17th Annual Biology Student Research Symposium
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Abstracts of Presentations

POSTER PRESENTATIONS

1.

Lizzie Grabowski (Biology, '17) and Dana Wohlbach.
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Cider Yeasts: An Exploration of Micro-environmental Terroir at Three Springs Fruit

Terroir is the concept that place and environment impart unique aesthetic characteristics onto the products they yield (Trubek 30). This study aims to quantify the terroir of Three Springs Fruit Farm in Aspers, PA through isolation and identification of the yeast strains naturally present in the orchard and found within orchard products. Known species of yeasts are quite diverse, genetically, ecologically, and physiologically: there is more genetic diversity within the yeasts than can be found in either vertebrates or flowering plants. Furthermore, yeasts are integral to the production of any alcohol, including cider, and their fermenting abilities have been harnessed for millennia in order to safely preserve food and drink. Since the first beverages were fermented, likely in 7000 BC China, brewing and wine-making have transitioned evolved from chore to art form (Sicard & Legras 230).
This autumn is the inaugural season for Three Springs' own commercial beverage operation, hard cider production. To assist Three Springs with their goal of transforming Adams County into a hard cider destination, we provided a characterization of the wild yeast found on their orchard. This information, and the accompanying glycerol stocks of yeast strains, informed cider production by identifying optimal conditions for each yeast strain as well as providing the orchard the information to standardize their different cider blends. Samples were collected from both completely wildly fermenting ciders as well as ciders that were fermented with a mix of wild and commercial yeasts. Additionally, domesticated apple and wild crabapple samples were collected to provide comparative yeast populations to those isolated from the ciders. Our contribution to Three Springs Fruit Farm will have implications beyond the Central Pennsylvania food community and contribute greater understanding of the microenvironments that influence production, taste, and terroir.

2.

Madison Parks (Neuroscience, '17) and Mary Niblock.
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Functional development of central chemoreceptors and onset of fetal breathing movements in embryonic and early postnatal mice

The central chemo reflex is the body's response to increased carbon dioxide in the blood. It is activated by peripheral and central chemoreceptor cells that increase respiration via connections to the ventral respiratory column, which in turn innervates the phrenic motor neurons that drive the contraction of the diaphragm. Previous in vitro studies have shown that parts of this pathway function before birth and that embryonic central

chemoreceptors respond to carbon dioxide/acidity in vitro, but no study has examined the functional development of the intact pathway in utero or in vivo. In our study, we are using whole animal carbon dioxide exposure of pregnant Fos-Tau-LacZ (FTL) mice or early postnatal FTL mice coupled with ultrasound scanning to determine when the central chemoreflex circuit develops and if the timing of its development correlates with fetal breathing movements. Because previous studies have found a sex difference in one region of the pathway, we will be studying male and female mice.

3.

Sadie A. Signorella (Biochemistry and Molecular Biology, '18), Zev Greenberg (Chemistry, Biochemistry and Molecular Biology, '16), and Kirsten A. Guss, Ph.D.
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Scalloped on the brain: using ChIP to identify targets of Scalloped function in the fruit fly central nervous system.

The *scalloped* gene encodes a transcription factor with a TEA domain that binds to DNA, and plays a role in the development of the embryonic neuromusculature and the adult wing of *Drosophila melanogaster*. The Scalloped protein is broadly expressed in the larval brain; however, its function in this tissue is unknown. Because Scalloped directly controls the expression of genes in the fly wing, we wanted to explore whether it is functioning as a transcription factor to control gene expression in the fly brain. To identify potential target genes in this tissue, we are using the Chromatin Immunoprecipitation (ChIP) assay. We have dissected over 200 brains from third instar larvae, and are purifying genomic DNA from these tissues. Next, the DNA will be incubated with a protein-specific antibody to recover sequences bound by Scalloped. These sequences will then be used to locate candidate genes in the genome. By the completion of this project, we hope to have identified specific nucleotide sequences to which Scalloped is bound in cells of the larval brain.

4.

Courtney Gamache (Biochemistry and Molecular Biology '18), Jamie George (Biology '17), and Dana J. Wohlbach.
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Domestication of Saccharomyces cerevisiae and Saccharomyces paradoxus yeasts

Although Saccharomyces cerevisiae has been domesticated for use in the production of food, beverages, and biofuels, as well as in the laboratory, there is limited understanding of the genetic changes associated with its domestication. This project aims to recapitulate laboratory domestication of yeast in order to determine the extent to which selection for biochemical changes and gene expression changes are drivers of evolutionary change during domestication. To test the variability of evolutionary trajectories, four different wild strains of S. cerevisiae and S. paradoxus are being domesticated in quadruplicate. Cultures are propagated in rich media containing glucose and incubated at 30˚C (standard laboratory conditions). Daily transfers are made approximately 22 – 26 hours after each previous day's transfer. Domestication will be continued for thousands of generations, with strains saved and examined approximately every 100 generations. The resulting evolved strains and their initial wild type counter parts will be sequenced and compared to map

notable genome changes associated with domestication. Over the duration of the experiment, we expect to find that as strains evolve to grow more efficiently in laboratory conditions, evolutionary trade-offs such as reduced stress resistance, will also emerge.

5.

Rinaldys J. Castillo '17 (Biology), **Tim O. Nieuwenhuis '17 (Biochemistry & Molecular Biology)**, **Rebekah S. Samuels '17 (Biology)** and Dana J. Wohlbach.
Department of Biology, Dickinson College, Carlisle, PA 17013

The wild side of yeast: Sequencing and characterization of novel species

One of the overarching goals of the Wohlbach Lab is to improve microbial fermentation-regulated biofuel production by the brewer's yeast *Saccharomyces cerevisiae* and other yeasts. The efficiency of such biofuel production is limited by poor fermentation of some sugars, as well as the stressful conditions of biofuel production. To investigate these topics, we isolated, identified, and characterized new species of wild yeast with the hope of discovering species with the capability to metabolize different carbon sources and/or novel stress response mechanisms. Yeasts were isolated from forest flora and fauna, underwent ribosomal DNA amplification and sequencing, and were computationally analyzed against all currently known species of yeast via the Basic Local Alignment Search Tool (BLAST) algorithm. In mycology, candidate novel species of yeast are defined as having more than 3% ribosomal DNA sequence divergence as compared to previously discovered species. We have successfully isolated and identified over 300 yeast strains, of which at least 15 are tentatively new species. Ongoing work is mainly focused on the description of candidates' colonies and cells and the characterization of carbon metabolism and fermentation. Future work will focus on characterizing nitrogen metabolism and stress responses.

6.

Sarah L. Schneid (Biology, '17) and Kirsten A. Guss, Ph.D.
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This fly's got nerve: investigating the role of *scalloped* in motor neuron patterning

Segmental nerve b (SNb) is a motor neuron with four branches that innervates the ventral muscles of the fruit fly *Drosophila melanogaster*. Previous studies have shown that SNb is shorter in embryos with a mutation in *scalloped (sd)* than in the wild type. We are investigating the role of *sd* in SNb patterning. We are using whole mount immunofluorescence to examine motor neuron structure using antibodies to FasII, which is expressed on the surface of all motor neurons, and GFP, to determine the genotype of *sd* mutant embryos. Through this process, we are investigating which branches of SNb are impacted in embryos carrying the *sd* mutant, and whether other segmental nerves are impacted, as well.

7.

Isaac Pitkow (Biochemistry and Molecular Biology '17), Devlynn Chen (Biology '17) and Tiffany Frey.
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The role of sex hormones in the expression of inflammatory microRNAs and tristetraprolin

The epidemiology of inflammatory lung diseases such as asthma implicate that females are

more at risk for these diseases. This connection suggests that female sex hormones such as estrogen and progesterone should be considered when treating diseases like asthma. In addition, it is well established that one way to regulate inflammation is by degradation of inflammatory mRNAs, a process mediated by both microRNAs (miRNAs) and Tristetraprolin (TTP), molecules that bind to the 3' UTR of inflammatory mRNAs. The goal of this project is to examine how sex hormones affect the expression of miRNAs and TTP. In order to achieve this goal, peripheral blood mononuclear cells (PBMCs) were exposed to either estradiol or progesterone overnight followed by stimulation with either LPS to activated monocytes or anti-CD3 and anti-CD28 to activate T cells. TTP expression was measured by Western blot and miRNA expression was measured by real-time polymerase chain reaction (RT-pcr). Data generated from this project will lead to a deeper understanding of these inflammatory processes.

8.

Alissa Resnikoff (Neuroscience '18), Rulaiha Taylor (Biology '18) and Anthony Pires.
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Effects of ocean acidification and salinity stress on growth and metamorphosis in larvae of a marine gastropod.

The earth's oceans are becoming more acidic due to absorption of atmospheric CO2. Ocean acidification decreases the concentration of carbonate ions in seawater, and imposes increased energy costs on organisms that deposit calcium carbonate shells and skeletons. Larval stages of these organisms are especially vulnerable to combined stresses of acidification, low salinity, and nutrition, which may have a common energetic basis. We investigated how acidification and low salinity affect growth of a marine gastropod during the planktonic larval period that precedes metamorphosis to the benthic juvenile stage. We also asked if acidification or salinity affected the induction of metamorphosis in response to a natural cue, as well as to pharmacological manipulation of nitric oxide signaling, a neurochemical pathway implicated in the metamorphosis of many marine invertebrate larvae. Our study species, the "slipper limpet" Crepidula fornicata, is widely distributed in temperate near-shore coastal environments, including estuaries where wide fluctuations in pH and salinity often occur. Larvae were cultured at defined combinations of salinity (20-30 ppt), and pH (7.5-8.0), with food level and temperature held constant. Carbonate chemistry of culture water was further characterized by titration of total alkalinity, and calculation of saturation states of the two mineral forms of calcium carbonate found in gastropod shells. Larvae grew more slowly at lower salinity and at lower pH. The effect of dilution on growth was magnified by acidification. Larvae raised in all conditions were competent for metamorphosis. However, larvae cultured at low pH and low salinity metamorphosed less frequently in response to inhibition of nitric oxide signaling. Experiments in the spring semester will focus on exploring consequences of acidification and salinity stress in juveniles during the period immediately following metamorphosis, and on "carryover" effects of larval stresses on juvenile performance.

9.

Amanda Jimcosky (Biology '17) and Dana J. Wohlbach.
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Computational analysis of the environmental stress response in Ascomycete fungi

Ascomycete fungi occupy diverse niches; some are free-living while others hold symbiotic or pathogenic relationships with host organisms. The environmental conditions of the various niches in which these fungi thrive often fluctuate rapidly; therefore, Ascomycete fungi must be able to rapidly adapt to changing conditions. The gene expression response to various stresses has been termed the environmental stress response (ESR) in Saccharomyces cerevisiae (S. cerevisiae) and a similar stress response has been documented in Schizosaccharomyces pombe (Sz. pombe). The ESR consists of approximately 300 induced and 600 repressed genes that activate metabolism and cellular signaling and downregulate protein synthesis. Regulation of the ESR is composed of both condition-specific regulators and general stress factors, which operate in various stress conditions. Data sets for gene expression under various stress conditions in S. cerevisiae, Candida albicans, Aspergillus nidulans, Aspergillus niger, and Aspergillus oryzae were acquired and analyzed to define a stress response in each species. The response in each species will be compared using orthologous gene lists in order to determine which modules are conserved throughout evolution and how the regulation of the ESR has evolved across the Ascomycete lineage.

10.

Courtney Gamache (Biochemistry & Molecular Biology, '18)[1], Zoe Irons (Biology, '18)[1], Erik Williams (Biology, '18)[1], John Henson[1,3], and C. Bradley Schuster [2,3]
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Super resolution imaging of contractile ring components in dividing sea urchin embryos

The process of cell division, or cytokinesis, has long been known to be driven by the interaction of actin filaments with the motor protein myosin II arranged within a structure termed the contractile ring (CR). However, despite a great deal of research effort, the precise structural organization of actin and myosin II filaments – as well as other protein components - within the CR remains unknown. In this study we have combined immunofluorescent labeling with antibodies with 3D structured illumination super-resolution microscopy (SIM) to examine the fine structure of proteins in the CR of cortices isolated from first division sea urchin embryos. The localization results suggest that early in cytokinesis myosin II filaments - labeled in a way that allows for the recognition of head and tail regions - are organized into discrete clusters arranged in a broad stripe. As division progresses, this arrangement transforms into an aligned linear array of myosin II and actin filaments that are oriented parallel to the CR long axis. In addition, in cortices isolated from highly contracted embryos the myosin II filament heads tend to be oriented towards the cleavage furrow. Interestingly, treatment with the actin filament disrupting drug Latrunculin A prevents this reorganization of myosin II filaments and inhibits division. This suggests the critical nature of actin and myosin II interaction. In other experiments the CR component proteins septin2 and anillin were localized with reference to myosin II. Images of septin2 localization suggest the presence of a gauze-like network of filaments in the CR in close association with myosin II. Anillin staining indicates that this protein is also codistributed with myosin II filaments in the CR. These results support the accepted purse

string model of cytokinesis and suggest that animal cell CR myosin assembly may derive from clusters in a manner that has been shown to occur in fission yeast.

11.

Surya Brown-Moffitt (Biology, '17) and Scott Boback.
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Kinematics of feeding behavior in the American toad: Vertical limits of tongue projection

Since 2011 The Dickinson College Farm has been used as a living laboratory for student researchers investigating organisms within an Integrated Pest Management (IPM) system. IPM is a technique used to control pest insect populations without the use of harmful pesticides. Recent research investigating the diet composition of the American toad suggests toads consume a variety of invertebrate organisms on the Dickinson College Farm including some pest insects. What we do not know, however, is what proportion of these pest insects are susceptible to predation by toads. The current project is designed to address this by studying the mechanics of toad feeding behavior to determine the vertical limits of prey capture. To measure these parameters toads were filmed using high speed videography, when presented with live prey at varying heights. Movement of the tongue and mouth were followed using Tracker video analysis software. Initial analysis indicates that predation of insects at higher levels requires different body positions relative to predation of insects on the ground. Preliminary observations also seem to suggest that toads may be less likely to consume elevated insects as it took longer for the toad to become interested in these prey items compared to those on the ground.

12.

Lindsey Zwecker (Biochemistry and Molecular Biology ’17), Tyler Llewellyn (Biology ’17), Eric Vogt (Biology ’17) and Tiffany Frey.
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Isoprenoid depletion alters inflammatory gene expression in human cells

Systemic autoinflammatory diseases (SAIDs) are characterized by episodes of increased inflammation with related symptoms such as fever, rash, and joint pain. SAIDs are caused by mutations in genes that control the innate immune system and are therefore categorized by genetic mutation. One category of SAIDs is the hereditary periodic fever syndromes. Examples include Familial Mediterranean Fever (FMF), TNF receptor-associated periodic syndrome (TRAPs), and mevalonate kinase deficiency (MKD). In FMF and TRAPs, the genetic mutations are found in genes that are clearly related to the production of inflammatory molecules. However, MKD is an interesting case since the genetic mutation in located in the mevalonate kinase gene. The mevalonate pathway is responsible for the production of both sterol and non-sterol isoprenoids, which play a role in many cellular processes including signal transduction, membrane fluidity and trafficking, and cytoskeletal structure. In order to understand the role of isoprenoids in inflammation, this disease was modeled by treating healthy donor peripheral blood mononuclear cells (PBMCs) with drugs that block isoprenoid production or function. PBMCs are a mixture of white blood cells including T cells, B cells, monocytes, and dendritic cells. Lipopolysaccharide (LPS) was then added to the cells to induce an inflammatory response and real time PCR was utilized to measure inflammatory cytokine mRNA levels. Significant

changes in TNF-a, IL-6, and IFN-y mRNA levels were observed in the PBMCs following isoprenoid depletion, which is similar to the changes in circulating cytokine levels that have been reported in MKD patients. In addition, increased levels of TNF-a and IL-6 were observed in purified monocytes following isoprenoid depletion, but not IFN-y. This result indicates that the monocytes are responsible for the increased production of some, but not all MKD cytokines. Future work will focus on the mechanisms of cytokine gene expression changes in different cell types.

13.

Charlotte Heroux (Biochemistry & Molecular Biology, 17')[1], Elizabeth Eckert (Clinical and Translational Science Track Ph.D. Program)[2] and Stephen J. Russell M.D., Ph.D.[2]
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Encoding Chemokines in Vesicular Stomatitis Virus to Enhance Oncolytic Virotherapy

Chemokines could potentially be encoded into Vesicular Stomatitis Virus (VSV) to enhance its oncolytic activity. VSV selectively replicates in cancer cells and lyses cells in its replication cycle, so it is a favorable virus for oncolytics. Chemokines are a unique kind of signaling protein that recruit immune system cells to the site of injury or tumor lyses through chemotaxis. There are four chemokines used for this project; mCCL2, mCCL5, mCXCL2, and mCXCL5. mCCL2 recruits monocytes, memory T lymphocytes and natural killer cells. mCCL5 recruits CD8+ T cells and natural killer cells. mCXCL2 and mCXCL5 both recruit neutrophils to the site of tumor lyses. The goal for encoding these chemokines, individually, into VSV, is to have specific immune system cells be recruited to the site of tumor lysis and aid in the killing of the cancer cells as well as help "clean up" the tumor microenvironment by ingesting dead and damaged cells. After cloning four new VSV viruses, each with one of the specific chemokines, two of the viruses confirmed the correct sequence; VSV-mCCL2 and VSV-mCXCL2. After transfection and passaging of VSV-mCCL2 and VSV-mCXCL2, ELISA's were performed and the presence of mCCL2 and mCXCL2 in VSV was confirmed. VSV-mCCL2 measured with a concentration of virally-encoded mCCL2 at a physiologically relevant concentration. This project will continue, with VSV-mCCL2 and VSV-mCXCL2 in the lab to determine if the chemokines expressed are functioning and if so, move on to in-vivo mouse model testing.

14.

Hannah L. Hartman (Biochemistry and Molecular Biology'18)[1], Dr. Amanda Stewart[2] and Dr. Rafael Quirino[2].
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[2]Department of Chemistry, Georgia Southern University, Statesboro, GA, 30458

Incorporation of Biomolecules in the Development of Innovative Materials: Novel Bone and Tissue Replacement Therapies

As the population of the United States continues to age, the need for advanced medical treatment for the elderly will continue to rise. For some of these patients, treatment options will include bone or joint replacement, especially for patients who experience a decrease in mobility as a result of age or injury. Currently, the devices used in bone and joint replacement operations are composed primarily of metal and petroleum derived

components, meaning that these devices are created from nonrenewable resources that are not inherently biocompatible. There exists a need to produce a material that offers the same opportunity to increase quality of life for patients while utilizing natural, more sustainable and biocompatible materials. This study aims to examine the mechanical properties and biocompatibility of a new material synthesized from Tung oil and collagen, both of which were selected based on their biological and structural properties, to determine the material's potential for use in bone and tissue replacement. Tung oil, which is readily available from the seed of the Tung tree, is a triglyceride in which each of the three fatty acid chains possess a 83-95% chance of being a-eleostearic acid. Structurally, a-eleostearic acid is a conjugated triene, polyunsaturated fatty acid. This structure allows Tung oil to polymerize rapidly and allows Tung oil to be successfully made into a thermosetting composite. Collagen, an essential component of connective tissue, is one of the most abundant types of proteins. Collagen is a structurally sturdy yet flexible fibrous protein which exhibits a triple helical structure established by the Gly-X-Y sequence constraint. The triple helix is composed of three supercoiled polyproline I-like helices. Previously, this research group endeavored to determine whether incorporation of collagen into Tung oil composites enhances the structural rigidity of the composite. Ultimately, it was hoped that Tung oil and collagen composites would demonstrate the properties necessary to be a candidate as a biocompatible material to be used in tissue replacement. After purification of collagen and incorporation of collagen into Tung oil composites, circular dichroism (CD) indicated an unexpected structure of purified collagen, specifically a random-coil structure as opposed to the anticipated triple helix. It became known that the presence of excess ions present in solution affected the collagen structure. In an effort to understand whether or not the composite strength is affected by the collagen conformation change caused by the presence of ions, collagen will be purified from beef tendons and then desalted to obtain the collagen triple helix. Once the proper triple helical structure is observed using CD, the desalted collagen will be incorporated into Tung oil composites at varying percentages. Composite sample properties will then be tested and compared to the properties of the composites prepared previously with ion bound collagen.

15.

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Characterization of microbial diversity in freshwater lakes of southwest Greenland

Increasing surface water temperatures have been observed in many large lakes around the world, with this change attributed to increasing air temperatures as a result of global climate change. These changes have been particularly rapid in the Arctic, and have potential impacts on species ranges, population dynamics, and food web interactions. In Arctic lakes, the breakdown of complex molecules into simple biologically available forms by microbial communities is a key process controlling lake nutrient dynamics. However, despite their potential importance in controlling how lake food webs are modified by climate-mediated changes in thermal habitat, relatively little is known about how microbial communities are altered by changes in lake habitat in this region. Here, we seek to assess microbial community taxonomic and functional diversity in Arctic lakes to understand how microbial communities in Arctic freshwater lake ecosystems may be both impacted by and

effectors of climate change. To begin to address these questions, we have surveyed microbial communities in several lakes across the Kangerlussuaq area of Greenland. Our data suggest that lakes with physical and chemical differences display distinct bacterial community structure. Future work will address functional differences in bacterial communities.