in Prof. Sharon Doty's lab and have been shown to promote growth in several other plant species as broadly diverse as rice, tomato, and Douglas-fir. One of the proposed reasons for this increased growth is that endophytes living within the plant fix nitrogen from the atmosphere and exchange it for a carbon source from the plant. Nitrogen is one of the most important nutrients for plant growth and fertilizers are heavily used worldwide to provide this nitrogen. However, fertilizers are costly to produce and have significant negative impacts on the environment so the goal of our lab is to replace these fertilizers with a consortia of natural endophytes. This study seeks to determine the genes necessary for nitrogen fixation in these endophytes by using random transposon mutagenesis. Endophytes will be selected for their ability to grow on nitrogen-free medium. Then, an E. coli donor strain will transfer a plasmid containing a transposon, a segment of DNA that can transpose into the bacterial genome, with an antibiotic resistance gene to our bacteria through conjugation. The bacteria will then be screened for resistance to that antibiotic to check if the plasmid was taken up by the bacteria, allowing for transposon integration at random areas of the genome. Transposon integration will disrupt the genome of the bacteria, creating mutants. Finally, the conjugated bacteria will be screened for their inability to grow on nitrogen-free medium. Bacteria that lost their ability to grow on this medium will then be sequenced in order to determine the location of the insertion into their genome and therefore the location of genes related to nitrogen fixation. This project will also result in the creation of many mutant endophytes that have lost their ability to fix nitrogen and we will therefore be able to compare their effect on plant growth to wild-type inoculated plants.

Microbial Endophyte Effects on Three Washingtonian Fruit Crops, *Malus domestica*, *Prunus avium*, and *Fragaria x ananassa*

Victor Ernest (Victor) Van Epps, Senior, Biology (Ecology, Evolution & Conservation)
Mary Gates Scholar, UW Honors Program
Nicholas Steven (Nick) Chandler, Junior, Biology (General)
Mentor: Soo-Hyung Kim, Environmental and Forest Sciences
Mentor: Hyungmin Rho, School of Environmental and Forest Sciences

Endophytes are symbionts that live in the intercellular spaces of host plants. They can be fungi, bacteria, actinomycetes and/or viruses. Initial investigations indicate that endophytes provide a number of benefits that promote plant growth including, but not limited to, dinitrogen fixation, plant hormone production, nutrient acquisition, stress tolerance and increased immunological response. In exchange, they receive domicile and photosynthates. Endophytic biological and ecological services hold great potential for bioengineering and evolutionary inquiries, particularly so for sustainable agriculture. Nitrogen has historically been the nutrient limiting factor in all crop production. Bioengineering endophytic biomes capable of alleviating input demands while ameliorating soils damaged from industrial agriculture stands to offer novel solutions. This two year student-independent research is evaluating the inoculation of three fruit species, *Malus domestica*, *Prunus avium*, and *Fragaria* $\times$ *ananassa* with an endophytic consortia isolated by the UW Doty Lab. Washington is a leading state in fruit crops with over a hundred thousand hectares dedicated to production. Sweet fruit crops require heavy nitrogen inputs to yield nutritionally and economically viable fruits. Our primary objective is to investigate the effect of inoculation on the aforementioned crop physiology and fruit production. We are examining these effects through ecophysiology metrics involving rates of photosynthesis, stomatal conductance, biomass accretion, chlorophyll content, fluorescence rate, drought response, fruit sucrose content and biostatistic analysis. We hypothesize that our inoculated fruit crops will out perform controls as indicated with greater total biomass, fruit production and quality. First season preliminary results point to greater fruit biomass with higher sucrose content in *Malus domestica*. With crops now well established after season one, we anticipate a continued trend of improved crop total yields, higher sucrose content and greater total biomass production in all three varieties.