Science Collaborative Research Program Symposium

Student Presentations

Friday, September 14, 2012

Ford 122

WILLAMETTE UNIVERSITY'S SCIENCE COLLABORATIVE RESEARCH PROGRAM SYMPOSIUM

Friday, September 14, 2012 * 3:30-7:00 PM

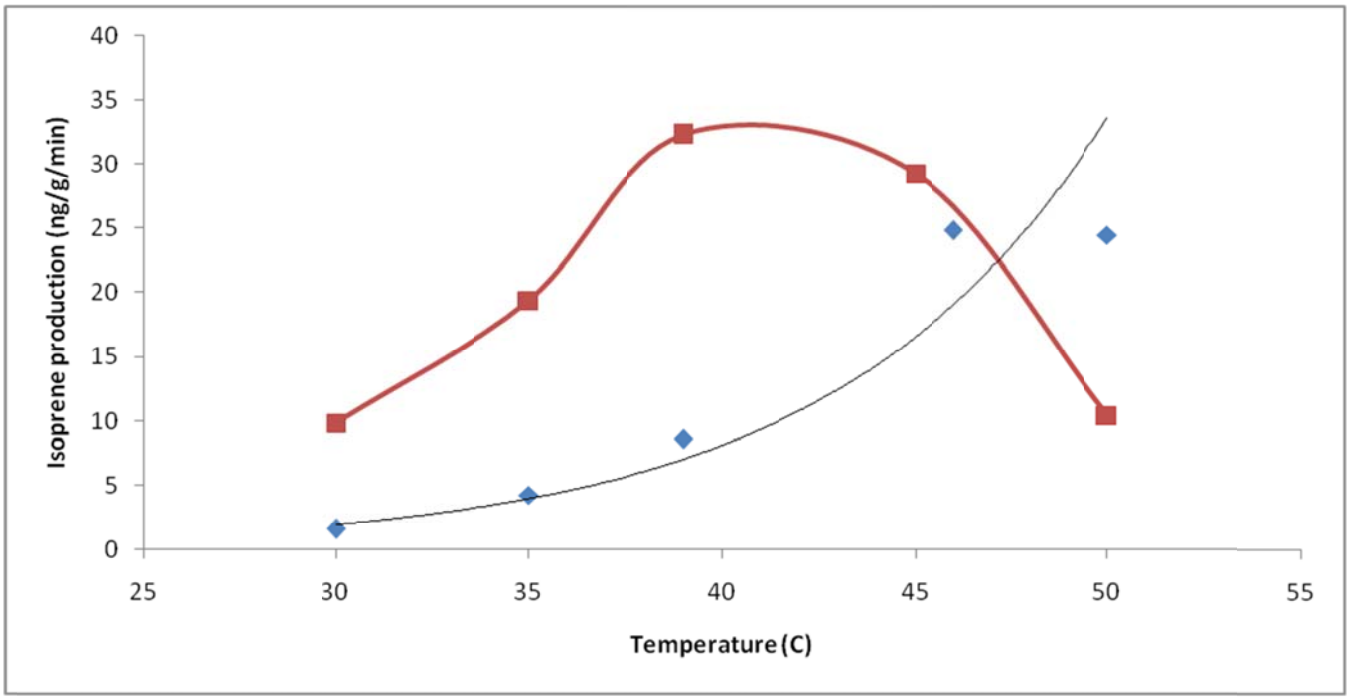
4:30-4:40 COFFEE BREAK

5:36-5:52 COFFEE BREAK & GROUP PHOTO

Alexandra Lantz. Characterization of a Novel Isoprene Synthase from *Campylopus introflexus*.

(*Advisor: Professor Alison Fisher, Chemistry)*

Isoprene is an important compound economically as a precursor to rubber and atmospherically as a catalyst for production of photochemical smog. Many plants, including certain pine, aspen, and kudzu enzymatically produce isoprene from dimethylallyl pyrophosphate (DMAPP). Isoprene production is most common in mosses, but to our knowledge no other isoprene synthase has been isolated and characterized from moss. Extract from *Campylopus introflexus* (heath star moss) had been shown by previous researchers to produce isoprene from DMAPP. In this research, the purification and characterization of the presumed isoprene synthase was continued. Partial purification was accomplished using a DEAE ion exchange column. Isoprene production of purified samples was determined using gas chromatography with a reducing gas detector. The production of isoprene was shown to be dependent on extract volume (R^2 = 0.99). Isoprene production of whole moss samples was 0.074 ± 0.003 μg/gdw/h, and was light dependent (p = 0.005). The temperature optimum of the enzyme was found to be 42 °C. Further research on this enzyme will help illuminate its relationship to angiosperm isoprene synthases, and may help research on the biological function of isoprene production.

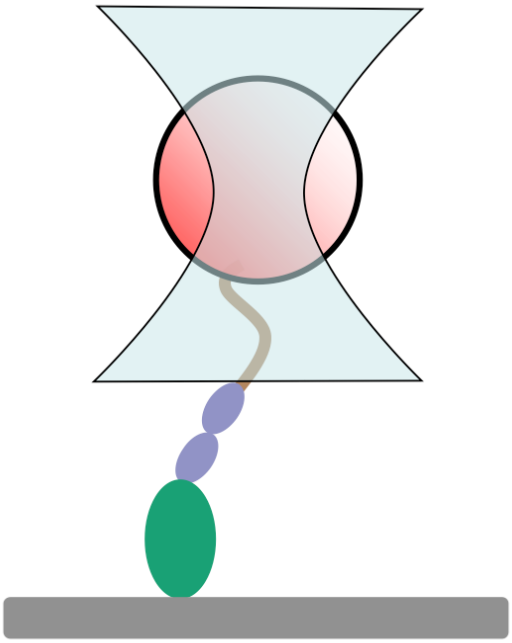


This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Jay Howard. Exploring the force-dependence of *Acanthamoeba* myosin 1c activity.

(Advisor: Professor David Altman, Physics)

Myosin motors reside in our cells and have the ability to convert chemical energy in the form of ATP into mechanical energy, thereby doing work within the cell. Different myosin classes perform different tasks, ranging from assisting in locomotion of the cell to trafficking cargo within the cell. We are examining the force-sensitivity of *Acanthamoeba* myosin 1c (AM1C) activity. While a functional response to external forces has been observed for other class 1 myosins, it is not yet known whether this same force-sensitvity occurs for AM1C. We completed the setup of an optical trap, a device we use to perform single-molecule experiments with myosins. We then developed a calibration technique that allows us to measure the nanometer-scale motions of a single myosin as well as the picoNewton forces experienced by a motor held in the optical trap. We are now poised to collect data of single AM1C motors moving against varying external forces to test whether the motor's function is regulated by force.



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Peter Ho. A quick guide to patching a cell.

(Advisor: Professor Emma Coddington, Biology)

The study of context dependent behaviors serves to identify how different scenarios influence decisions made. While the neuromodulators and brain regions associated with context dependent courting behavior are well defined, the electrical and physical characteristics of the neurons involved are not well understood. Through electrophysiology, many of the cell's structural features can be revealed, and it becomes possible to better understand how they receive and integrate information to influence this behavior. One of the many techniques of electrophysiology is the patch clamp. By sealing off a piece of the cell's membrane against a recording electrode and varying either the current or voltage across it, recordings of the cell membrane's electrical properties are acquired. In this presentation, I will provide a further backdrop into electrophysiology by identifying the key concepts, techniques, and procedure behind patching a cell membrane. These will be placed within the framework of the research our lab is pursuing and the questions I am to answer using electrophysiology.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Alexandria Parsagian. Design of a calcium oven and Zeeman slower for use in cooling and trapping calcium atoms.

(Advisor: Professor Michaela Kleinert, Physics)

Ultracold heteronuclear molecules are of special interest to the scientific community for their applications in ultracold chemistry, precision spectroscopy, tests of fundamental symmetries, and quantum computation. We are working toward the creation of the novel molecule RbCa. This molecule possesses both a permanent electric and magnetic dipole moment, making it ideal for the study of strong long-range dipole-dipole interactions. For the laser cooling and trapping of calcium, an oven capable of reaching the necessary temperature of ~800K, as well as creating a collimated beam must be designed. Additionally, the beam must be slowed down to ~60 m/s before it can be trapped, making a Zeeman slower necessary. In the past, these have been designed using a current-carrying coil. The idea of a permanent magnet Zeeman slower is relatively new, but has various advantages, in that it does not require water cooling or high currents. We designed our own permanent magnet Zeeman slower using neodymium magnets at varying distances from the calcium beam. This setup will allow us to have great control over the magnetic field and the slowing of the calcium atoms. I will be talking about the design and construction of the calcium oven and Zeeman slower. Together, these components will allow for the cooling and trapping of calcium atoms, as well as the cooling and trapping of RbCa.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the National Science Foundation.

Hayley Serres. Mucoidy acts as a defense against bacteriophage infection in *Caulobacter crescentus*.

(Advisor: Professor Melissa Marks, Biology)

The ϕCR30 bacteriophage (virus) infects the fresh water bacterium *Caulobacter crescentus* by binding to a protein complex on the cell surface. Variability in phage susceptibility is conferred the presence of a mobile element in the genome. Interestingly the delta (Δ) mobile element (ϕ) allele has fixed in some laboratory populations suggesting it may be energetically expensive to maintain. Cultures were infected with ϕCR30 and survival was monitored and quantified over 18 hours by measuring optical density (OD). When challenged with ϕCR30, a non-mucoid strain (NA1000Δϕ) is less susceptible to infection than a mucoid strain (NA1000+ϕ) (t = 10.750, p < 0.0001). Based on the predicted function of the genes encoded in the mobile element, we predict that the mucoid produces an exopolysaccharide (EPS) layer that obscures binding of ϕCR30 to cells. To more closely monitor the process of infection and determine the stage at which the protective effect of the EPS layer is manifested, we developed a q-PCR assay to measure ϕCR30 adsorption and replication. Preliminary data are consistent with higher rates of adsorption to NA1000Δϕ than NA1000.

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Geri Laudenbach. Repress the repressor? Ethylene's regulation of flowering time in *Arabidopsis thaliana* plants.

(Advisor: Professor Alison Fisher, Chemistry)

The timing of the floral transition in *Arabidopsis thaliana* plants is regulated by both endogenous and environmental cues. These cues are signaled via multiple genetic pathways that converge on few, well-studied genes. These genes include the floral repressor *FLOWERING LOCUS C* (*FLC*) and the floral integrators *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*). The Fisher lab studies the role of the plant hormone ethylene in regulating the floral transition in *Arabidopsis*. In this project, we used *Arabidopsis* plants with mutations in the ethylene signaling pathway to determine whether ethylene signaling regulates the expression of *FLC*, *SOC1* and *FT*. We used reverse transcription (RT) of mRNA coupled with quantitative polymerase chain reaction (PCR) to measure the expression of *FLC*, *SOC1* and *FT* in ethylene signaling mutants and wild-type plants. In both 10-day-old and 20-day-old plants, expression of *FLC* was higher in the ethylene signaling mutants relative to wild-type plants, while expression of *SOC1* was lower relative to wild-type plants. Analysis of *FT* expression proved more difficult. Our results suggest that ethylene signaling promotes the floral transition in *Arabidopsis* by reducing transcript levels of the floral repressor *FLC*, allowing SOC1 (and likely FT) to actively promote flowering.

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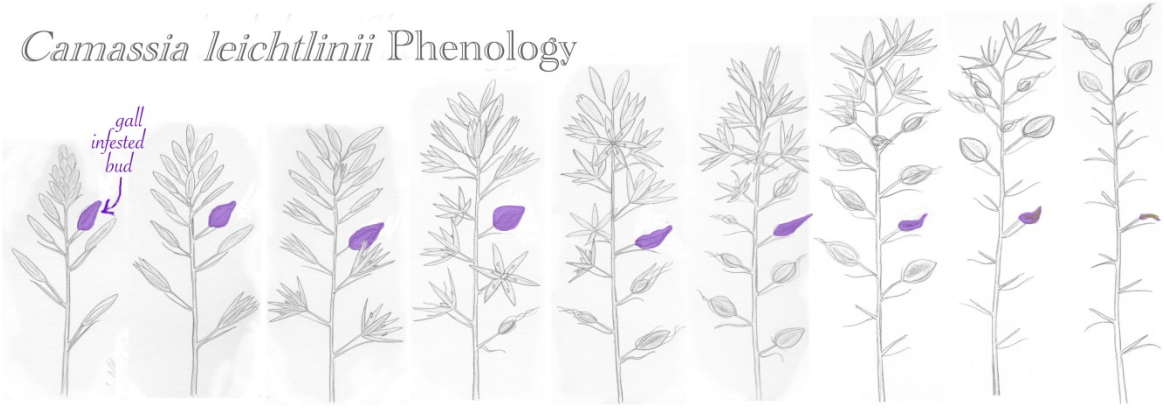
Theresa Ann Barosh & Linnea Jean Hardlund. Examining phenology and herbivory of *Camassia* in the Willamette Valley.

(Advisors: Professor Susan R. Kephart and Post-doctoral Scholar Dr. Kathryn E. Theiss, Biology[1])

Species in the genus *Camassia* offer an opportunity to explore species boundaries through genetics, morphology, phenology, and ecological variation. *Camassia quamash* and *Camassia leichtlinii* often co-occur sympatrically in the Willamette Valley; however, they are distinctly different species with varying phenologies. Phenological traits in flowering time can determine species boundaries. In *C. leichtlinii* detailed assessments led to the discovery of unusual galling midges, herbivores that may significantly influence plant morphology and phenology. We evaluated reproductive isolation via phenological traits, including the number of open buds throughout the flowering season and asked three questions: 1.) Do differences in phenology help maintain species boundaries in *Camassia*? 2.) Does herbivory affect flowering phenology therefore influencing reproductive fitness? 3.) What *Camassia* traits are associated with this galling midge? We collected phenological data on *C. leichtlinii* and *C. quamash* from four sites in the Willamette Valley to track floral development over time by noting the time and form of galling and flowering at reproductive nodes. We compared the phenologies of each species at all sites, using the extent of flowering overlap to estimate the amount of reproductive isolation between them. We also compared the percentage of herbivory by cecidomyiid galling midges. We detected a trend for smaller percentages of galls per stem in lower elevation sites. To understand the ecological implications of the interaction, we also examined the traits of camas plants with and without herbivory. Both height and size of the camas plant seem to have little to no correlation with frequency of herbivory. Future studies on the genus *Camassia* should explore phenology as it relates to climate change as well as alternative explanations for species boundaries and galling midge plant preference.



[1] The coauthors and mentors would like to give special recognition to team member Surabhi Mahajan who contributed to our field team success with her time, expertise and enthusiasm.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the National Science Foundation.

Patrick Reynolds. Determining hybrid morphology in Joshua trees.

(Advisor: Professor Christopher Smith and Ramona Flatz, Biology)

Joshua trees, *Yucca brevifolia,* and Yucca moths, *Tegeticula*, are joined into an obligate pollinator mutualism where one could not reproduce without the other. With two separate Joshua tree types and sister species of Yucca moth that co-occur in Tikaboo Valley Nevada we find trees that are morphologically and genetically hybrid. I ask the question how do the important reproductive features of hybrid trees differ from their parental tree types in hopes of determining genetic and evolutionary forces effecting hybrid trees. Microsatellite data was extracted from leaf tissue to determine tree type. The results of an anova test between tree type and style length reveals that hybrids are statistically different from both parental tree types and average out to have intermediate style length. While a histogram of style length separated by tree type reveals that hybrid morphology are spread throughout the style length spectrum. This suggests that style length is a polygenetic trait in that it is determined by multiple genes. I plan on running a future hybrid zone analysis graphing how tree genotype and style length change over the distance of the hybrid zone. The inflection point and steepness of this graph can be used to determine sectional strength on style length.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Jordan Loos. Use patterns of a 5,000 year old building: Portable X-ray fluorescence analysis of in situ floor deposits of Structure 8 at the Ness of Brodgar in the Heart of Neolithic Orkney, Scotland.

(Advisor: Professor Scott Pike, Environmental and Earth Sciences)

Non-destructive elemental analysis using portable X-Ray Fluorescence (pXRF) spectroscopy is a rapidly growing practice in archeology. The current study is part of a broad research program to assess the utility of pXRF on active excavations. A Bruker Tracer IIISD pXRF was used to analyze in situ floor deposits of Structure 8 at the Neolithic site of the Ness of Brodgar in Orkney, Scotland. Multiple analyses of over 100 gridded sample points were analyzed using two different instrument configurations. The resultant spectra were quantified and the data normalized to the elastic rebound value of Rh. Spatial comparisons of the resultant ratios suggest different use patterns of the different spaces in the building. The results will assist archaeologists with interpreting the activity patterns of the Neolithic occupants of this unparalleled, unique structure and provide important geochemical information to assist the excavators in recognizing stratigraphic layers.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Alexandra Schrimp. Genetic identifications of Joshua trees predict moth visitation in Tikaboo Valley.

(Advisor: Professor Christopher Smith and Ramona Flatz, Biology)

Joshua trees (*Yucca brevifolia*) are exclusively pollinated by yucca moths, which in turn require the tree to provide food and shelter for their developing larvae. In Tikaboo Valley, there is exists a hybrid zone where two species of Joshua tree, and two species of yucca moth, *Tegeticula synthetica,* and *T. antithetica*, coexist. It is believed that the yucca moths typically pollinate only one species of tree. However, it has been observed that within Tikaboo Valley both moth species are visiting both Joshua tree species. This study aims to determine whether particular tree features influence pollinator specificity. We measured moth visitation to individual trees using sticky cards based at the base of the inflorescence to capture visiting moths. Moths were identified to species based on morphology and DNA barcoding. We measured tree vegetative and floral morphology, genotyped trees using microsatellites, and compared floral scent using gas chromatography mass spectroscopy. We then evaluated how well each of these features predicts moth visitation using a stepwise linear regression. The results showed that the genetic identifications of the Joshua trees are the only variable necessary to explain moth visitation in the hybrid zone of Tikaboo Valley. As we determined, yucca moths are able to determine which tree species they are pollinating. Previous studies suggest that moths have a reproductive benefit when they pollinate the correct tree. Therefore being able to identify which tree will give them a reproductive benefit will enable them for reproductive success.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Emily Abraham & Audrey Davis. Investigating the role of cannabinoid-1 receptors in vasotocin-OG Internalization in clasp-controlling hindbrain neurons of *Taricha granulosa*.

(Advisor: Professor Emma Coddington, Biology)

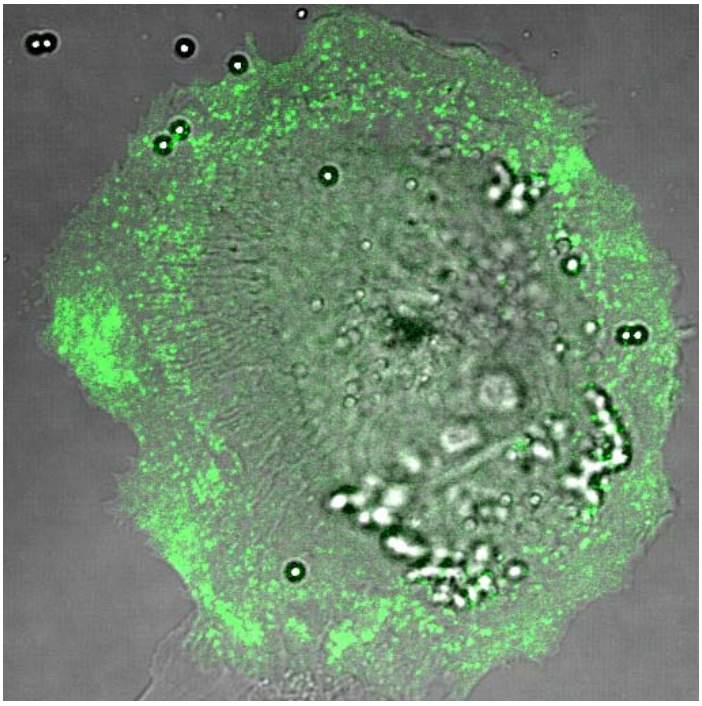
We understand very little about how hormones affect neurons and behavior, yet the ability of the nervous system to execute context-dependent behaviors is a crucial survival mechanism for animals in the wild and is dependant upon hormone and neuron signaling. Such context-dependent behaviors have been a topic of ongoing research in the *taricha granulosa* model, in which the hormone vasotocin (VT) has been shown to enhance clasping behavior and responsiveness of medullary neurons that respond to cloaca stimulation. Corticosterone (CORT) and cannabinoid (CB) application, however, have been shown to suppress this clasping behavior and the excitability of clasp-controlling hindbrain circuits. CORT's effect is likely mediated through increased CB production, but the mechanism through which CBs alter VT signaling and clasping behavior remain uncertain. Recent data suggests that CBs prevent the internalization of VT into Rf neurons. The mechanism through which CBs are suppressing VT internalization is unknown, but the CB1 receptor has been implicated in behavior and electrophysiology studies in which application of a CB1 Antagonist (AM281) prior to CB prevented CB-induced suppression of clasping and neuronal excitability. The purpose of this study is to test the role of the CB-1 receptor in CB-mediated suppression of VT internalization. AM281, CB agonist, and VT tagged with the Oregon-green fluorophore (VT-OG) were applied directly to the hindbrain regions of 24 newts. Following the experiments the brains were fixed, frozen, sliced, and imaged using a Zeiss Confocal microscope to produce 3-D images of DAPI-stained nuclei and internalized fluorescent VT-OG. These images will be quantitatively analyzed to assess and compare VT internalization among groups.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the M.J. Murdock Charitable Trust (EA) and the Mary Stuart Rogers Foundation (AD).

Bianca Nagata. The role of myosin VI and VIIa in the internalization rates of retinal pigment epithelial cells.

(Advisor: Professor David Altman, Physics)

Retinal pigment epithelium (RPE) cells phagocytose waste shed by rod photoreceptor cells. This is important for the health of the eye, and failure to complete this process results in retinal degeneration due to the accumulation of waste. We studied the role of molecular motors in the phagocytic process. Specifically, we tested the hypothesis that motor proteins myosin VI and VIIa generate the forces and motion required for the internalization of rod cell debris. We examined the contribution of these motors to the speed of internalization of phagosomes by RPE cells. Preliminary data shows that the disruption of myosin activity resulted in reduced rates of internalization. This result suggests that these motors play a role in RPE phagocytosis, and are thus necessary to the proper function of these cells.



This work was completed as part of the Willamette University Science Collaborative Research Program. Bianca Nagata volunteered her time for this project.

Kayla Johnson. A novel mutation in the *Drosophila slingshot* gene identifies a requirement for its function in the maintenance of synapse morphology.

(Advisor: Professor Jason Duncan, Biology)

Nerve cells employ a microtubule-based transport system to bidirectionally transport proteins, vesicles, and organelles between the cell body and the synaptic terminal through the axoplasm. We have undertaken genetic screens to identify novel genes required for axonal transport. Among the candidate genes isolated, we have identified a novel mutation in the ADF Cofilin gene *slingshot (ssh)* that regulates actin filament dynamics. Slingshot mutant larvae exhibit a posterior paralysis and tail-flip phenotype, which are hallmarks of impaired axonal transport. However, immunohistological analysis of axons from the peripheral nervous system of *ssh* mutant larvae fail to reveal focal swellings and accumulations of transported components, indicating that axonal transport is not disrupted or altered. Given the role of *ssh* as a regulator or actin filament dynamics, we hypothesize that the aberrant crawling behavior observed in *ssh* mutant larvae is the consequence of a requirement for its function in the maintenance of synapse morphology. Analysis of glutamatergic motor neuron synapses from *ssh* mutant and wildtype larvae reveals a significant difference in the size of synapses, as determined by total number of boutons and synapse area. Future work is directed at further characterizing the ssh^{WU7} allele in light of these unexpected results.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Dylan Goldade. Population genomics of Joshua trees: Identifying signatures of selection in the Y*ucca brevifolia* genome using next-generation sequencing.

(Advisor: Professor Christopher Smith and Ramona Flatz, Biology)

The advent of next-generation DNA sequencing has made it possible to complete genomic studies for non-model organisms with few genomic resources. Additionally, complexity reduction techniques, which involve sequencing only small "snapshots" of the genome, make it possible to compare genome-scale data between multiple individuals using an approach that is faster, cheaper, and less computationally intense than conventional genome sequencing. The Joshua tree (*Yucca brevifolia*,) is both an iconic species of the Mojave Desert and is a model system for studies of coevolution. Joshua trees occur in a western morph (*Y. b. brevifolia*) whose flowers feature longer stylar canals, and an eastern morph (*Y.b. jaegeriana*) whose stylar canals are shorter. Two species of Yucca moth (*Tegeticula synthetica* and *T. antithetica*) selectively pollinate the western and eastern trees, respectively, while they lay their eggs inside the flower with ovipositors matching the length of their preferred tree's stylar canals. The genetic basis of differences in floral morphology that is crucial to this coevolutionary relationship is unknown, and it is unclear whether these differences are the result of divergent natural selection. We used RAD tag sequencing—a procedure that combines restriction digestion with next-generation sequencing—to genotype 30 individual trees, representing the two morphotypes of Joshua trees, at ~20,000 loci. From these loci we identified ~3,000 single nucleotide polymorphisms. We used an F_{ST} outliers method to identify signatures of positive selection using two different programs. To compare signatures of selection in expressed versus non-transcribed regions of the genome, we BLASTed the RAD markers against the *Y. brevifolia* transcriptome. To identify putative genes that may be under selection, we BLASTed loci showing evidence of positive selection against the *Arabidopsis thaliana* genome. A very small fraction of the genome showed evidence of positive selection (214 loci), but proportionally more of the transcriptome shows signs of positive selection than the entire genome (p =0.0024). The most significant BLAST hits (e-values ≤ $1.00e^{-10}$) on the *A. thaliana* genome coded for functions such as cell transport, kinase activity, and DNA binding. This study demonstrates that interesting and meaningful findings can result from using a combination of bioinformatics and next-generation sequencing to answer questions about the genetic component of documented biological phenomena.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Arthur A. Wilson Research Scholarship Award.