UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

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Evolution, Genetics, and Biochemistry of Plants, Algae, and Fungi

Session Moderator: Richard Olmstead, Biology, Burke Museum

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3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Flower Color Gene Implicated in Pollinator-Mediated Speciation of Monkeyflowers

Riane Calida (Riane) Young, Senior, Biology (General)
Mary Gates Scholar
Mentor: Toby Bradshaw, Biology

Darwin and Wallace recognized natural selection as an essential force in evolution over 150 years ago. Today, we still investigate the processes that drive evolution and the origin of new species. Two sister species of wildflower in the genus Mimulus (monkeyflowers) present an ideal case for studying the genetic and ecological basis of speciation. M. lewisii and M. cardinalis coexist in overlapping ranges in the Sierra Nevada Mountains, and although they successfully interbreed in the laboratory, hybrids are extremely rare in the wild. Each flower species is visited by a unique pollinator – M. cardinalis by hummingbirds, M. lewisii by bumblebees. This indicates that traits influencing pollinator choice and pollination success account for reproductive isolation. We hypothesize that these interspecific differences can be traced to single genes with large effects on flower traits, such as color, that pollinators use in making the choice of which species to visit in the field. If our hypothesis is supported, this would demonstrate that single genes of large phenotypic effect are directly responsible for the origin of new species. Field work indicated that hummingbirds prefer red flowers (high amounts of purple and yellow pigment) while bumblebees prefer pale pink (small amounts of purple pigment). Using a genetic technique called positional cloning, my main contribution to the project was to locate a novel gene that regulates the amount of purple pigment (anthocyanin) in Mimulus flowers. We named this gene ROSE INTENSITY1 (ROI1), verified its identity and function by creating transgenic plants carrying the alternative M. lewisii and M. cardinalis alleles, and showed that ROI1 represses transcription of the genes that encode enzymes for anthocyanin biosynthesis. Our findings are being published in the May issue of Genetics. Future directions include quantifying the effect of ROI1 on pollinator visitation in the Sierra Nevada Mountains.

Primer Development for the Pentatricopeptide Repeat Gene Family for use in the Large Plant Group Lamiales

Benjamin Paul (Ben) Meersman, Junior, Biology (Ecology, Evolution & Conservation)
Mentor: Richard Olmstead, Biology, Burke Museum
Mentor: Patricia Lu-Irving, Biology

Phylogenetics is the study of evolutionary relationships and in order to infer these relationships in plants, various loci that are phylogenetically useful in nuclear as well as chloroplast DNAs should be utilized. Presently, the use of chloroplast loci is widespread in phylogenetic studies whereas the nuclear genome is still somewhat under-utilized. The pentatricopeptide repeat (PPR) gene family is a large group of protein coding nuclear genes that has been shown to be highly informative in the inference of evolutionary relationships among closely related species. There are multiple reasons that make these genes useful to these types of studies. The PPR gene family is very large which offers researchers multiple loci that are available for phylogenetic analyses. This is important because having multiple loci is imperative to answering important phylogenetic questions. A large portion of PPR genes are intronless making it possible to sequence and align data with little or no difficulty. They also have a high rate of evolution, and are single-copy in most plant genomes. 127 of these loci have been identified as phylogenetically useful but of these only 5 have been developed. This research looks to develop 10 more loci that will specifically target the Lamiales. To do this, 10 primer sets were tested using a sampling of species across the Lamiales. The primers were used to amplify these loci in order to ascertain their usefulness for a wide range of species and to obtain sequence data for each species. The sequences obtained were aligned and the alignments were used to design new primers. These new primer sets will allow for new data to be more easily collected that has been either dif-

ficult to obtain or previously unavailable and will increase the number of nuclear loci available for phylogenetic studies.

GIGANTEA Mediated Stabilization of CONSTANS in Photoperiodic Flowering

Daniel Andres (Daniel) Estrada, Senior, Biology (General)
Mentor: Takato Imaizumi, Biology
Mentor: Young Hun Song, Biology

Flowering in plants is induced and tightly regulated through the use of internal circadian clocks that respond to changes in day length and light quality. In the model plant, *Arabidopsis thaliana,* the molecular mechanism behind flowering induction relies upon expression of the *CONSTANS* (*CO*) gene and subsequent CO protein stabilization. Daytime *CO* expression occurs strictly under long day (LD) conditions and is reliant upon removal of the transcriptional repressor CYCLING DOF FACTOR 1 (CDF1) from the *CO* promoter by a complex consisting of FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) and GIGANTEA (GI). Following FKF1/GI mediated induction of *CO* expression, CO protein is stabilized in the LD afternoon through direct binding to FKF1. Our most recent unpublished data show CO stabilization is also crucially dependent on the presence of GI protein. However, the mechanism by which GI interacts with CO has yet to be determined. We hypothesize that GI is involved in CO stabilization through either binding to CO independently of FKF1 or via an FKF1/GI complex. Through the use of molecular genetics and nuclei-specific protein analysis, we aim to elucidate the mechanism by which GI interacts with CO, and furthermore, establish GI as a regulator not only in expression of *CO* mRNA, but also in stabilization of CO protein.

Fungal Symbionts in Genus *Rhododendron*: Evaluation of Ericaceous Mycorrhizal Relationships

Katie L. (Kate) Jenks, Senior, Biology (Plant)
Mentor: Michelle Stitzer, Biology
Mentor: Benjamin Hall, Biology
Mentor: Joe Ammirati, Biology

The presence of fungal symbionts residing in the root tissue of plants is a well-documented occurrence, yet questions regarding the identification and comparison of fungal partners in mycorrhizal relationships have been largely unanswered. Ericoid mycorrhizae, an example of a mycorrhizal relationship, are found in host plants within the order Ericales. Ericales, which contains such familiar species as persimmon, blueberry and Rhododendron, are able to persist in edaphic conditions due to their fungal symbionts. These symbionts form hyphal coils inside plant cell membranes, and thereby exchange crucial nutrients with the host plant. This project aims to evaluate the specificity between fungal communities and their host Rhododendron species, with the expectation that differing communities may exist, even in closely related hosts. Using known techniques to extract fungal DNA from the root systems of Rhododendron species in varying conditions and proximity, this DNA is then used to generate species based communities within specific Rhododendron hosts. By using type-cultures and genomic sequencing, comparisons of the presence or absence of fungal species within host roots can be made. This will shed light on infection intensity, and specificity between roots and fungal symbionts. Anticipated results of high levels of specificity between host plant and fungus could prompt questions regarding the importance of fungal symbionts in genus Rhododendron, especially with regards to the speciation between individual host plants.

Evolution and Distribution of Protochlorophyllide Oxidoreductases in the Heterokont Algae

Tejinder Singh (Tj) Randhawa, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Rose Ann Cattolico, Biology
Mentor: Heather Hunsperger, Department of Biology

The diatoms are a prolific and productive group of phytoplankton belonging to the phylum Heterokontophyta. They are responsible for ˜30% of oceanic primary production and their high photosynthetic capacity depends on their abilities to synthesize chlorophyll. The penultimate step in the biosynthesis of chlorophyll is catalyzed by the enzyme 'light-dependent protochlorophyllide oxidoreductase'. To understand the evolutionary history of the gene (*por*) encoding this enzyme (POR) we have constructed an algal *por* gene tree using diatom *por* genes as well as those from sister taxa and distantly related taxa. This process involved the cultivation of various strains of algae, DNA extraction, PCR, gene sequencing, data mining from public databases, and bioinformatic analysis. We found two distinct por genes (*por1* and *por2*) within the diatoms and a single haptophyte alga. We propose three hypotheses as to the origin of dual *por* genes in diatoms: (1) a *por* gene duplication event occurred before the divergence of the Heterokonts and Haptophytes; (2) a *por* gene was laterally transferred to a common ancestor of these two algal taxa; or (3) reciprocal lateral gene transfers occurred between these two algal taxa. Whereas the first two hypotheses are more parsimonious, we are unable to determine which scenario is correct with the current dataset. Nonetheless, these data provide evidence for a shared common ancestor between the heterokonts and haptophytes, a hypothesis that is currently hotly debated. Furthermore, the presence of these two *por* genes in various algal taxa presents a rich opportunity to study the evolutionary forces that either modify or maintain cellular gene function after gene duplication or lateral gene transfer. Thus, the results of this study will soon be complemented with research regarding the enzymatic function and regulation of the two *por* genes in diatoms.

The Discovery and Characterization of the Phytochelatin Synthase Gene in Conifers

Robert Tournay, Senior, Environmental Science, UW Tacoma
Mary Gates Scholar
Mentor: Erica Cline, Sciences and Mathematics, Interdisciplinary Arts & Sciences

While necessary for many biological functions, essential heavy metals, such as copper and zinc, are highly toxic when elevated, requiring their concentrations to be tightly regulated. This need to maintain homeostasis has led to a variety of adaptive mechanisms that chemically sequester excess metals, such as biosynthesis of phytochelatin by the enzyme phytochelatin synthase (PCS), found in plants, algae and several fungal species. Induced in cells of organisms exposed to elevated heavy metals, phytochelatins also bind non-essential heavy metals such as cadmium and arsenic, offering protection against anthropogenic toxins. In previous research, Douglas-fir (*Pseudotsuga menziesii*) seedlings exposed to municipal solids containing heavy metals also had elevated phytochelatins. The objective of this project was the discovery and characterization of the PCS gene in conifers. Degenerate primers were designed based on published sequences for PCS from other non-conifer plants including *Arabidopsis*, then used with the polymerase chain reaction method to search for the PCS gene in genomic DNA extracted from several conifer species. PCS gene segments of 267 and 133 base-pairs in length were successfully amplified and sequenced from ponderosa pine (*Pinus ponderosa*) and lodgepole pine (*Pinus contorta*), respectively. More recently, using an alternative approach employing complimentary DNA (cDNA) and DNA primers that were designed based on this newly obtained sequence data, an additional 354 base-pair PCS gene segment was amplified and sequenced from Douglas-fir. Protein alignments between the newly obtained conifer sequences and published PCS sequences from other plants revealed a strong degree of consensus, suggesting that our newly obtained sequences were indeed from the PCS gene. This constitutes the first report of PCS gene sequences from conifers. Ultimately, a better understanding of the PCS gene in conifers could enhance the use of phytochelatins as bioindicators of metals stress in conifer forests exposed to anthropogenic metals pollution.

Lipid Production and Potential Stress Response Mechanism in Freshwater Haptophyte *Chrysochromulina sp.* under Salinity Stress

Boris Mikhail (Boris) Rozenberg, Senior, Biology (Physiology)
Mentor: Stephanie Brunelle, Biology

Lipid bodies are cellular organelles involved in lipid production and storage within cells. Until recently, lipid bodies were thought of as inert storage units rather than complex organelles that play a large role in lipid biogenesis. The freshwater haptophyte *Chrysochromulina sp.* has served as an excellent model organism for research on lipid bodies because of its high growth rate, fully sequenced genome and two large, distinct lipid bodies. In algae and other eukaryotes, heat shock proteins can be investigated to detect when the cell is stressed. In many species of algae, when the cell population is placed under stress, the cells produce large lipid bodies. A classic stress response has also been detected in Chrysochromulina sp. after discovering genes coding for heat shock proteins (HSP 70 and HSP 90). Previous studies show that HSPs appear to be induced under high salinity stress in Chrysochromulina sp. The current study examines two important aspects of the correlation between salinity stress and lipid body production: what concentration of salt and what time frame is needed to cause haptophyte cells to become stressed enough to produce large lipid bodies. Previous experiments show that adding salt to algal media during log phase of growth fails to produce large lipid bodies while adding salt initially to media does not produce significant biomass to allow protein assays. Since HSPs are induced under high salinity concentrations, the second part of this study aims to determine a correlation between lipid body size, lipid production and how/when HSP proteins are induced. This study uses flow cytometry, the neutral lipid stain BODIPY 505/515 and Western Blotting to examine the relationship between lipid bodies and HSPs. Future research projects may include manipulation of stress response pathways that are relevant for biofuel applications.