2013

Women in Science 2013

Clark Science Center's Summer Research Fellows Program

SMITH COLLEGE

2013 Women in Science

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INTRODUCTION

Research is a hallmark of scientific education at Smith College. Introductory courses get students thinking like researchers. Labs and special studies give students opportunities to build research skills. Sustained projects give young scientists room to study subjects in detail and challenge them to acquire the discipline and practices to sustain independent research. Honors theses and other capstone work create space for grappling with complexity and taking responsibility for reaching noteworthy outcomes. Projects of summer research, like those detailed in this book, reflect the substance of science done at Smith College and the multiple ways that our students make science their academic home.

"Women in Science" summarizes research done by Smith College's Summer Research Fellowship (SURF) Program participants. Ever since its 1967 start, SURF has been a cornerstone of Smith's science education. In 2013, 167 students participated in SURF, supervised by 57 faculty mentor-advisors drawn from the Clark Science Center's fourteen science, mathematics, and engineering departments and programs, and associated centers and units. At summer's end, SURF participants were asked to summarize their research experiences for this publication.

We have many reasons to be proud of our 2013 SURF researchers.

- They worked on some of the biggest research challenges of our times, including eradicating human disease, rethinking life sciences through a genetic lens, understanding climate change, and harnessing renewable energy.
- SURF research took place in Smith labs as well as out in the wider world: including, locally (study of Northampton's water source and local forests), nationally (projects on the Atlantic, Pacific, and Gulf coasts with NOAA scientists), and internationally (examination of coral reefs in Belize and geological formations in the Bahamas).
- Technical know-how, quantitative literacy, and presentation skills grew as students used state-of-the–art instrumentation, interpreted their data with specialized software assistance, readied results for presentation to others, and explained the "so what" of their research in lab meetings, posters, and conference presentations.
- SURF students became part of a research team: for all, learning how to work with mentors and peers and, for some, learning how to take a student research leadership role.

We are excited about what SURF participants say they learned from SURF.

- *"Summer research is where I was able to use … knowledge that I obtained in biology classes …. I now don't see what I learned during the semester as abstract ideas, which is great!"*
  SURF research introduces students to laboratory and other types of disciplinary research. Scientific research requires active problem-solving, patience, and persistence.
- "*Summer research allows you to completely immerse yourself in your project.*"
  Each student commits substantial time to SURF research: typically, 30-40 hours per week for 8-10 weeks of the summer. Often, students make a distinct contribution to a faculty member's on-going large research project.
- *"The summer research allows the student to explore and take charge of their learning." "I work much more independently during summer research."*
  In many cases, SURF research contributes to the particular student's honors thesis or continues a special studies project.

Thank you, Smith College students, faculty, staff, friends, and benefactors. It truly takes a diverse and dedicated community to sustain a program like SURF.

NOTE: Smith College SURF participants were asked to take the SURE III survey in August 2013. The student quotes on the previous page are anonymous comments drawn from the survey summary provided to Smith College by the survey administrators at Grinnell College. See the following for published reports of the SURE survey data across a large number of colleges and universities: Lopatto, D. (2004). Survey of Undergraduate Research Experiences (SURE): First Findings. *Cell Biology Education*, 3, 270-277 and Lopatto, D. (2007). Undergraduate research experiences support science career decisions and active learning. *CBE – Life Sciences Education*, 6, 297-306.

We are pleased to recognize the generous contributors who provided funding for the 2013 Summer Research Fellowship (SURF) Program and extend our deep appreciation to them.

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Smith College offices and units:

Botanic Garden & Friends
Center for the Environment, Ecological Design & Sustainability (CEEDS)
Committee on Faculty Compensation and Development (CFCD)
Provost's Office

Some Undergraduates were able to participate in summer research internships away from campus with support from the PRAXIS Internship Program.

We wish to recognize and express gratitude to the faculty members and staff who provided supervision, guidance, encouragement and support to SURF participants in the lab, doing field research, on-campus, and away from campus. SURF would not be possible without your devoted and generous contributions.

For further information, please contact:

Margaret Lamb, Ph.D, Administrative
Director, Clark Science Center

Table of Research Topics (Classified by Supervisor's Field of Study)

Astronomy

Biochemistry

Biological Sciences

Biological Sciences (*continued*)

Biological Sciences (*continued*)

Biological Sciences (*continued*)

Chemistry

Chemistry *(continued)*

Computer Science

Engineering

Environmental Science and Policy

Environmental Science and Policy *(continued)*

Geological Sciences

Mathematics and Statistics

Mathematics and Statistics *(continued)*

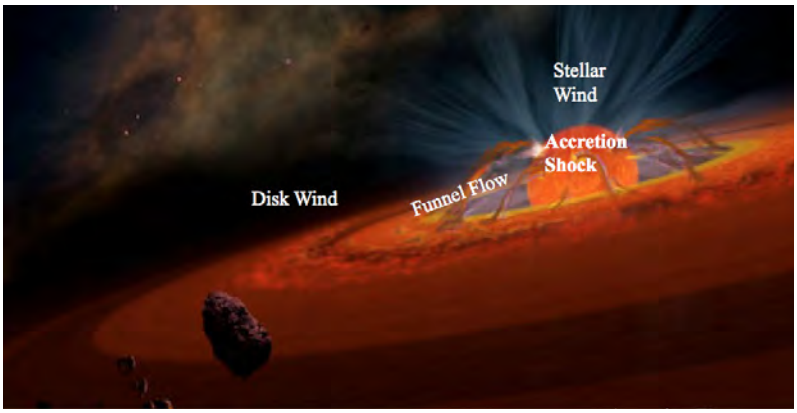
Neuroscience

Neuroscience *(continued)*

Psychology

Discovering Accretion Properties in Young Stars

Jenny Podel/2014



T Tauri stars are only one million years old and are still surrounded by accretion disks that are in the early stage of planet formation. My goal for the summer was to look at emission lines that formed in the inner disk, where the disk and star meet, to find the environmental conditions in that region. We would then like to see how this affects the angular momentum of the star and future planet formation.

I used a collection of high-resolution spectra of young stars simultaneously taken with the Keck Telescopes using the optical HIRES and the infrared NIRSPEC spectrographs. I analyzed emission lines from the Hydrogen Paschen series, He I and the Calcium II infrared triplet.

My approach was to decompose complex line profiles into Gaussian components. In general the Gaussian components could be broad (70 – 400 km/s) or narrow (10-70 km/s). These fits allow us to identify the multiple kinematic components of the star-disk region. Earlier work[1] suggests that the narrow emission comes from the accretion shock region, where material falling from the disk hits the star, and the broad emission comes from a combination of regions with high velocities, such as the funnel flow, disk wind, stellar wind or Keplerian disk.

The data I am using is 1.5 X better spectral resolution than used before, allowing better definition of kinematic components. I have found unexpected kinematic Gaussian components, and some important similarities and differences between the three lines. The next step involves comparing line ratios to physical models. I have done this for the Calcium II emission and find that the narrow component is formed in a region that is more optically thick than the broad component, consistent with formation in accretion shock. All the stars have broad components in two to three of the emission lines; these broad components vary in line width by a factor of three. This suggests there is a fundamental difference in the regions where this emission is formed.

(Supported by the Schultz Foundation)

Advisor: Suzan Edwards, Astronomy

Resources:

HELIUM EMISSION FROM CLASSICAL T TAURI STARS: DUAL ORIGIN IN MAGNETOSPHERIC INFALL AND HOT WIND

GEORGINA BERISTAIN, SUZAN EDWARDS, AND JOHN KWAN 2001, Astrophysical Journal

Exercise and Estrogen Receptors: A Look at the Biceps Brachii of the Common House Mouse

Justine Gelzinis/2014

Everyday our muscles are exercised even if only walking from point A to point B. A group of three important proteins that can be found in skeletal muscle are the estrogen receptors (ERs), which include ERα, ERβ, and G-protein coupled estrogen receptor (GPER). Estrogen receptors are part of the nuclear receptor family of transcription factors that bind the steroid estrogen.[1] In the classical (genomic) ER pathway estrogen is bound to an estrogen receptor and the complex activates transcription when bound to DNA.[1] However, estrogen receptors can also be activated through signal transduction pathways (nongenomic), such as the MAPK signaling pathway, rather than just the classical pathway.[1] My research focuses on the three ERs in the biceps brachii of the common house mouse, *mus musculus,* to determine how the expression of the three receptors is affected by exercise and whether any gender dimorphism is evident, as it is known circulating estrogen levels are higher in females.

Murine biceps were removed under protocols approved by the Smith College IACUC. A high ionic strength extraction buffer was used to solubilize the muscle samples for total protein estimation (modified Lowry assay). Separation by molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was followed by immunoblot analysis on PVDF membranes.

Quantitative immunoblot analysis allows for the detection of a particular protein by using an antibody specific for that protein. Three different antibodies were used for the immunoblot analysis of ERα, ERβ and GPER (Santa Cruz Biotech). MCF7 cell lysate was used as a standard (Santa Cruz Biotech) as it is known to express all three ERs. So far, I have been able to standardize the antibodies and find the optimal dilution for each, as well as the optimal protein concentration to use for the MCF7 standard for each antibody and samples.

Preliminary results show GPER is present in both male and female adult murine skeletal tissue and it seems to be slightly higher in females compared to males.

My research will continue over the next academic year. The next step will be to observe the differences of each estrogen receptor between 8-week-old male and female mice at five different time points after a bout of downhill running. An ELISA assay will be used to determine how much steroid (estrogen, progesterone and testosterone) is actually present in the blood at each interval of exercise.

(Supported by the Blakeslee Fund in the Biological Sciences)

Advisor: Stylianos P Scordilis, Biological Sciences

[1]Bjornstrom, L. and M. Sjoberg. 2005. Mechanisms of Estrogen Receptor Signaling: Convergence of Genomic and Nongenomic actions on target genes. Molecular Endocrinology, **19(4):** 833-842.

Carbohydrate Metabolism Transcriptomics of C2C12 Cells during Myogenesis

Anagha Inguva/2015

The goal of this study was to determine changes in the transcriptome of the enzymes involved in carbohydrate metabolism during the three different stages (day 0 (myoblasts), 4 (early myotubes), and 9 (late myotubes)) of myogenic development of murine C2C12 cells. A carbohydrate metabolism qRT-PCR microarray was used to examine the mRNA levels of 84 different enzymes involved in carbohydrate metabolism.

qRT-PCR arrays were used to determine mRNA levels of 84 different enzymes involved in the regulation of carbohydrate metabolism (n=4 per stage). RNA was isolated from cells during each of the three stages of differentiation and assessed for purity. From the pure RNA cDNA was synthesized and used for the qRT-PCR arrays. Results were analyzed using Microsoft Excel, JMP 10 (statistical analysis), DAVID and MeV (graphical analyses).

The RT-PCR arrays showed that 64 genes showed changes in mRNA levels that were statistically significant (ANOVA, $p<0.05$) from the control group (day 0). Of these 64 genes, 39 genes increased or decreased by two fold or higher and were considered to be biologically significant changes (see figure 1 for a heat map presentation of these data). Fifteen genes were involved in the TCA cycle, 9 genes were involved in glycolysis, 8 genes were involved in glycogen metabolism, 4 genes were involved in the pentose phosphate pathway and 3 genes were involved in gluconeogenesis. Isoenzyme mRNA level shifts were also present during the three stages of myogenesis.

These data indicate that the TCA cycle, glycolysis and glycogen metabolism pathways were significantly altered during myogenesis. Further research includes determining isoenzyme shifts of mRNA levels using RT-PCR arrays. Protein expression of genes that had significant differences in mRNA levels during myogenesis will be determined using proteomic techniques. These data along with further research show the glucose metabolism profile of C2C12 cells during myogenesis.

(Supported by the Howard Hughes Medical Institute)

Advisor: Stylianos P. Scordilis, Biological Sciences

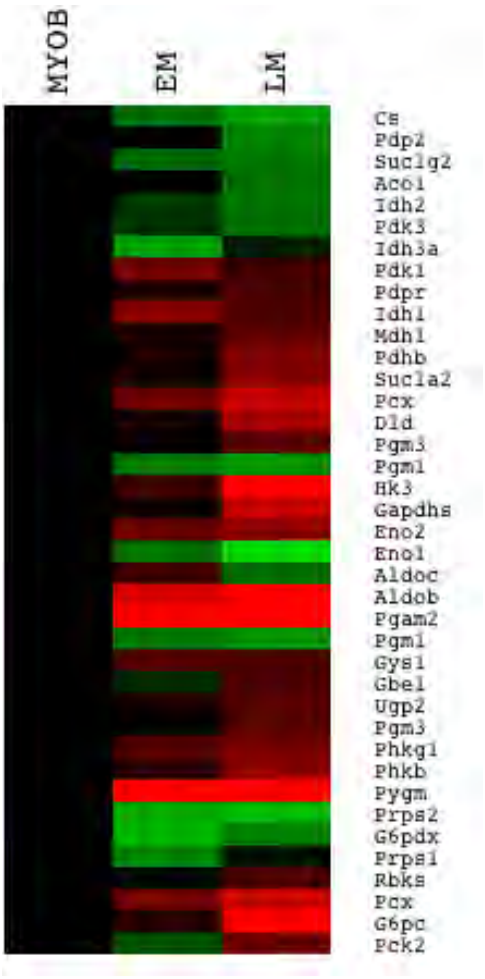


Figure 1: Heat map of genes that changed by two fold or greater during myogenesis. Myoblasts (MYOB) were used as the control group to compare to the early myotube (EM) and late myotube (LM) stages. Green indicates down-regulation while red indicates up-regulation of mRNA expression. Gene name abbreviations are given in the rightmost column.

Mitogen-activated Protein Kinase Signaling during C2C12 Myogenesis

Nhu Nguyen/2014J

C2C12 is an immortal murine skeletal muscle cell line derived from the thigh of an adult mouse (Yaffe and Saxel, 1977). C2C12 differentiates rapidly *in vitro* and recapitulates myogenesis that demonstrates three cell stages. Myoblasts are dividing embryonic progenitor cells that can fuse, leading to the formation of non-dividing multinucleated cells, early myotubes; then as late myotubes, they initiate myofibrillogenesis (Yoshida et al., 1998). The mitogen-activated protein kinases (MAPKs) are a system of enzymes that regulate many intracellular responses of eukaryotic cells (Kyriakis and Avruch, 2012). Upon receipt of an extracellular signal through a transmembrane receptor, a MAPK signaling cascade is activated, which is capable of regulating cell function, gene transcription, cell cycle control, differentiation, cell survival, or apoptosis. The three major MAPKs are: extracellular signal-regulated kinase (ERK or SAPK), c-Jun NH2-terminal kinase (JNK), and p38. Each of these enzymes is activated by specific phosphorylations. The process of cell fusion during myogenesis is considered to be a cellular stressor and the cells may adapt to the stress by regulating the activity of the MAPKs. This project investigates whether the expression, using antibodies specific to each enzyme but not to their phosphorylation sites, and activation of ERK1/2, JNK1/2, and p38, determined by antibodies specific to the phosphorylation sites for each enzyme, vary significantly during C2C12 myogenesis. Activation is inferred by the ratio of the blot intensity of the phospho-antibody band to the total enzyme band intensity.

C2C12 myoblasts were grown in 10% fetal bovine serum/Dulbecco's Modified Eagle's Medium (DMEM) and harvested at 90% confluency "Day 0". The medium was replaced with 5% horse serum/DMEM and induced differentiation to early and late myotubes on "Day 4" and "Day 9", respectively. The protein concentration was estimated by the Lowry assay. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and quantitative immunoblotting were used to compare the expression and activation of each MAPK in the three stages of myogenesis.

The results show that ERK1 and ERK2 activation significantly increases in late myotubes.

The activation of p38 only increases in early myotubes and JNK1 activation significantly decreases in early and late myotubes. There is no change in the activation of JNK2 during myogenesis. Further analysis of these results will be carried out during the fall of 2014 as my Honors Thesis.

(Supported by the Howard Hughes Medical Institute)

Advisor: Stylianos P. Scordilis, Biological Sciences

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Yoshida, N., Yoshida, S., Koishi, K., Masuda, K., and Nabeshima, Y. (1998). Cell heterogeneity upon myogenic differentiation: down-regulation of MyoD and Myf-5 generates 'reserve cells'. *J Cell Sci, 111*(Pt 6), 769-779.

Regulation of Desmin during Myogenesis

Aunaly Palmer/2014J

Desmin, a type III intermediate filament protein is part of the eukaryotic cytoskeleton. In skeletal muscle, desmin filaments usually associate with the Z-disk and help maintain the structure of the muscle cell by forming a framework around the Z-disk and attaching it to the cytoskeleton and other organelles[1]. Filament formation is regulated by the post-translational modifications (PTM) of ADP-ribosylation and/or phosphorylation. The addition of an ADP-ribose group from NAD^+ to Arg^{48} or Arg^{68} in desmin disassembles the filaments and disabling repolymerization until the ADP-ribose groups are removed[2]. Desmin phosphorylation occurs at Ser^{12}, Ser^{60}, or Thr^{17}; this halts the process of subunit polymerization[3]. Myogenesis, the process by which proliferating embryonic muscle cells exit the cell cycle and fuse to form myotubes is studied *in vitro* at three stages: myoblasts, early myotubes and late myotubes. The various post-translationally modified species and relative abundance of desmin at these stages of myogenesis was determined.

Myogenesis was studied in the murine cell line, C2C12. Using mammalian cell culture techniques cells were grown to all three stages of myogenesis. Cells were harvested, isolating the total protein of the cells. Proteomic techniques were then utilized; at each stage seven two-dimensional gels were run, five of which were stained with Coomassie Brilliant Blue R-250 for protein and PTM identification and two were transferred to a PVDF membrane for immunoblotting. One blot was developed using the anti-desmin DE-U-10 antibody clone as the primary antibody and the other was developed using the B-7 antibody clone. The developed blots were imaged using QuantityOne (BioRad) and the resulting images of the stained gels and the immunoblots were overlaid (see figure). The spots found in common were manually cut to from the gels. A total of 172 spots were excised and high pressure liquid chromatography-coupled mass spectrometry (LC-MS) is being used to identify the amino acid sequence and the position and nature of post-translational modifications of the proteins in the excised spots. These results demonstrated a clear disparity between the specificities of the two desmin antibodies; LC/MS sequencing should resolve this issue and allow for the precise identification of the desmin species and the antibodies' specificity.

(Supported by the Howard Hughes Medical Institute)

Advisor: Stylianos P. Scordilis, Biological Sciences

[1]Pauline, D., & Li, Z. (2004). Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. *Experimental Cell Research*. **301:** 1-7.

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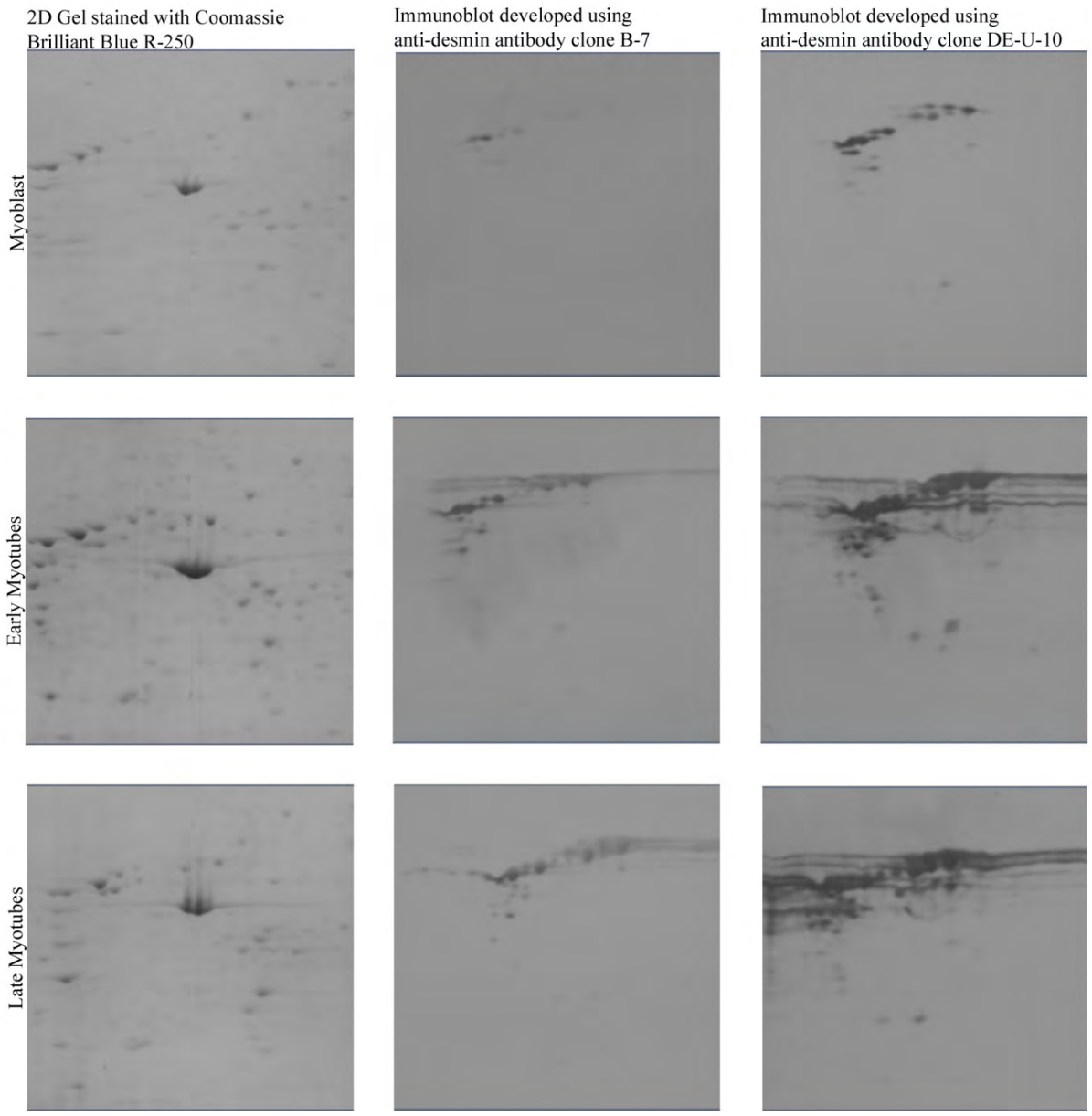

2D Gel stained with Coomassie Brilliant Blue R-250
Immunoblot developed using anti-desmin antibody clone B-7
Immunoblot developed using anti-desmin antibody clone DE-U-10
Myoblast
Early Myotubes
Late Myotubes

Slit-Roundabout Signaling in Commissure Formation during Zebrafish Forebrain Development

Abigail Antoine/2015 and Nina Wren/2016

The Barresi lab studies neural development. During embryonic development, neurons extend their axons in order to make necessary connections with other neurons. This project focuses on the formation of commissures, bundles of axons that cross the midline. Here, axons are guided in part by Slits, extracellular signaling molecules, and their respective Roundabout receptors. It has been demonstrated that Slit1a promotes interaction between axons and glia via its attractive role while Slit2 and Slit3 are known to act as chemorepellents. Using zebrafish as our model, the goal is to determine how different Slit guidance cues and Roundabout receptors promote commissure formation in the forebrain, specifically the Post-optic commissure. We plan to continue our research using a combination of gain and loss of function approaches and specifically determine which Robo receptor (Robo1, 2, 3, or 4) mediates which Slit function.

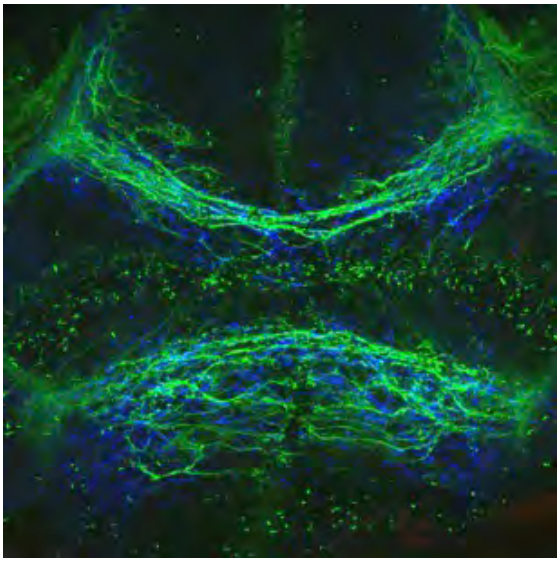
Our project uses various genotypes in order to perform desired gain and loss of function experiments. For example, a You-Too mutant is characterized by a *gli2* mutation in the Sonic Hedgehog pathway that results in the expansion of Slit2 and Slit3 and reduction of Slit1a cues. As mutant fish are not viable into adulthood, we genotype our adult fish to confirm that they are heterozygous for the You-Too allele. Consequently, 25% of the progeny will be mutant. This summer we genotyped many of our lines for Astray (loss of Robo2 function) and Twitch-Twice (loss of Robo3 function) genes.

This summer we also conducted two experiments with the goal of further characterizing Slit1a and determining if Robo1 mediates the Slit1a signal. We injected half of the embryos in each group with a Robo1 morpholino (knocking down the Robo1 receptor). Then we fixed embryos from each group at 25hpf, 27.5hpf and 30hpf (hours post-fertilization). The first Post-optic commissure axon crosses the midline at 24hpf while we see a fully formed Post-optic commissure at 30hpf. Therefore, different stages in commissure development can be seen between our test groups of 25hpf, 27.5hpf and 30hpf.

We were able to eliminate the expression of Robo1 (a hypothesized Slit1a receptor) in a transgenic line of fish that globally expresses the Slit1a signal. This allows us to inspect whether or not the Slit1a signal was received through that specific receptor. Using a laser scanning confocal microscope we obtained high resolution images of the Anterior and Post-optic commissures in our experimental and control groups. The following image illustrates

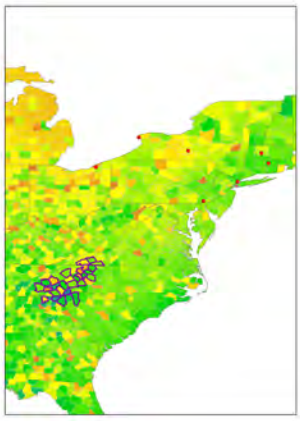
(Supported by the National Science Foundation, Antoine, and the Howard Hughes Medical Institute, Wren)

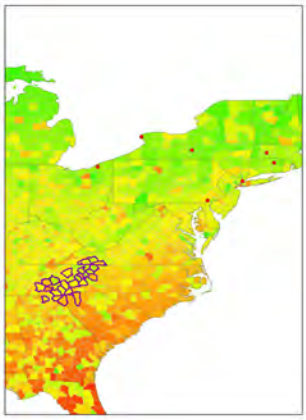
Advisor: Michael Barresi, Biological Sciences



The Impact of Climate Change on Plant Ranges

Emily Barbour/2014





http://www.ncwildflower.org/index.php/plants/details/diphylleia-cymosa/

This summer I helped continue the long-term research studying the ability of *Diphylleia cymosa* to grown outside its native range. *D. cymosa*, commonly known as the umbrella leaf plant, is an endemic species native to higher elevations in the Appalachian Mountains in North Carolina and Southern Virginia[1]. This species may be threated by warming brought on by climate change because it relies on the cooler temperatures of the higher elevation and their only means of escape is to transition to higher elevations[1]. However, because they are native to the higher elevations of these mountain ranges they only have so much farther to transition and their seed dispersal mechanism is not sufficient to start new colonies in suitable climates[2].

For this experiment seeds were collected from plants found in the native range and planted in 5 test sites outside of their native range. On a weeklong road trip in mid-June I traveled to the Virginia, Maryland, Pennsylvania and New York sites and helped to collect data by tracing an outline of the leaf of each plant along with any herbivory damage onto a notecard. Once we brought these notecards back to the lab I cut the trace of the leaf out and scanned it to find the area. After I found the area I cut out areas of the leaves damaged by herbivory and scanned them again, in order to calculate percent herbivory damage as well as the growth rate.

In addition to my work on this project I assisted in several other ongoing projects in the Bellemare lab, mostly concerning the effect of the decline in hemlock trees on their ecosystem at MacLeish field station. I also took some of my time this summer to prepare for my thesis this coming year concerning germination rates of *D. cymosa*. I have begun research and drafted the proposal, as well as created a map using ARCMap showing the native range of *D. cymosa*. These maps visually represent the locations where it has been known to survive in horticulture, as well as layers depicted the average high and low. temperature and precipitation by county to compare the native range to the areas of growth in the north.

(Supported by B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Jesse Bellemare, Biological Sciences

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Neurogenesis: The Effects of Radial Glia Cell Ablation on Neural Cell Populations in a Zebrafish Model

Jessica Barragan/2015 and Victoria von Saucken/2016

Correct development of the central nervous system is based upon an intricate interplay of astroglial cells, axons, neuronal migration, and cell signaling. Remarkably, the complexity of basic mechanisms concerning the formation of the embryonic nervous system remains unknown. In the latter decades, it has become clear that glia cells possess key properties in synapse formation and maintenance, communication with neurons and other glial cells, and can serve as neural stem cells with gliogenic, neurogenic, and regenerative abilities (Götz & Huttner, 2005; Kriegstein & Alvarez-Buylla, 2009). The versatile nature of these cells constitutes a major area of research that lends itself to examining the implications that neuron-glial interactions have on the formation of the nervous system. To directly test how radial glia are required for neurogenesis and axon guidance during embryonic development, we have employed a prodrug dependent cell ablation model to specifically remove radial glia from the nervous system in zebrafish.

Prodrug dependent cell ablation is a technique that induces cell apoptosis in a controlled, discrete manner as it targets specific cell populations (Pisharath & Parsons, 2009). The specificity of cell ablation comes from using the E.Coli nitroreductase B (*nfsb*) gene encoding the nitroreductase enzyme (NTR), which can convert a prodrug into toxic cellular metabolites to mediate cell ablation in cells specifically expressing the *nfsb* gene while leaving neighboring cells unharmed (Curado, Stainier, & Anderson, 2008). In order to visualize the cell ablation, the *nfsb* gene is fused to a fluorescent protein (Pisharath et al., 2007). In order to drive cell specific expression of NTR within radial glial cells, collaborators have placed the *nfsb* gene under the control of the zebrafish *gfap* promoter, which is expressed only in radial glia. The zebrafish model system was adapted for our research due to the optical transparency and quick external development which lends to live microscopy and the ability to easily test the effectiveness of our cell ablation techniques.

With the use of our transgenic *Tg(gfap:nfsb-mCherry)* line, we tested the specificity of our chemically induced cell ablation model by exposing zebrafish embryos to the prodrug Metronidazole (MET) at 8 hours post fertilization (hpf), prior to expression of *gfap*. In the presence of NTR, MET will be converted from an antibiotic into a cytotoxin in all astroglial cells. To ensure that MET on its own is harmless, *Tg(gfap:nfsb-mCherry)* embryos were also treated with the vehicle control dimethyl sulfoxide (DMSO) and assayed for cell death. Lastly, to ensure that the cell ablation visualized was due to presence of the *nfsb* gene, transgenic embryos not carrying the *nfsb* gene, *Tg(gfap:gfp)*, were also treated with DMSO and MET. Prior studies in our lab indicated that cell death began as early as 36hpf with the most death seen around 54hpf and 60hpf. However, in order to investigate at what hour(s) cell death is ubiquitous in the spinal cord, embryos were fixed at 24hpf up to 60hpf at six-hour intervals. Embryos were also labeled for anti-activated caspase-3 to visualize cell death.

We observed an increase in cell death in older NFSB MET samples (42-60hpf) as compared to NFSB DMSO, GFP MET, and GFP DMSO sample groups. The cell death present in the NFSB MET samples (42-60hpf) was widely distributed throughout the spinal cord, whereas in the NFSB DMSO, GFP MET, and GFP DMSO groups exhibited little to no cell death. This study will be continuing throughout the 2013-2014 school year to determine the specific cell populations affected by the removal of radial glia.

(Supported by Science Foundation, Barragan, and the Howard Hughes Medical Institute, von Saucken)

Advisor: Michael Barresi, Biological Sciences

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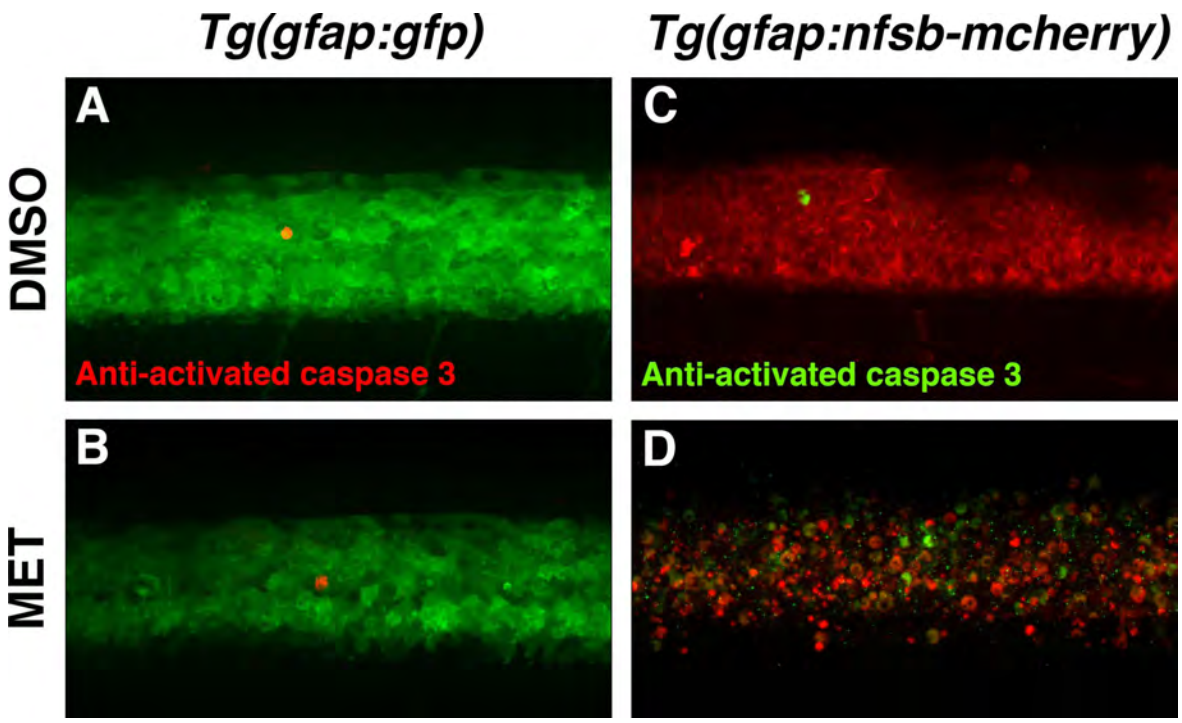

Figure 1: Metronidazole induced cell ablation of radial glia within the spinal cord. (A-B) *Tg(gfap:gfp)* embryos treated with either vehicle control (DMSO) (A) or Metronidazole (MET) (B) showed little to no cell death as labeled by anti-activated caspase-3 (red) at 42hpf. (C-D) *Tg(gfap:nfsb-mcherry)* embryos treated with DMSO (C) or MET (D) and labeled with anti-activated caspase-3 (green). An increase in cell death was seen following MET treatment (D, green labeling) as compared to its control at 42hpf.

The Effect of Temperature on Small Regulatory RNA in the Commensal K-12 Escherichia coli

Natalie Belkov/2016

E. coli (Escherichia coli) is a gram-negative, facultative anaerobic bacterium that can cause gastrointestinal and urinary tract infections in warm-blooded organisms.[1] Like any infectious bacteria, *E. coli* has adapted to possess different capabilities allowing it to navigate both intracellular and extracellular environments as well as the process of traveling between the two. Entering a host cell presents a multitude of potential stressors for an *E. coli* bacterium such as osmotic pressure, fluctuation in iron levels, nutrient deprivation and varying temperatures. Sigma factors, specific proteins that are involved in bacterial transcription, can be activated in response to different environmental circumstances. RpoS (RNA polymerase,sigma S) is a specific sigma factor protein responsible for adaptations to stress in *E. coli.* RpoS expression is determined at the translational level when small regulatory non-coding RNA (sRNA) sense environmental stresses and subsequently increase RpoS translation to allow the cell to adjust to those specific stresses.[2] My research is focused mainly on the roles of different sRNAs in an environment mimicking the temperature stress an *E. coli* cell faces entering and exiting a host cell.

In order to observe the effects that different sRNA have on cell activity in a stress environment, I first needed to expose cells to that environment and harvest samples to further analyze. The specific stressor we chose to observe is temperature. We mimicked the entrance into a warm-blooded host by shifting bacteria grown at 23˚C at 37˚C. Both the wild type and rpo*S* mutant MC4100 strains were used. At specific time points (t= 0.5, 1, 2, 3, 4, 6, 8, 10 hours), cells were centrifuged and RNA was subsequently extracted. Quantitative real-time polymerase chain reaction (qRT-PCR) testing on the isolated RNA determined the effect of temperature on sRNA expression.

I ran a qRT-PCR using forward and reverse primers for the sRNA RprA. This specific sRNA is required for the production of RpoS in response to general stress.[3] I predicted that the level of RprA expression would decrease when I shifted the wild type sample from 23˚C to 37˚C because the lower temperature presents a more stressful environment. Therefore, the cell would need to produce more RpoS in response to that stress. My data supported this hypothesis, presenting a two-fold decrease in expression of RprA at 37˚C as compared to cells grown at 23˚C. On the other hand, the mutant strain presented no significant change in expression which also aligns with my prediction since the mutant strain is void of RpoS. MicF, RybB, omRA and spot 42 sRNA were also tested. Each of these primers deals with osmotic stress and/or stress on the cell envelope[4567] therefore I predicted that there would be no significant change in expression. The data supported this supposition showing no sizable decrease or increase in expression of any of these four primers.

I hope to continue my work in Dr. White-Ziegler's lab delving into further research on sRNAs, understanding specific functions and observing how each reacts to the stresses of temperature changes. With a broader understanding of how specific sRNA respond to temperature and help bacterium to adapt, these can be used as targets for drug therapy or disinfection strategies. From a biological engineering standpoint, I could attempt to design membrane-embedded structures on host cells that would directly inhibit the bacterium's ability to survive the stress experienced when living outside of a host cell as well as traveling into one. In many cases, certain sRNA are triggered by more than one source of stress. In observing commonalities between different sRNA, I could achieve a greater understanding of the decision of the bacterium to either be motile or form a biofilm, both important processes for colonization, survival and virulence. I look forward to continuing my work in Dr. White-Ziegler's lab where I will eagerly explore more of these approaches.

(Supported by the Howard Hughes Medical Institute)

Advisor: Christine A. White-Ziegler, Biological Sciences

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Image: http://2012.igem.org/wiki/index.php?title=Team:OUC-China/Project/DesignMaking/Background&oldid=294863

Metagenomics

Louise Bodt/2014 and Hangyi Pan/2015

As the former group leader is graduating, the metagenomics group has been under transition. We decided to change the experimental subjects from horses to mice, in an attempt to study the microbiome of the GI tract in mice broad scale while minimizing individual differences. We were using the experimental subjects' stool sample to extract DNA of the microbiota inside subjects' GI tract. By analyzing the DNA sequences, the genus and species of the bacteria could be identified, thus the microbiome inside mice's GI tract would be revealed. During our SURF period, we tested various preservatives in order to find the best way to transfer samples while preserving the DNA. Additionally, we tested the primers that were designed for the amplification of a variable region of the 16S ribosomal subunit on the mouse DNA that we had extracted from various preservatives. This would indicate whether the DNA extraction was successful as well as the success of the primers on mice samples.

The stool samples that we tested in the past month were from the animal quarters. To mimic the actual process of transferring samples for a long distance, four different types of preservatives were prepared for preserving the samples: RNAlater, 95% ethanol, propylene glycol as well as DET. In addition to freezing (with no preservatives inside the conical tube), 3 sets of samples (each consists of 5 tubes with different preservative methods) were collected.

DNA extraction was performed after 1 day, 4 days and 7 days of sample collection to test out the effectiveness of each preservatives. PCR amplification were performed to test whether the DNA extracted were the target DNA that we were looking for. Gel electrophoresis was designed to test the presence of the 16S amplicon.

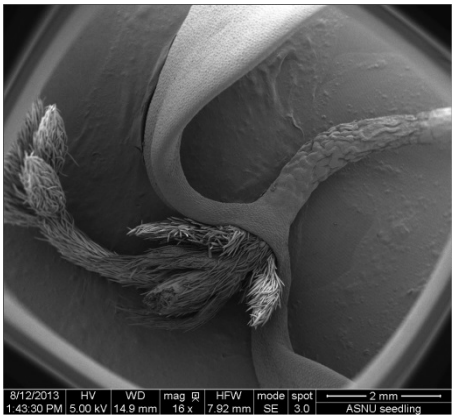
By the end of our SURF period, we were able to identify that the samples preserved in RNAlater yielded the most target DNA. In the coming fall semester, we are expecting to get more samples using RNAlater as a preservative from a facility with mice or gerbils to continue our experiment. We will also begin to start analyzing the metagenome in mice's GI tract before and after infection. This will be tested finally by taking the 16S amplicon and sequencing with the nextgen miseq sequencer.

(Supported by the Howard Hughes Medical Institute, Bodt and the Smith College Provost's Office, Pan)

Advisor: Steven A. Williams, Biological Sciences

Physiology of Invasive and Native Desert Annual Plants

Kyle Boyd/2015



Invasive species can alter ecosystem dynamics in many ways. This summer I investigated the relationship between the non-native invasive plant species, *Erodium cicutarium* (ERCI), and the native plant species, *Astragalus nuttallianus* (ASNU). *Erodium cicutarium* and ASNU are desert winter annual plants found in the Chihuahuan Desert ecosystem, located in southeastern Arizona. Long-term study plots have been established and monitored the changes in species abundance since 1988. A dramatic increase in the abundance of ERCI was observed after 1996. Most native species have since declined, except for ASNU which has instead increased in abundance since the irruption of ERCI. *Astragalus nuttallianus* and ERCI appear to have a beneficial relationship that is uncommon among native and non-native species. Since long-term shifts in the ecosystem could be due to germination changes we investigated the relationship between the non-native and native species at this stage of plant development.

Our lab investigated the relationship between the non-native ERCI and the native ASNU in a controlled environment by growing our seeds in the growth chambers at Ford Hall. This allowed us to mimic the winter desert environment while staying at Smith College. We placed seeds in different treatments and recorded information about their germination. This allowed us to compare the germination success of both species while in either inter- and intra-species competition environments.

In addition to comparing the timing and amount of germination we used the resources at the center of microscopy to look at the seeds and seedlings of both species under high magnification and high resolution. This allowed us to compare the morphology of both species, which may help to show us how the species are interacting.

Our initial results showed that both ASNU and ERCI appeared to do better with inter-species competition experiments. *Erodium cicutarium* appeared to germinate faster and at higher levels while surrounded by ASNU. On the other hand ASNU appeared to germinate faster while with ERCI. Future research in our lab will build upon these results and investigate this relationship further.

Our research supports the notion that there is a beneficial relationship between the native ASNU and non-native ERCI species. Both appear to germinate at higher rates when grown with the other species. This supports what was seen in the field, where they both increased in abundance almost simultaneously. Ultimately, advancing this research will help us understand changes in the native plant community and ecosystem dynamics.

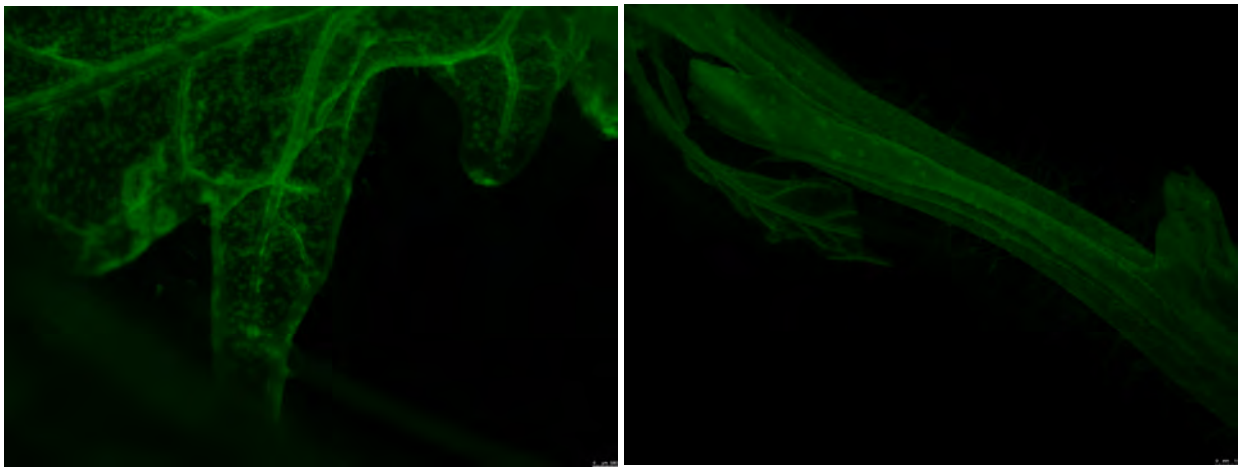
This work was a continuation of a special studies project that I did last semester. This research will be continued in the coming academic year by my fellow lab mates.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Danielle Ignace, Biological Sciences

Phyllotaxis: mPS-PI Staining on Plant Vasculature

Karen Chau/2016



Phyllotaxis is the arrangement of organs around a plant's stem. If the left and right handed spirals formed by the organ arrangement are counted, the numbers will often be adjacent members of the Fibonacci sequence. When two leaves are aligned such that they form a vertical line, the pair of leaves belong to an orthostichy. It is presumed that the vascular traces to leaves of an orthostichy are derived from a common vascular bundle, discrete columns of vascular tissue arranged within the stem. However, the patterns of vascular tissue connections to leaves are not well understood. In addition, the phyllotactic spirals may change because of plant development, resulting in a transition to different adjacent numbers in the Fibonacci sequence. In my research, I am using fluorescence microscopy to examine how the pre-existing vascular bundles accommodate changes in phyllotaxis. A distinctive feature of plant vascular tissue is cells with walls reinforced by lignin (xylem cells) and sieve cells with callose deposits in sieve end plates (phloem cells). Staining techniques exist to visualize both of these features, but observing them in three dimensions has not yet been successfully accomplished.

The study subjects used were *Linum perenne*, *Linum usitatissimum*, and *Lycopersicon* (pictured). These were chosen because reports in the literature indicated that all three species show phyllotaxis transitions over relatively short temporal and spatial intervals and all are suitable to growth in a chamber. Plants were grown in 16:8 L:D at 23:16°C. We tried several staining and clearing procedures, but the most successful used a modified Schiff reagent with propidium iodide (mPS-PI) as described in Truenit et al.[1] with the additional step after fixation of 10-30 minutes in 80% ethanol at 65-70°C. After staining, the plant samples were cleared using the Clear(T^2) protocol following Kuwajima et al.[2]

Samples were viewed with a Leica205A fluorescent microscope using the mCherry filter that excites in the range of 587-610 nm because the peak excitation wavelengths of the propidium iodide stained callose in phloem cells are around 536 nm with peak emissions around 617 nm. By conducting a z-stack through the depth of the stems, partially focused images of the vascular bundles were obtained, with the most vibrant signal coming from leaf vascular bundles leaving the stem. Branching of vascular bundles within the stem remains unclear.

Therefore, the mPS-PI staining is a promising staining technique, but two problems remain. The first is increasing the depth of tissue that can be imaged in three dimensions. The second is to clarify the vascular tissue imaging by removing background noise. One option to further improve the quality of the imaging is to differentiate the signal of the fluorescence coming from the vascular bundles from that in background tissues. During the academic year, I plan to explore confocal microscopy and alternate sectioning and imaging techniques to refine the results.

(Supported by the Schultz Foundation)

Advisors: Denise Lello and Christophe Golé, Mathematics and Statistics

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Deep Water Horizon Oil Spill Crude Oil Disrupts Specific Developmental Processes During Zebrafish Embryogenesis

Diane Chen/2014 and Michelle Deadwyler/2014

In April of 2010, the Deepwater Horizon Spill released 200 million gallons of crude oil into the Gulf of Mexico. Although the Deepwater Horizon Spill was capped, the hydrocarbons and dispersants released into the waters still have a large and negative impact on the ecosystem. The project we work on uses zebrafish as a model system to assess the precise effects of the known components in the oil on vertebrate development. This summer we focused specifically on the development of the inner ear and early populations of cranial neural crest cells that enter the transient structures known as the pharyngeal arches in zebrafish. Politicians attempting to strengthen the guidelines that govern offshore drilling could use our documentation of the severe phenotypic effects suffered by developing embryos.

In order to determine how the inner ear is affected, we used the Arl13B-GFP transgenic zebrafish line, which is a cilia marker used in identifying cilia in zebrafish. By using this line, we characterized inner ear defects using oil-treated wild-type embryos. About 50 % of the oil exposed fish showed differences in otolith morphology as compared to the control. However, we were unable to re-characterize the oil treated zebrafish using the Arl13B-GFP transgenic fish because the oil we have directly sampled at the time of the oil spill has been losing potency. Therefore, the subsequent trials have shown a dramatic decrease in the phenotypes we had characterized earlier. Exposure to light and oxygen and volatiles escaping from the container may be decreasing the potency of the crude oil.

While we worked on a new crude oil exposure protocol to protect the oil from losing potency, we exposed the zebrafish to the first four polyaromatic hydrocarbons (PAHs) the United States Environmental Protection Agency (EPA) deems as priority pollutants. We tested naphthalene (present in the Macondo crude oil), acenaphthylene, acenaphthene, and fluorene. Zebrafish exposed to the compounds show gross morphological defects similar to fish exposed to the Macondo Crude Oil[1]. Ancenapthene above 100µM concentration continued to kill 100 % of the zebrafish embryos within 8 hours after exposure. Our focus shifted toward defects in pharyngeal arch morphology. We used *fli*-GFP fish, a transgenic fish line with fluorescing endothelial cells in order to study the pharyngeal arch structure of oil-exposed fish embryos at 30 hours post fertilization (hpf). Pharyngeal arches are transient structures that allow cranial neural crest cells to migrate to their later permanent destinations to form the many craniofacial elements of the developing embryo. We saw that 30 % of the *fli* transgenic fish had either morphed pharyngeal arches and/or had only four of the five pharyngeal arches (Figure 1). The phenotypes we saw are similar to the results seen in the Barresi et al. 2010 paper[1]. Pharyngeal arch defects may contribute to the vascular and craniofacial defects we observed in oil-exposed fish.

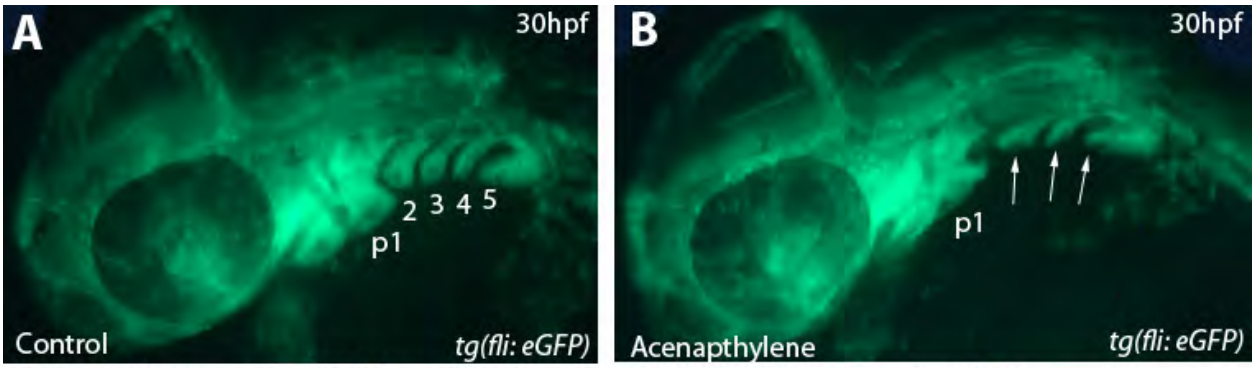

Figure 1 Pharyngeal arch defects incudced by acenaphthylene exposure (A-B)
(A-B) *Fli* driven expression of GFP transgenic zebrafish show cranial neural crest forming pharyngeal arches (1-5) at 30 hpf. (A) DMSO control zebrafish show five visible pharyngeal arches (1-5). (B) A pharygneal posterior arch is missing in acenapthylene exposure (B, arrows) as compared to controls.

We currently have results showing a pharyngeal arch defect and in the coming fall semester we will continue to run more trials using the next set of EPA PAHs. We will also be using molecular tools to identify which affected genes play a role in causing the pharyngeal arch defect. We will apply the microarray data we ran last year to further investigate the Arl-hydrocarbon pathway and its affect on early development of neural crest cells. We will be doing *in-situ* hybridizations to visualize the early population of neural crest cells in EPA PAH exposed embryos.

We had the opportunity to attend the 17th International Congress of Developmental Biology in Cancun, Mexico from June 16th to June 20th this year to present our SURF summer research.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences, Chen and the Howard Hughes Medical Institute, Deadwyler)

Advisor: Michael Barresi, Biological Sciences

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Therapeutic Effects of *Quisqualis Indica* on Lymphatic Filariasis

Naihan Chen/2014

Lymphatic Filariasis (LF) caused by the filarial nematodes (*Brugia malayi, Brigua timori,* and *Wuchereria bancrofti*) affects 120 million individuals worldwide and is the second leading cause of disability. Due to the resistance to current medications displayed by animal models and their limited range of biological activity, new drugs are imminently needed. Because *Quisqualis indica* (Figure 1 and 2), primarily found in Southern and Southeastern Asia, has been used as an anthelmintic by locals in a form of tea to expel parasitic worms in children for over 1700 years, it is likely that the plant can be used to kill filarial parasites. Therefore, during this summer, I tested the effect of *Q. indica* water extractions on the mortality of *Brugia pahangi* (adults and microfilariae) and examined its acute toxicity against the crustacean *Artemia salina* (i.e. Brine Shrimp Test) in order to determine its pharmaceutical potential as a drug candidate against LF.

Q. indica was extracted in water following the protocol used in traditional Chinese medicine, and extracts of different concentrations (2.642, 2.945, and 3.243 mg/mL) were applied to *B. pahangi in vitro* cultures. Mortality for microfilariae and adult worms was recorded at various time points after treatment, and a dose-response curve was generated. Levamisole was used as the positive control for its strong anthelmintic properties based on a similar study by Houzangbe-Adote et al. studying Trychostrongylus colubriformis L3s.[1]

Unlike L3s (an earlier life stage of *B. pahangi*) where 100% mortality was achieved by 40 hours after treatment at the highest concentration tested (3.243 mg/mL), complete mortality of adult worms at the same extract concentration was reached by 12 and 17 hours, respectively, for females and males, with first death observed within 4 hours post treatment for both sexes. These data show differences in drug action and differential impact of the extract on various life stages and genders of *B. pahangi*.

Acute toxicity of *Q. indica* extract was also examined using Brine Shrimp Test. Brine shrimps (*Artemia salina*) were tested in extract concentrations of 2, 3, 4, and 5 mg/mL in triplicates. The death rate was calculated at 24, 48, 72, 96, and 120 hours post-treatment, with acetic acid (42 μg/mL) used as the positive control.

Very few deaths (around 10%) were observed before 96 hours across all concentrations, and the majority of death occurred between 96 and 120 hours, with some (between 30% and 60%, dependent on the concentration) dying after 120 hours. The near absence of any death within 48 hours after treatment suggests the lack of acute toxicity of the *Q. indica* water extract against brine shrimp, indicating its potential use as a drug candidate.

My plan is to pursue this project as an honor thesis in which I will examine the efficacy and toxicity of this plant extract. The clinical effectiveness and therapeutic dosage will be analyzed using gerbils in collaboration with University of Georgia. This animal model testing will also help evaluate the plant toxicity *in vivo*. Cellular and molecular interactions between the extract and the organism will be later studied by analyzing differential protein expression after treatment. Although *Q. indica* seeds can be incorporated directly into diet, the molecular mechanism by which the compound exerts its anthelmintic effect will elucidate the activity pathway, which if altered may improve specificity and effectiveness of the active component(s) of *Q. indica*.

(Supported by the Howard Hughes Medical Institute)

Advisor: Steven A. Williams, Biological Sciences

Figure 1. *Quisqualis indica* is a vine primarily found in Southern and Southeastern Asia.[2]

Figure 2. Dried fruit of *Quisqualis indica* with anthelmintic effect.[3]

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Dengue Fever Primer Design

Faith Donaher/2015

Dengue fever is caused by four different serotypes of an RNA virus. Serotype specific diagnostic tools are useful to the treatment of dengue fever as reinfection with a different dengue serotype poses an increased risk of dengue shock syndrome and dengue hemorrhagic syndrome.[1] Serotype specific diagnostic tools can also be used to determine which serotypes are present in a given region. While several PCR tests already exist for the diagnosis of dengue, as the virus changes over time and a wider variety of sequences becomes available, it is important to continue to develop new tests.[2]

This project was started in the 2013-2014 academic year with the participation of Ridwana Fairuz, Luvana Chowdhury, Susan Haynes and Faith Donaher. All complete genome sequences available on Genbank were collected for each serotype. To minimize the difficulty of dealing with a large number of sequences, a Python program was used to remove sequences that had over 99% similarity to another sequence. Subsequently, the sequence sets for each serotype were examined using Mesquite and potential primer sites were located within each serotype.

Primers were designed with several features in mind. The final products were to be from 200-400bp long, with a difference of at least 25bp between the length of each serotype's product, so that product generated by each serotype could be distinguished using gel electrophoresis. Primer sites were specifically selected to contain a region extremely conserved within the serotype of around 20base pairs long that could potentially be used as a probe site.

Unfortunately, it was not possible to obtain samples of RNA from all four serotypes this summer. Dengue 2 RNA was available, so only the primer for this serotype was tested. PCR was conducted on the sample both with a set of fairly standard conditions and with an increased concentration of either MgCl2 or primer. As the PCR conducted with an increased concentration of MgCl2 was the most promising, it would be advisable to perform further PCR tests adjusting the concentration of MgCl2. Furthermore, once samples of Dengue 1, 3 and 4 become available those primer sets should be tested, with the final goal of determining PCR conditions under which all four primer sets function.

(Supported by the Howard Hughes Medical Institute)

Advisor: Steven A. Williams, Biological Sciences

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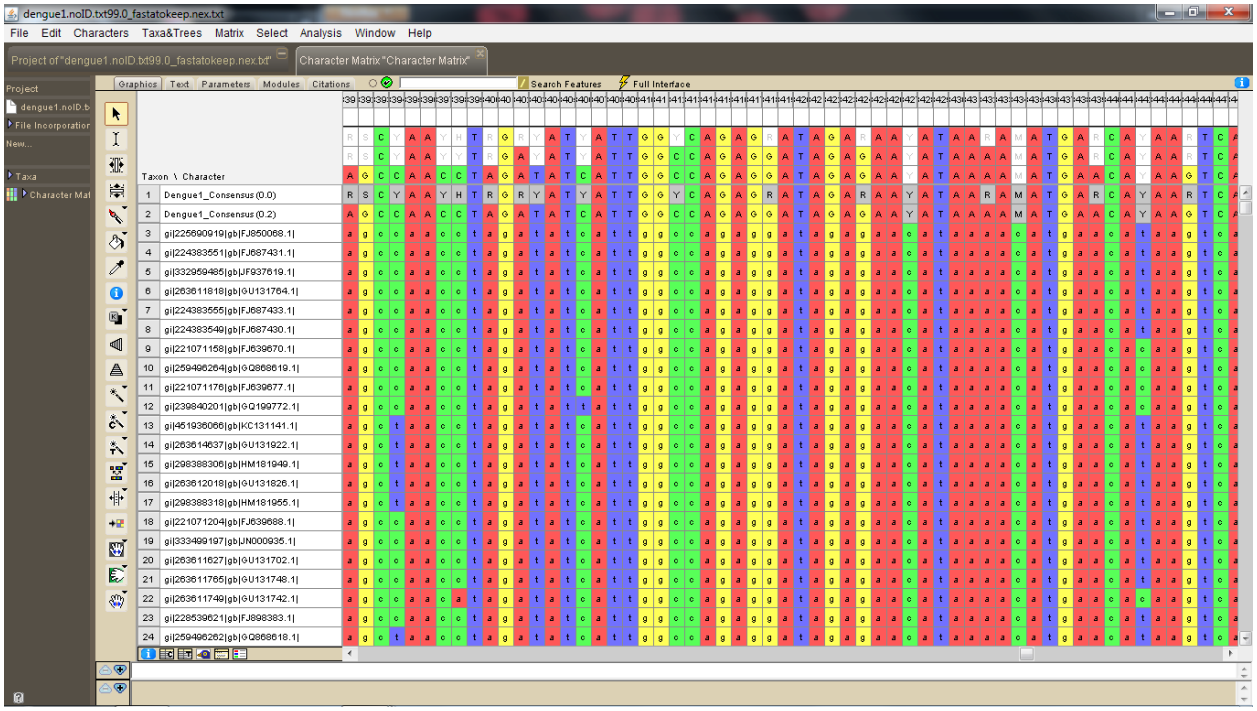
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Exploring Physiological Traits of Native and Non-native Plant Species

Allison Ferreira/2014

Studying long-term fluctuations in ecological communities is crucial to understanding the mechanisms of assembling communities and shifting community dynamics. In the Chihuahuan Desert ecosystem in southeastern Arizona the non-native species, *Erodium cicutarium*, irrupted simultaneously with a decline in the native winter annual plants. Several environmental factors could have conceivably contributed to these ecological variations including drought, temperature shifts, and competition factors. The lab sought to explore the dynamics of *E. cicutarium* growth that allowed its seeds to germinate and establish resources prior to native winter annual species in the field. Growth chambers were utilized to examine and compare germination rates and patterns of growth between the invasive *E. cicutarium* and the native annual *Astragalus nuttallianus*. Conditions essential to sustaining life in the Chihuahuan Desert were simulated including 11:13 photoperiods with 3 hour ramping as well as simultaneous daily temperature adjustments. Several experiments were replicated investigating *E. cicutarium* and *A. nuttallianus* in a competition environment. In our results, both *E. cicutarium* and *A. nuttallianus* appear to germinate at a higher rate when surrounded by their own species, while they struggle when surrounded by the other species. Additionally, manipulating water availability allowed us to determine optimal conditions for consistently high germination rates.This research will provide additional information and support to the long-term research of the invasion of *E. cicutarium* and the impact of environmental shifts in southeastern Arizona.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Danielle Ignace, Biological Sciences

Reproductive Patterns Between Talus and Non-talus Dwelling Pika

Abbey Fleming/2014

Pikas belong to a single genus, *Ochotona*, within the family Ochotonidae. Pikas live primarily in Asia, but some species exist in North America. Ochotonids can be split ecologically into talus and non-talus dwelling species.[1] Talus dwellers live within crevices between rocks in mountainous areas, while non-talus dwellers live in habitats such as meadows, usually at high elevations. In general, talus-dwelling species tend to be heavier than non-talus dwellers, though this difference is not significant.[2] Research has suggested that a pattern between body size and reproduction exists within the order Lagomorpha, of which Ochotonids belong.[3] Seeing as talus-dwelling pikas have typically larger body mass than non-talus dwelling Pika, I wanted to look at potential differences in reproductive patterns between the two ecological groups. In general, larger mammals give birth to smaller litters due to the added energy costs of reproduction.

As expected, talus-dwellers have smaller litter sizes than non-talus dwellers (Fig. 1A). Non-talus dwellers exhibit a positive relationship between litter size and female body mass, which was not as expected. As body mass increases, we would expect to see litter size decrease but this pattern is not seen in non-talus dwelling pikas. Talus-dwelling pikas show little variation in litter size within the group.

Also, talus-dwelling pikas have fewer litters per year than non-talus (Fig. 1B). Within the group, non-talus dwellers show no relationship between litters per year and body mass. Within talus-dwellers, a slight positive relationship exists between body mass and litters per year. Again, this positive relationship is not expected as generally larger mammals give birth to fewer litters per year.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Virginia Hayssen, Biological Sciences

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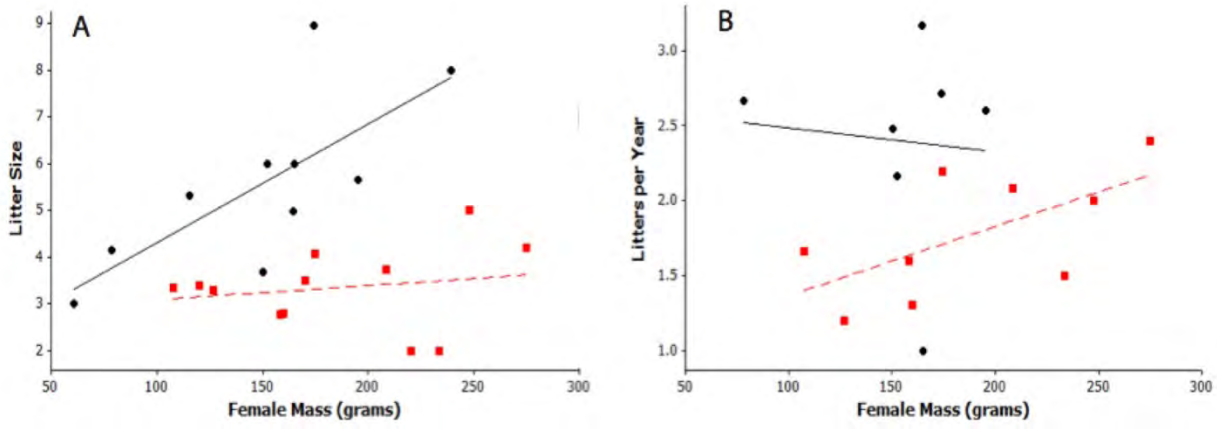


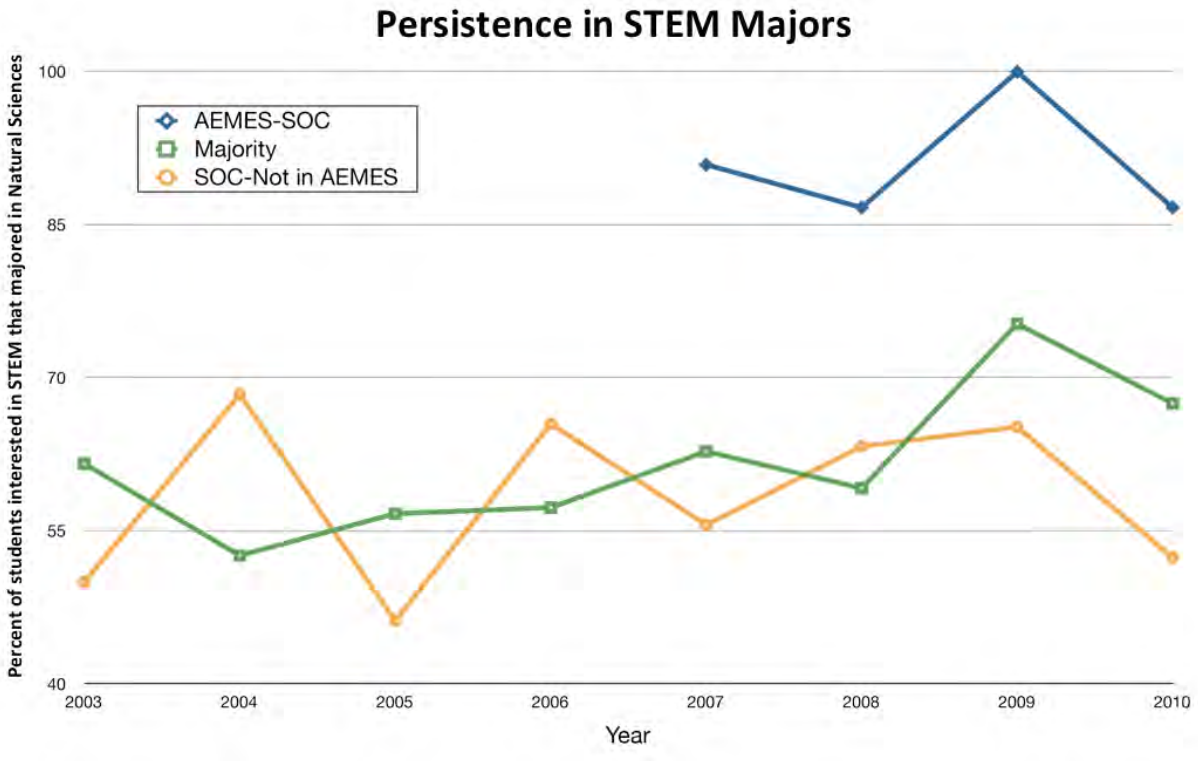
Figure 1A: Litter size versus female body mass among talus and non-talus dwelling pika.
Figure 1B: Litters per year versus female body mass among talus and non-talus dwelling pika.
Key: Non-talus represented by circles and talus represented by squares.

Improving Outcomes for Students from Underserved Communities Interested in STEM Majors: the AEMES Program at Smith College

Yadira Flores/2015

In 2007, Smith investigated STEM migration among its students, or how often a student who intended to major in a STEM discipline switched to non-STEM disciplines, and *vice versa*. The conclusion from this study was, "Overall, Smith College showed a small net gain in STEM majors with a slightly larger percentage of students migrating into STEM fields than dropping out. However, the opposite is true for underrepresented minorities at Smith – they appear to be more likely to leave STEM than to be recruited in from another field."[1] In 2007, the AEMES Scholars Program was also launched to address this and other issues that were impacting underrepresented (minority and/or first generation college students) students in STEM (science, technology, engineering, and mathematics). This summer we conducted an analysis in order to evaluate the progress the AEMES Scholars Program has had on its participants and the broader community of underrepresented students at Smith.

In the end, our results yielded several observations including that students of color (SOC) who participate in the AEMES Scholars program have a higher persistent rate in STEM than either students of color not in AEMES Scholars program and majority students. In Biology and Chemistry gateway courses, we see a trend that students of color who are AEMES Scholars tend to have GPAs higher than students of color not in AEMES Scholars but have lower or equal GPAs to majority students, though these results are not statistically significant.



These results show that the AEMES Scholars Program is effective in keeping diverse students in the STEM pipeline. Future analysis on individual parts of the program will help to determine what is effective as will assessing the role of mentoring on the broader community.

(Supported by the Schultz Foundation)

Advisors: Laura Katz, Biological Sciences and Katie Lipp, Program Director, Health & Mentor Program, Clark Science Center

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Enrolled Student Survey Report, Office of Institutional Research, Smith College

Effects of Elevated CO_2 on the Coccolithophore Emiliania huxleyi: Implications of Ocean Acidification

Christina Goethel/2013

Ocean acidification, due to increasing atmospheric CO_2, is particularly detrimental to calcifying organisms, such as molluscs and corals, because it reduces rates of calcification; lower pH also results in declining saturation states of $CaCO_3$ in seawater, leading to shell dissolution. Some important primary producers, such as coccolithophores, are also at risk because they secrete $CaCO_3$ coccoliths. Thus, understanding their response to elevated CO_2 is important for predicting long-term effects of ocean acidification on marine food webs.

A North Atlantic strain CCMP2668 of the coccolithophore, *Emiliania huxleyi*, was grown in culture under three levels of CO_2 (~335 ppm, ~620 ppm, and ~810 ppm) to study the effects of elevated CO_2 (and lower pH) on calcification and morphology. I measured particulate organic carbon (POC) and inorganic carbon (PIC), cell and coccolith size, number of coccoliths per cell, and percentage of malformed coccoliths. This summer I completed analysis of Scanning Electron Microscopy (SEM) images initiated as part of my Honors thesis.

Cells grown under control conditions had a significantly higher growth rate than cells held under enriched CO_2. Cultures were neither light nor nutrient limited, indicating a stress response (lower growth rate) to acidification (control pH= 8.1, enriched pH= 7.75). I found a significant inverse relationship between cell size and number of coccoliths per cell under varying CO_2 levels suggesting that elevated CO_2 results in a decrease of number of coccoliths per cell at least for strain CCMP2668. Significantly more malformed coccoliths were observed under CO_2 enrichment (~15%) compared to the control treatment (~3%). Malformations of coccoliths are strain specific and trend geographically. The strain of *E. huxleyi* that I studied matched other strains of the North Atlantic, displaying more malformed coccoliths under elevated CO_2.

The rate of inputs of atmospheric CO_2 is escalating (22% increase from 1958-2010); therefore, calcifying organisms that may have adapted in the past to altered ocean chemistry may not be able to adjust to contemporary accelerated rates. This study revealed how important oceanic primary producers like *E. huxleyi* might respond to changing levels of CO_2 and ocean acidification, potentially altering oceanic food webs. I presented my results at the National Conference for Undergraduate Research in April 2013.

(Supported by the Nancy Kay Holmes Fund)

Advisor: Paulette Peckol, Biological Sciences

Examining Diversity of *Chilodonella uncinata* in a Spatial and Temporal Context

Carmen M. Hernández/2013

Diversity in microbial lineages is far more expansive than what is found in animal lineages. Within a single microbial species, diversity becomes less focused on morphology but more directed on the genetic level, which can potentially even challenge its classification as a single species.[1,3] A group of taxa that exemplifies uniformity in morphology but divergence in their molecular profile is the ciliates.[2] They are characterized by their cilia, present in at least one part in its life cycle, genome rearrangement, and nuclear dimorphism, in which they have a somatic macronucleus and a germline micronucleus (the macronucleus is transcriptionally active during most of its lifecycle while the micronucleus is only active during conjugation).[2] For this project, I worked extensively with the ciliate morphospecie *Chilodonella uncinata* (class: Phyllopharyngea), by examining a region of its genome, the divergent mitochondrial small subunit ribosomal DNA (mtSSU-rDNA) (up to 8.0%).[3] In previous work of the Katz lab, 28 cells of *C. uncinata* were isolated from collecting sites on the Smith College campus and nSSU r-DNA/mtSSU r-DNA sequences were found. Phylogenetic analyses indicated that all sequences clustered with other known sequences of *C. uncinata*, and interestingly enough, for any location at any point in time only there was only one genetic type present.[4] However there was not an emphasis on how genetic types changed over time in the course of one day. In order to reconcile this lack of information denaturing gel gradient electrophoresis (DGGE) in conjunction with *C. uncinata* specific mtSSU primers are to be used to detect the genetic types potentially present at any point in time in a particular location. Preliminary data using this methodology resulted in remarkable patterns, with genetic haplotypes exclusively appearing during the nighttime and almost completely vanishing during the daytime. Additionally I was able to discern new genetic haplotypes. Work during this summer was to focus on collecting more samples, increasing the scope of how many genetic haplotypes are found and validating whether the first preliminary observation is true. While no further data was collected, protocols on how to obtain data were solidified. Further work now includes increasing the areas of sampling and collecting samples that yield higher quality community DNA. Hopefully the results from these collections will continue to show the same pattern as before or they may show a different pattern challenging assumptions on the seasonality of ciliate population structure.

(Supported by the Nancy Kay Holmes Fund)

Advisor: Laura Katz, Biological Sciences

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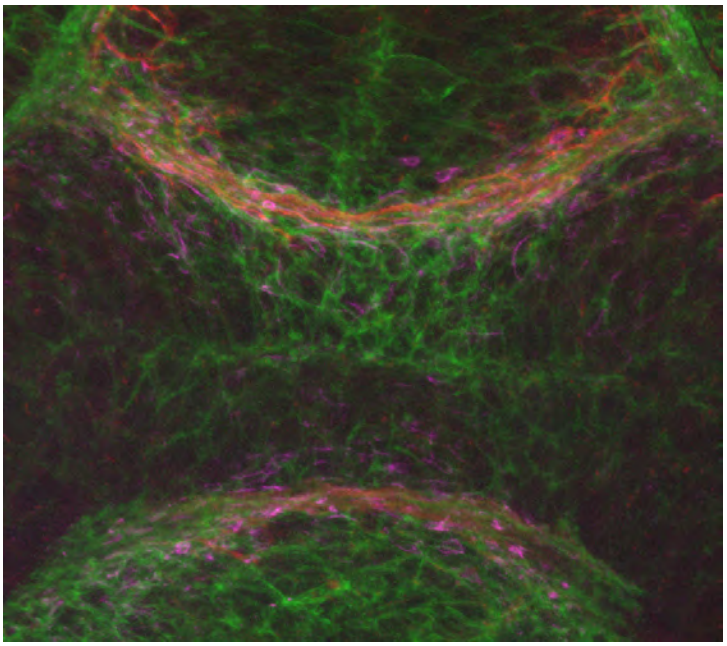
Cellular and Molecular Characterization of Post-Optic Commissure Formation in the Zebrafish Forebrain

Tanya Husain/2016 and Tatenda D. Mahalanza/2016

The focus of our research is to characterize the formation of the postoptic commissure (POC) in the forebrain of a developing zebrafish embryo. During early embryonic brain development, axons cross the midline in order to establish connections (commissures) between the two sides of the central nervous system. The postoptic commissure, the anterior commissure and the optic nerve are three such connections found in the vertebrate forebrain. During commissure formation, axons contact astroglia, which play a role in the guidance of the axons to their target locations.

Transgenic fish lines, created by injecting DNA constructs into the embryo at the one-cell stage, can be used to visualize axons and glia by expressing fluorescent proteins such as green fluorescent protein (GFP) and mCherry, in either the cell membrane or nucleus. The embryos express these DNA constructs and proceed with normal development. Four of these transgenic fish lines are currently used, all of which carry the regulatory regions of the *glial fibrilary acidic protein* (*Gfap*) promoter, which drives specific expression in astroglial cells.

Using these lines we sought to characterize the positioning and timing of astroglial cells development in the embryonic zebrafish forebrain. We used these lines paired with immunocytochemistry to visualize the simultaneous positioning of axons and other glial markers. This approach will allow us to visualize axon-glial interactions with fluorescent microscopy. We isolated homozygous transgenic fish through pairwise mating. From these embryos we successfully collected transgenic progeny expressing their specific fluorophore (GFP or mCherry), and we show that the expression does fall within the diencephalic glial bridge, which correlates with the positing of the POC. We propose to use these transgenic fish lines in the future to conduct live cell imaging using the Laser Scanning Confocal Microscope.



(Supported by the National Science Foundation, Husain, and the Schultz Foundation, Mahalanza)

Advisor: Michael Barresi, Biological Sciences

The Bioaccumulation of Mercury in the Avery Brook Watershed

Clare Jacobson/2016

Methylated mercury is a dangerous neurotoxin that is accumulating in water sources across the globe. Elemental mercury is produced naturally on the Earth as well as from sources such as coal burning power plants. This elemental mercury is then deposited into the environment where it is methylated and bio-accumulates up the food chain In my research, I focused on the Avery Brook Watershed in West Whately, MA. This watershed contains a series of beaver ponds, which are hotspots for mercury bioaccumulation

Sulphate-reducing bacteria (SRB), the main methylaters of mercury, are abundant in the sediment of beaver ponds. Therefore, using a core sampler I sampled and analyzed four different beaver ponds from the watershed. The bottom, middle, and water-sediment interface levels of the core were run through the Hydra C mercury analyzer to determine the ppm of mercury in each sediment.

The data collected with the Hydra C demonstrated that there is the most mercury in the water-sediment interface level of the sediment, then mid layer, and the lowest concentration was the bottom layer. This is demonstrating my hypothesis that the source of the mercury, the SRB, is in the water sediment-interface layer. They are methylating the mercury and it is then dissipating into the sediment.

To definitively tell if SRB are the source of the mercury I will sequence the DNA found in the water-sediment interface. This will let me speciate any bacteria found in order to look at the presence of SRB. Two sets of primers were designed for a MiSeq next-gen sequencer and they were designed to sequence variable regions of the *dsrA* and *dsrB* genes, which are the genetic markers for sulfur reduction and subsequent mercury methylation in SRB. MegaAlign software from DNAStar was used to align various *dsrAB* sequences from SRB to find conserved and hyper-variable regions within the gene. Primers were then designed to bind to conserved regions bracketing the hyper-variable ones, one pair on each gene. I intend to sequence these in the fall when I continue my research.

(Supported by the Howard Hughes Medical Institute)

Advisor: Robert Merritt, Biological Sciences

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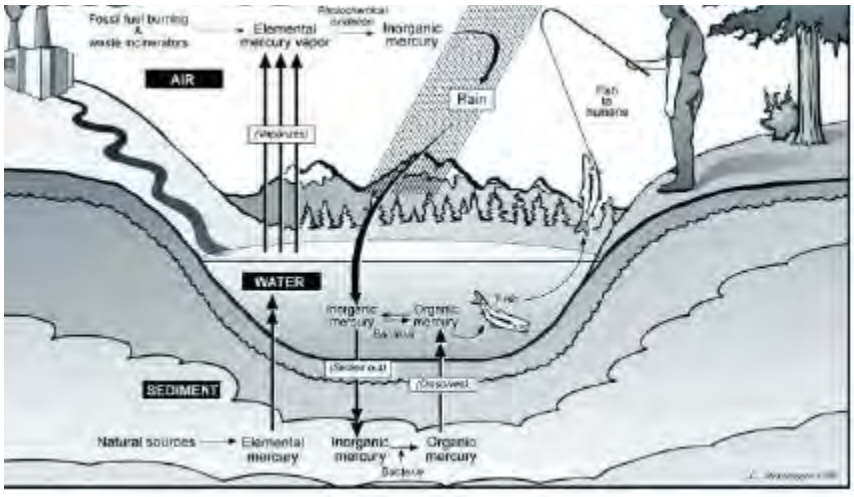

Developing a Southern Blot Protocol for Detecting an Inversion on the X Chromosome

Lindy Jensen/2014

In Eutherian mammals, the Xq28 region of the X chromosome has been found to contain a pair of highly-conserved long inverted repeats surrounding two genes, Emerin and Filamin.[3] Due to the unique conditions that sex chromosomes undergo during meiosis, the presence of these inverted repeats can cause the order of the two genes to flip during recombination. The first successful method used to detect a humans genotype at this locus was a radioactive Southern Blot.[1] My research focused on developing and improving a non-radioactive Southern Blot assay.

A Chemiluminescent Southern Blot involves digestion of a genome, electrophoresis fragment size selection, transferring these fragments to a membrane, and then visualizing the desired fragments using fluorescently labeled probe. The probe is made using PCR amplification and fluoresces different sized bands for different inversion genotypes.

I took steps to improve the existing protocol by first examining other Southern protocols. Comparison yielded a number of possible alterations and improvements. Because the membrane used is positively-charged nylon, changing the buffer from high-salt to alkaline causes the DNA fragments to covalently bond to the membrane and reduces the blot time from overnight to two hours as well as eliminating the need for UV crosslinking and baking.[2] These protocols were tested side by side and compared. Due to issues with stringency washes before visualization, neither method turned out a readable result. There was insufficient time to adjust wash stringency or visualize more membranes due to time spent troubleshooting the probe.

The probe protocol encountered problems involving all aspects of the process. These problems were identified and addressed by trial and error with systematic and logical adjustments to the PCR program and purification process. The PCR program was changed twice but was still unsuccessful at amplification by the end of my ten weeks. The purification process was found to be entirely unnecessary so long as PCR was successful and was removed from the protocol.

Developing this non-radioactive assay is important because it allows students try this experiment in lab. To understand why this inversion occurs and what this chemiluminescent assay detects, a multitude of key topics in molecular biology must be learned. This provides a unique teaching opportunity to weave those topics into an experiment that will tell a student their own genotype and teach them advanced molecular biology lab techniques.

(Supported by the Howard Hughes Medical Institute)

Advisor: Robert Merritt, Biological Sciences

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Exploring the Biogeography of Sulfate Reducing Bacteria (SRB) Communities in the Avery Brook Beaver Ponds

Rachel Kaminsky/2013

We conducted a study of the SRB community located in the sediments in a system of beaver ponds in the Avery Brook stream system located in Conway, MA. This subcatchment forms part of the Mill River watershed and drains into the Northampton Reservoir, the main source of drinking water for Northampton.[1] This summer, we focused on distinguishing how the SRB community diverges both within and between ponds.

The methods used for sampling the sediments involved coring vertically into the sediment and segmenting off the top 3 cm of the core for molecular analyses. Three ponds were chosen for this study, and three samples were taken from each pond, yielding a total of nine samples. Genomic DNA was extracted from the samples using the PowerSoil® DNA Isolation Kit from MoBio Laboratories, Inc. Denaturing gradient gel electrophoresis (DGGE) was performed on bacterial dsrB sequences that were amplified using PCR. PCR products of the same length can be separated by DGGE based on nucleotide composition. This approach is useful for creating a genetic profile of environmental communities and can also be used to identify abundant taxa, which is very difficult using other methods given the hyper-diverse nature of sediment environments.2 Bands of interest were excised from the gel and sequenced. Sequences were entered into the BLAST database to determine their identities.

Preliminary data shows that there is a great deal of sulfate reducing bacteria diversity in the ponds. Many of the sequences represent novel, or previously uncharacterized, and rarely found taxa. The majority of dsrA sequences were derived from members of the Deltaproteobacteria and the Firmicutes. The data also shows that sulfate reducing bacterial communities diverge between ponds, but not as much within ponds. This project will be continued into the 2013-2014 school year and will expand to include techniques to assess mercury and other geochemical indicators in the ponds.

(Supported by the Schultz Foundation)

Advisors: Robert Merritt and Laura Katz, Biological Sciences

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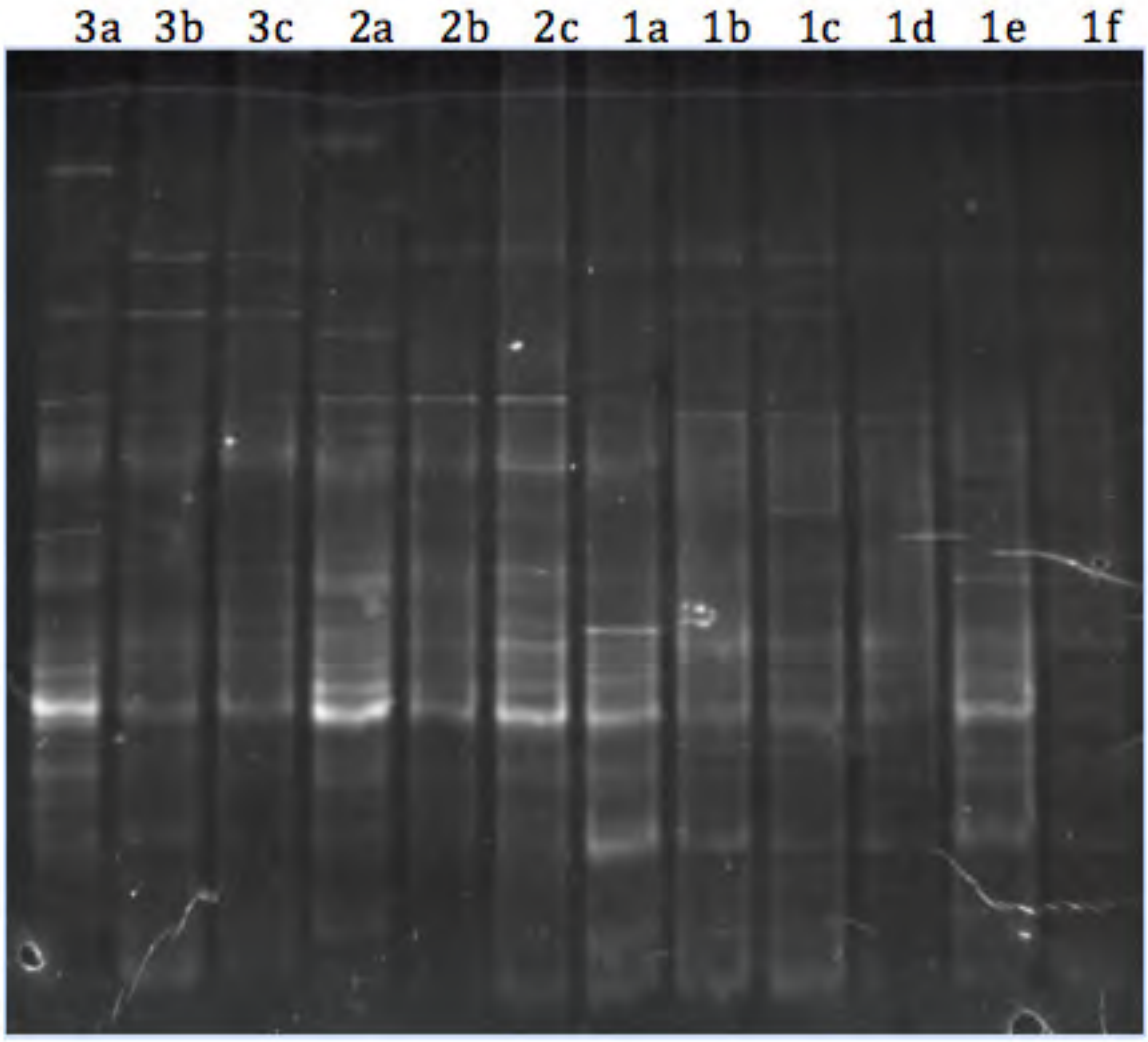

Figure 1: DGGE gel of sulfate reducing bacteria-derived dsrB sequences derived from twelve sediment samples from three beaver ponds. 1d-1f are derived from samples taken in October 2012.

The Open Tree of Life Project

Hana Kanee/2015

The Tree of Life tells us about the evolutionary history of all organisms of life and depicts shared ancestry among these organisms. Understanding the relationships between organisms gives us a deeper knowledge of phylogenetic relationships, which is essential for comparative analyses across biological disciplines.

My SURF work had two focuses: working on the Open Tree of Life project and working with upperclassmen in the Katz lab to learn a diverse set of biodiversity research tools. For the Open Tree of Life project, I worked on augmenting archaeal data for the Encyclopedia of Life (EOL), a public website that depicts the evolution of three domains from a single origin. The people that I contacted contributed their knowledge and an image of the organism that they work on. The other focus of the Open Tree of Life was analyzing a plethora of genetic trees collected from Phylografter, a database of trees and their genetic information derived from published studies. I analyzed the data by using SeaView, a program that visualizes genetic data in the form of a tree. I was able to study the trees and look for gene transfer among specific organisms through this program. I went through a number of preliminary trees and found ones that showed gene transfer from bacteria to eukaryotes, indicating lateral gene transfer. My project stemmed from that, where I looked for gene transfer among groups of monophyletic eukaryotes nested within bacterial taxa. I focused my work on the metazoan group; it includes all animals except for sponges and protozoans.

After looking through the metazoan trees that I had collected, I found that there was definitely lateral gene transfer among different species. I then noticed some recurring patterns in gene transfer between some of the species, which lead me to believe that there is a possibility to look further into these genes and tell a story that has yet to be told. As for my work on the EOL website, the website includes a number of archaeal and bacterial lineages that are now available for the public to see. I intend on continuing my research with the Open Tree of Life, hopefully leading to some answers about the metazoan gene transfers once I have more data to work with.

(Supported by the National Science Foundation)

Advisor: Laura A. Katz, Biological Sciences

Methanococcus maripaludis

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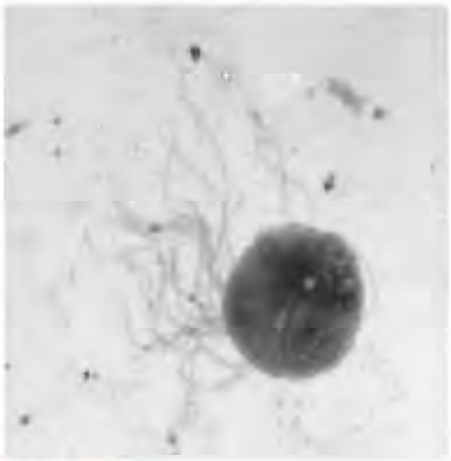

Methanococcus maripaludis
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IUCN threat status: Not evaluated

Brief Summary

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Methanococcus maripaludis

Methanococcus maripaludis is a mesophilic, methane producing member of the third Domain of life, the Archaea. Methanogens play important roles in the terminal step of anaerobic decomposition of organic matter. *M. maripaludis* was originally isolated from a salt marsh in South Carolina, USA. Like all methanogens, it is a stringent anaerobe. It derives energy for growth by the formation of methane gas, an important greenhouse gas, from carbon dioxide and hydrogen and uses CO_2 as sole carbon source. It can also fix nitrogen. As the name implies, it has an irregular coccoid shape. Its cell wall is composed solely of a protein coat termed the S-layer. The cells are weakly motile by means of a large number of archaella, the archaeal version of flagella. They also have other surface appendages, called pili, which aid in attachment of cells to surfaces. *M. maripaludis* is a model for study of Archaea due to its fast growth, high plating efficiency, a completely and publicly available sequenced genome and a comprehensive set of genetic tools.

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Found in 3 classifications

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Taxon recognized by World Register of Marine Species (WoRMS)
Archaea +
Euryarchaeota +
Methanococci +
Methanococcales +
Methanococcaceae +
Methanococcus +
Methanococcus maripaludis Jones, Paynter & Gu
Methanococcus aeolicus Kendall, Liu, Sieprawska-Lup
Methanococcus vannielii Stadtman and Barker 1951
Methanococcus vollae Balch, Fox, Magrum, Woese &

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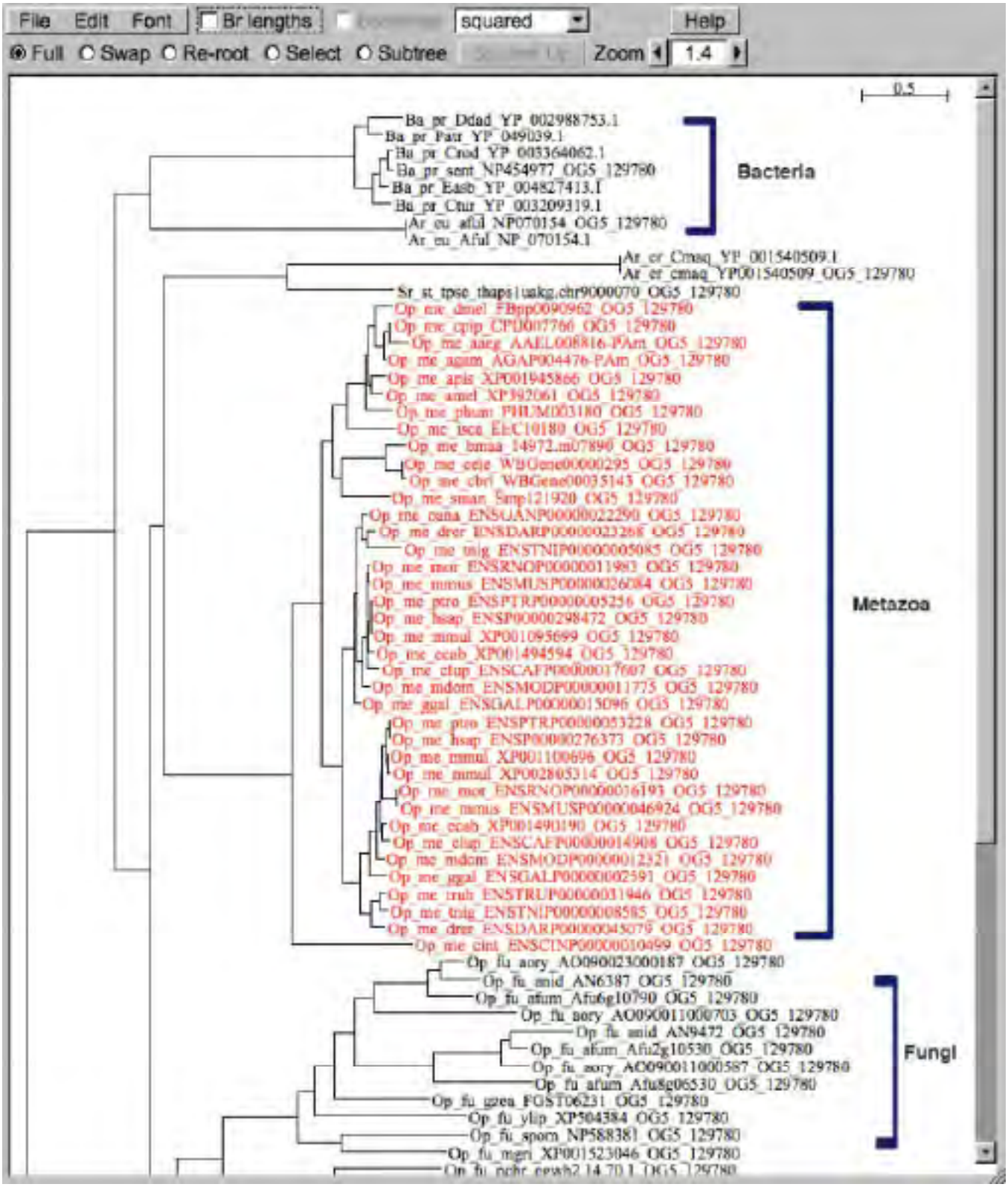
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Fungi

PCR- Sequence-Specific Primer Typing and of HLA Class II Alleles

Djene Keita/2014J

Human leukocyte antigen (HLA) class II alleles are part of the human major histocompatibility complex protein (MHC), a region on chromosome 6 essential to the immune system.[1] MHC molecules are important for the initiation of an immune response to a foreign antigen. The binding of HLA molecules to an antigen is essential for the activation of antigen specific T cells.[2] T cells are part of the adaptive immune response. They bind to the epitopes within the antigen and initiate an immune response. The binding of T cells to epitopes cannot occur without the binding of HLA molecules to the antigen. T cells are recruited to the site of infection with the binding of HLA molecules to the antigen. HLA typing is necessary for transplantation, vaccine design and others procedures. HLA typing is important for the design of vaccines for the eradication of Lymphatic Filiarisis (LF), a neglected tropical disease that has incapacitated millions of people in Africa and Asia. Determining the HLA haplotypes of populations endemic for LF is essential for understanding the mechanism by which pathogens of LF (Brugia Malayi, Timori and W. bancrofti) escape the immune response. Different typing systems have been developed to identify HLA haplotypes. These include diagnostic kits, primer sequence specific polymerase chain reaction (SSP-PCR), and others. Although the use of diagnostic kits is the fastest and effective way to identify HLA alleles, it is expensive. Research in endemic areas for LF has limited funding and cannot afford such typing system. PCR typing methods such as SSP- PCR are not only cost-effective but they also have been proven efficient for HLA typing. Performing lower cost typing systems such as SSP-PCR can help in the fight of understanding why the research community cannot make yet make an effective vaccine against these metazoan pathogens. My research thus focused on using the SSP-PCR to test the effectiveness of specific HLA class II primers obtained from literatures using DNA from check cells.

Primers sequences for HLA class II alleles DRB, DPB, DQB alleles were obtained through literature.[3] The primers were ordered through Invitrogen. DNA samples were extracted from cheek cells and purified using Phase Lock Gel. The molecular weight and purity of the samples were determined using the Qubit and the Nanodrop instruments. PCR was performed for each haplotype for each sample. The PCR products were checked on agarose gels. The gels were photographed for data analysis.

The results of the experiments were negative for DRB1, DRB3, DRB4 and DQB1 alleles for all samples. No bands were observed on the gel photographs. These results are inconclusive because they either indicated that the subjects did not have the alleles they were tested for or the primers used were not good at identifying the alleles. The next step of my research has been to test the same individual for the same HLA class II alleles using a diagnostic kit for the same haplotypes.

(Supported by the Howard Hughes Medical Institute)

Advisor: Steven A. Williams, Biological Sciences

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The Potential Absence of *Wolbachia* from Seal Heartworm, *Acanthocheilonema spirocauda*

Caroline Keroack/2014 and Caroline Decker/2009

Many filarial parasitic worms harbor the bacterial endosymbiont *Wolbachia*. This bacterium is believed to provide metabolic assistance to the worm, although the relationship between worm and bacterium has not yet been fully established. The presence or absence of *Wolbachia* in filarial worms is fairly inconsistent within genera.[1] All tested members of filarial worms of the genus *Acanthocheilonema* have been shown to lack *Wolbachia.*[2] We have obtained several marine mammal parasites, including seal heartworm (*Acanthocheilonema spirocauda*). Very few sequences for seal heartworm exist in the databases, so we used polymerase chain reaction (PCR) to amplify and then sequence several genes including COX1, SSU, 12s, 5s, and DIDR to confirm the identity of the worm. The phylogeny of *Acanthocheilonema* is poorly defined, so these sequences can provide insight into the evolutionary relationships between these filarial worms. Further, we decided to screen the worm for *Wolbachia* using primers to look for the *Wolbachia* surface protein (wsp) gene. This PCR was run several times, giving inconclusive results. Further *Wolbachia* screening PRCs were performed including the bacterial protein gene *fstZ*, 16s ribosomal gene for bacteria in general, and a specific *Wolbachia* 16s gene. These PCRs also gave inconclusive results, although the evidence points more towards the absence of *Wolbachia* in seal heartworm. Cloning was performed to try and amplify the wsp gene. Cloning was successful but sequence analysis revealed that PCR amplification was a result of nonspecific primer hybridization. Further research would include a western blot to confirm the current hypothesis that *A. spirocauda* lacks *Wolbachia.* Interestingly, a PCR for Porphobilinogen deaminase (PBGD) gene was positive. Sequence analysis revealed this to be a degenerative *Wolbachia* gene, indicating that this is a pseudogene transferred from the bacteria to the worm's genome. This indicates that *Wolbachia* was probably present in *Acanthocheilonema spirocauda* at some point in the distant past. Further research could elucidate how worms have adapted for survival without *Wolbachia.*

(Supported by the Shultz Foundation, Keroack)

Advisor: Steven A. Williams, Biological Sciences

Acknowledgements: I would like to thank Sea Rogers Williams VMD of the National Marine Life Center and Frederick Wenzel of NOAA, Northeast Fisheries Science Center, Woods Hole, Ma. for providing me with parasite samples.

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Characterizing Sulfate Reducing Bacteria Communities in Avery Brook Beaver Ponds

Nida Khan/2015

Beavers influence stream dynamics by creating dams, which retain organic material. This dramatically alters the chemical properties of water as well as sediment and organic material being transported downstream. As a result, beaver ponds are referred to as biogeochemical hot spots and the microbes inhabiting these areas remain highly unexplored. The purpose of this study was to characterize sulfate-reducing bacteria communities located in the sediments in a system of beaver ponds in the Avery Brook stream system. This subcatchment forms part of the Mill River watershed and drains into the Northampton Reservoir, a main source of drinking water for Northampton. Sulfate reducing bacteria are responsible for the conversion of inorganic mercury into methylmercury and are prevalent in the Avery Brook Stream system. Methylmercury is a neurotoxin that binds to proteins, which allows for the assimilation of mercury into animal tissue. This prevents it from being eliminated from the body, which poses a problem for humans due to its bioaccumulative property (Figure 1).

Sulfate reducing bacteria use sulfate as the terminal electron acceptor in anaerobic respiration and are characterized by the presence of dissimilatory sulfate reductase, an enzyme coded for by the alpha and beta subunits of the dsr operon. Because there are many bacteria that may have the dsrAB gene, but don't express it, RNA was isolated from soil samples in order to identify active taxa. The extracted RNA was converted to cDNA by RT-PCR and the resulting cDNA was PCR amplified at 350 base pairs. Afterwards, the PCR product was cloned into E. coli and the colonies that contained the insert were miniprepped and sequenced. The next step is to run these samples by denaturing gradient gel electrophoresis (DGGE), a fingerprinting approach that generates a pattern of genetic diversity of sulfate reducing bacteria. By comparing the DGGE gels of both the DNA samples and RNA samples, I can compare the diversity of the species of bacteria with the dsr gene that are actively expressing it with those that are not.

(Supported by the Howard Hughes Medical Institute)

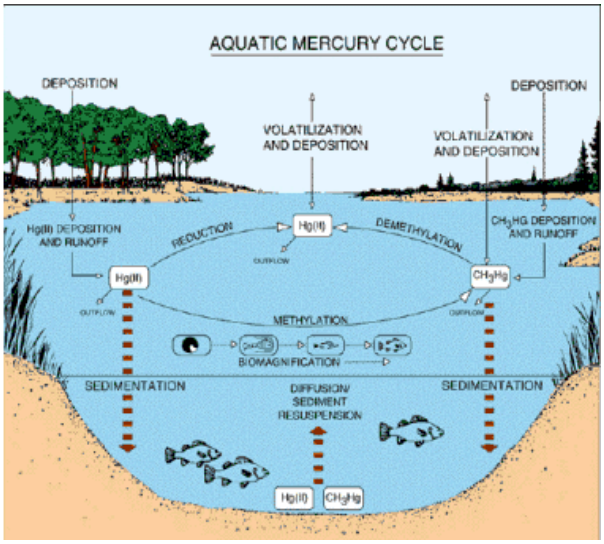

Figure 1. Beaver ponds exhibit conditions that allow for the favorable formation of methylmercury, a potent human neurotoxin. Because of its bioaccumulative properties, methyl mercury biomagnifies in the ecosystem and can severely impact developing fetuses of pregnant women.

Advisors: Robert Merritt and Laura Katz, Biological Sciences

Grazing Preferences and Behavior of the Periwinkle *Littorina obtusata*

Julia Kurys/2016

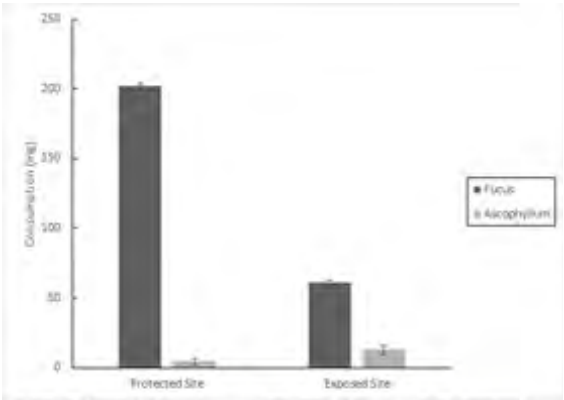

Fig. 1. Mean (±SE) consumption (mg) of Fucus vesiculosus and Ascophyllum nodosum from protected and exposed sites by the periwinkle Littorina obtusata.

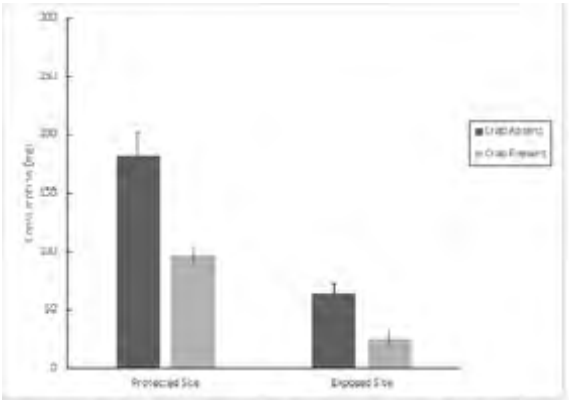

Fig. 2. Mean (±SE) consumption of Fucus vesiculosus by Littorina obtusata in the presence and absence of the green crab, Carcinus maenas.

I investigated trophic interactions of the invasive predator green crab, *Carcinus maenas,* and its prey, the herbivorous periwinkle, *Littorina obtusata.* I conducted grazing experiments to determine whether *L. obtusata* prefers *Fucus vesiculosus* from a protected area or wave exposed site (higher wave and current surge). I found that snails consumed significantly more *Fucus* from the protected site. The fronds of the protected population were broader than the streamlined blades of exposed *Fucus,* suggesting a morphological component to the snail's preference. Samples were frozen for analysis of phlorotannins (secondary compounds found to be herbivore deterrents). Because some research suggested that *Ascophyllum nodosum* was the preferred food source and habitat of *L. obtusata*[1,2], I offered a choice of *Ascophyllum* and *Fucus* from protected and exposed sites. The snails showed significant food preference of *Fucus* over *Ascophyllum* (Fig. 1), and snails spent more time on *Fucus* (35%) than on *Ascophyllum* (5%). Further, snail egg masses were found on *Fucus* (and not on *Ascophyllum*) in the field as well as on *Fucus* fronds in the laboratory suggesting habitat preference. Notably, snails collected on *Ascophyllum* from northern Maine sites also had a significant preference for *Fucus* over *Ascophyllum* in my choice experiments. I investigated the effect of the predator *C. maenas* on grazing rates of *L. obtusata* by securing green crabs in bags in the presence of snails. *L. obtusata* consumed significantly less *Fucus* in the presence of green crabs (Fig. 2). In the presence of crabs, the snails were emergent for much of the time, suggesting an escape response. However, in crawl out experiments (testing snail response to chemical stimuli in the water), snails in the water treated with crab effluent crawled out less (37%) than snails in the control treatment (57%). My experiments suggest that *L. obtusata* in the field are more likely to eat *Fucus* than *Ascophyllum* and grazing rates are affected by the presence of their predator. As the invasive green crab migrates northward with increasing water temperatures, its increased presence may modify grazing rates of *L. obtusata* and result in changes in macroalgal abundance in the rocky intertidal habitat.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Paulette Peckol, Biological Sciences

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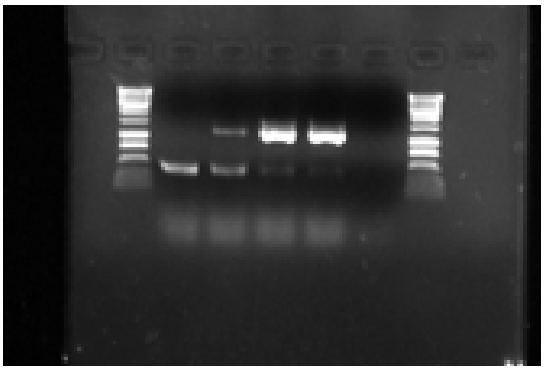
Understanding the Ciliate Community Linked to Water Mass

Cynthia Masai/2016



Gel picture of one of my PCRs

Microbes dominate the Earth, yet little is known about many microbial species. Studying the microbes will enable us to understand our ecosystem better, understand the role these microbes play in the ecosystem and understand the different disease caused by microbes. This summer the research I did mainly focused on ciliates, a group of eukaryotic unicellular organisms. I was matched with an ocean team that works on a project observing how the ciliate community in Long Island Sound is linked to water mass. Using samples collected from Long Island Sound at different stations, the team used PCR and DGGE techniques to observe the link between the ciliate community and water mass.

After being matched with the group, I worked mainly at doing PCR from different stations in order to observe the relation between the ciliate communities. My hypothesis was that the probability of finding same community at two different stations that are near each other is higher than finding same community in two different stations that are further apart. By the end of the summer, I mastered PCR and other techniques necessary for this project.

In addition to working on the Ocean project, I also learned basic molecular techniques needed to study microorganisms. These include cloning and DGGE. I also learned basic bioinformatics approaches to analyze molecular data. These include learning how to use Megalign, SeqMan and seaview. I also learned basic light microscopy in the lab, where I helped in picking Chilodonella uncinata, I will continue working in the same lab, doing a related project to what I did during summer.

The research experience was very helpful because I was able to gain a better understanding of molecular approaches to Biology. This experience will help me as I study science in the classroom during my sophomore year.

(Supported by the Schultz Foundation)

Advisor: Laura Katz, Biological Sciences

The Role of Wnt5b in Radial Glia Cell Division of the Zebrafish Spinal Cord

Chelsea Moriarty/2014

Wnt5b is a glycoprotein morphogen in the non-canonical Wnt pathway. Various non-canonical Wnts are involved in creating planar cell polarity, triggering intra-cellular calcium release, and antagonizing the canonical Wnt signaling. Wnt5b has been shown to play a role in neurogenesis. This study aimed to characterize the role of Wnt5b in regulating radial glia cell division and subsequent cell patterning in the Zebrafish spinal cord. Previous studies have suggested that a knockout of Wnt5b causes an increase of radial glia cell numbers. Zebrafish embryos were fixed at various times (15 hpf, 24 hpf, 30 hpf, 36 hpf, 42 hpf, 48 hpf). Immunocytochemistry and fluorescent microscopy were utilized to quantify the number of radial glia cells ($GFAP^+$) and cells in mitosis ($PH3^+$) within somite 10-12 of the spinal cord. Polymerase chain reaction was used to distinguish the genetic identity between wild type $Wnt5b^+$ and mutant $Wnt5b^-$ zebrafish embryos. Preliminary results suggest that there is an increase in $GFAP^+$ and $PH3^+$ cells in mutant $Wnt5b^-$ embryos as compared to wild type $Wnt5b^+$. These results may suggest a halt of mitosis. Radial glia cells accumulate within the spinal cord because they are prevented from completing cell division. Radial glia cells give rise to several other neural cell types, including oligodendrocytes precursors, motorneurons, and interneurons. A halt of mitosis in radial glia cells may cause a decrease in oligodendrocytes precursors, motorneurons, and interneurons. A time-lapse fluorescent microscopy study will show the correlation between a knockout of Wnt5b and a decrease of neural populations.

(Supported by the Howard Hughes Medical Institute)

Advisor: Michael Barresi, Biological Sciences

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Forest Reintroduction with the American Chestnut

Greylin Nielsen/2014

Before the devastating chestnut blight destroyed populations along the east coast, the American chestnut, *Castanea dentata*, accounted for 1 in 4 trees in eastern hardwood forests. These trees were widely considered to be a keystone species and their removal altered essential habitats. The manifestation of blight destroys the tree above the point of infection, leaving the root collar unaffected and meaning that new chestnut trees can sprout from the stump. Though chestnut trees are considered functionally extinct, they are still present, constituting about 1% of their original area. The few remaining trees and sprouts provided genetic material for researchers who have spent decades producing hybrid chestnut trees that are genetically about 94% American and 6% Chinese chestnut, ideally retaining blight resistance from the Chinese chestnut.

Now that nuts with blight resistance are available we are faced with the challenge of reintegrating once-dominant chestnuts into existing hardwood communities. Do we need to alter the forest and provide space for the chestnut to sprout and prosper? Is a gap in the forest canopy required to provide light for the chestnuts? Is it necessary to protect the chestnut from predators to ensure survival? This research project examines these and other questions related to the reintegration of chestnuts into a forest setting. We established plots in the woods at the MacLeish Field Station with a central point and nuts planted in the cardinal directions. The experimental plots have varying gap sizes created by felling existing trees, while control plots were left undisturbed. At each site half of the nuts are protected by tubes while the other half were planted directly into the ground. This experiment will take time to produce results. As the season turns we will collect data on survivorship in the forest plots to use as a baseline. We are hopeful that results from this experiment will be instructive for widespread reintroduction of these important trees into forests of the Appalachian region.

(Supported by the Center for the Enviroment, Ecological Design & Sustainability)

Advisors: Paul R. Wetzel and Reid W. Bertone-Johnson, Center for the Enviroment, Ecological Design & Sustainability

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Photo 1: Sprouting Chestnut nut.

Photo 2: Chestnut nut protected by plastic tube.

Longer Female Tail Lengths: A Comparison of Two Small Carnivores

Paula Noonan/GR2013

Sexual dimorphism among the carnivores usually refers to males that are larger than females and is especially striking among the Mustelidae. However, rare exceptions occur when the extremities are examined in comparison to body lengths. For instance, when the relative tail lengths of males and females are compared, reverse sexual dimorphism is revealed in a few species. Of the mustelids, longer female tails occur in *Eira barbara*; another example is the closely related small carnivore *Mephitis mephitis* (Mephitidae) (Table 1).

Table 1. Relative tail length compared to head-body length in two closely related small carnivores (data from specimens from the American Museum, Field Museum, and Smithsonian).

Species	*Eira barbara*	*Mephitis mephitis*
Female/male tail/head-body ratio	1.0917	1.0864
Number of subspecies	7	13
p-value	0.014	0.015

Clearly, the tail of *Eira barbara* differs strikingly from that of *Mephitis mephitis*. The habitats and locomotion of these two species also differ markedly. *Eira barbara* is both arboreal and terrestrial (Presely 2000); its tail aids in its life in the trees, including pursuit of prey. *Mephitis mephitis* is primarily terrestrial; its elevated tail is a notorious warning to predators (Wade-Smith & Verts 1982).

In a review of tail function, one author discusses the tail "as a surprisingly multi-purpose and important appendage." He divides its functions into mechanical functions such as balance and defense, behavioral functions such as warning and courtship, and physiological functions such as thermoregulation (Hickman 1979, p. 143).

With regard to mechanical function, the author notes, "the tail need not be prehensile to be useful to forms which are at least occasionally arboreal," such as arboreal forms of *Peromyscus* compared to terrestrial ones (Hickman 1979, p. 145). Significantly longer tails are also found in tree and flying squirrels, with females having longer tails than males (Hayssen 2008). Therefore, *Eira barbara* females may face the challenges of other arboreal female mammals: maintaining balance and efficiently obtaining food while carrying the weight of fetuses.

Long, thickly furred tails may serve to insulate the body of mammals such as foxes (Hickman 1979). While *Mephitis mephitis* can use daily torpor in winter, males in groups do not undergo torpor although females do. While males may need to stay alert to defend females, females may benefit from reserving body fat for successful reproduction (Hwang et al. 2007). Thus, longer tails may help females with thermoregulation during winter.

As intriguing as these explanations are for longer female tails, they don't necessarily address why males would have shorter tails. For *E. barbara* males, the pursuit of prey in trees requires equal agility. For *M. mephitis* males, a larger tail may also be advantageous for thermoregulation. An examination of species in which males have relatively longer tails (such as *Mustela erminea*) may provide some insight. However, given the diverse functions of tails, the explanation may point to yet another source for adaptation for differences in male and female tail lengths.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Virginia Hayssen, Biological Sciences

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Photos: Frans Lanting Photography; USFS

Quantitative Analysis of Commissure Formation Using GIS and GeoDa

Jin Sook Park/2015

Axon-glia interactions in the developing brain are caused by Roundabout receptors detecting Slit proteins, which are known to act as repulsive cues. These interactions guide bundles of axons, known as commissures, along the glia substrate to form the necessary connections within the nervous system.[1] Among the Slit proteins, Slit1a appears to have a distinct role from Slit2 and Slit3 as an attractive cue in axon guidance.[2] How is commissure formation affected when one or more components of the Slit/Robo signal-receptor pathway are disrupted? How can the different phenotypes be analyzed efficiently and objectively? Using a zebrafish model system, we studied embryonic forebrains to determine the role of the Slit/Robo pathway and to see if certain mutations resulted in specific phenotypes, especially in the formation of the post-optic commissure (POC). We needed to quantify the POC to compare and contrast different commissures, and possibly measure the amount of deviation a specific mutation may produce. We utilized geographic programs, known as GIS and GeoDa, to develop a method to quantify the POC using an unbiased perspective.

With help from the Spatial Analysis Lab, we quantified the POC based on the intensity of the pixels of the forebrain images. We established a uniform grid that compared the same aspects of one POC to another. We measured the mean pixel intensity of the POC and performed a cluster analysis, which informed us in a numerical and visual way, how the axons formed. The calculated Moran's I index showed if there was a pattern of either clustering or dispersion in the POC; this value can be used as a measurement of comparison between the commissures. We also determined the average distance that wandering axons traveled from an ideal wild type commissure.

When developing the GIS analysis, I learned, most importantly, that approaching the problem by combining two seemingly unrelated fields of study was unconventional, but innovative and perfectly acceptable. I learned how to extract useful information I would need for a thorough analysis from the images while also excluding excessive pieces of data. A few of the analyses we performed showed greater dispersion and wandering in the POC in heatshocked transgenic embryos when compared to a control. Currently, we are conducting more trial analyses on different images in order to gather more substantial data on different phenotypes.

The quantitative analysis using GIS provides an objective method to examine phenotypes and to compare one commissure to another. We can establish what a standard wild type commissure looks like, in addition to creating standards for the different transgenic and mutant phenotypes. This would make comparisons of phenotypes more meaningful and easier to visualize. During my research experience this summer, I was able to present the work with GIS at the Northeast Arc Users Group Spring Meeting. I also attended the 17th International Congress of Developmental Biology conference in Cancun, Mexico where my lab partner and I presented a poster on the research done on the role of the Slit/Roundabout pathway on axon guidance.

(Supported by Howard Hughes Medical Institute)

Advisor: Michael Barresi, Biological Sciences

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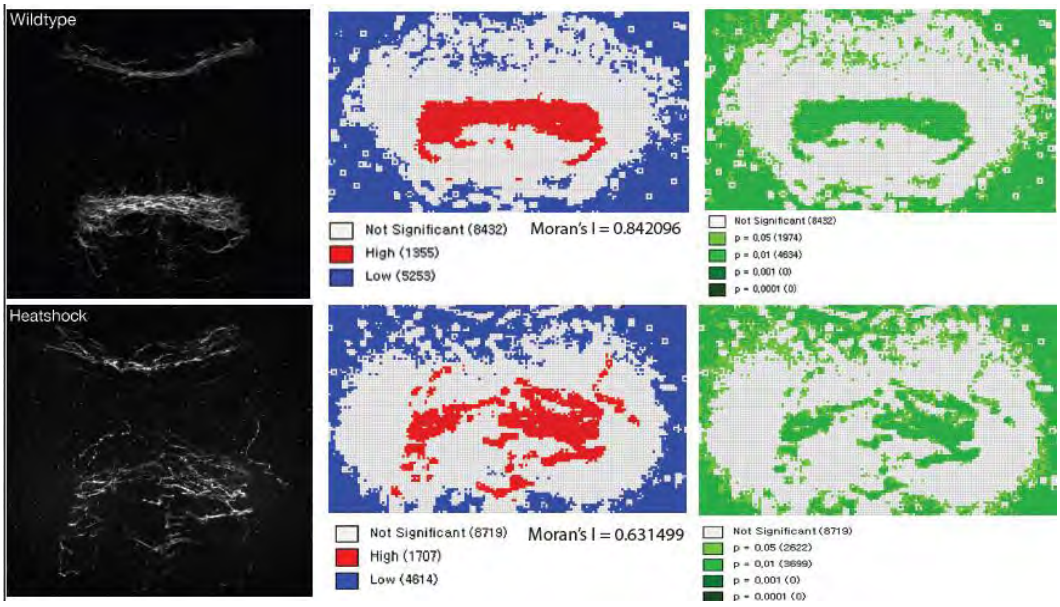
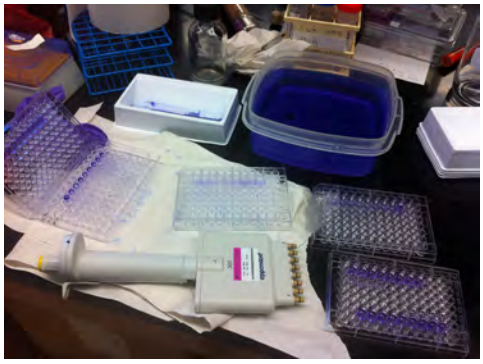

Wildtype
Not Significant (8432)
High (1355)
Low (5253)
Moran's I = 0.842096
Not Significant (8432)
p = 0.05 (1974)
p = 0.01 (4634)
p = 0.001 (0)
p = 0.0001 (0)
Heatshock
Not Significant (8719)
High (1707)
Low (4614)
Moran's I = 0.631499
Not Significant (8719)
p = 0.05 (2622)
p = 0.01 (3699)
p = 0.001 (0)
p = 0.0001 (0)

Biofilm Formation in *Escherichia coli* Commensal and Uropathogenic Strains

Gemma Regan-Mochrie/2015



Escherichia coli can be both beneficial, as with the commensal strain K-12 that contributes to human health, or pathogenic, such as CFT073 (UPEC) that causes urinary tract infections in the human host. In this project we examined the ability of both of these strains to form biofilms at room temperature (23˚C) and human body temperature (37˚C). A variety of microorganisms form biofilms; biofilms occur when these microbes attach to a surface and secrete a matrix, primarily made of polysaccharides that helps to protect them from antimicrobial molecules.[1] Biofilm formation can occur on catheters and in artificial implanted devices and is also integral to the progression of urinary tract infections, which occur when *E. coli* colonize the bladder epithelium cells.[3] Biofilms are more protected from the immune system and can be far more damaging when they occur in patients who have already suffered trauma. Beyond the effect of temperature this project also examined the role of the role of motility, substrate, and the RpoS general stress response regulator on biofilm formation.

In previous experiments, qRT-PCR showed that in K-12 there was increased biofilm gene expression at 23˚C whereas in UPEC there was increased biofilm gene expression at 37˚C. It was hypothesized that biofilm formation would follow these gene expression trends. This project involved growing biofilms under static conditions and quantifying the biofilm formation through crystal violet staining at various time points. Biofilm growth was corrected for differing growth rates at the two temperatures

Temperature was found to influence biofilm formation differentially in each of the two strains. The K-12 wild type showed 5-fold more biofilm formation at 23˚C whereas UPEC formed biofilms more readily at 37˚C. This was expected based on our previous research and literature sources.[4] The motile K-12 strain formed about 16-fold more biofilm than the non-motile strain at 23˚C, adding to other studies where motility and flagella have been shown to be critical for this process.[4]

The difference between the two substrates was not significant in the wild-type strain but there was more growth on polystyrene at 23˚C in the non-motile strain. The RpoS mutant strain formed far less biofilm than the wild-type strain, which was unexpected, based on the literature.[2] For UPEC both the wild type and the RpoS truncated strain formed more biofilm at 37˚C. The truncated RpoS strain shows greater biofilm formation and this is seen more drastically when the cells are grown on PVC. The temperature and RpoS results were expected based on our previous research. I will continue this research as part of a special studies in the up coming semester.

(Supported by the National Institutes of Health)

Advisor: Christine A. White-Ziegler, Biological Sciences

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Tracking Horizontal Gene Transfer Events to Fungi with Single-Gene Phylogenomics

Emma Holt Sawyer/2014

The presence of gene inheritance unrelated to reproductive cycles amongst bacteria and archaea has been widely accepted. This horizontal gene transfer (HGT) allows one species to pass genetic code to a second species that successfully incorporates it into their genome. HGT creates nonlinear pathways of inheritance and can allow species to gain new protein coding abilities in a more rapid manner. New research suggests that HGT is also widespread within the eukaryotic domain.[1] Details on HGT events to eukaryotes could lead to greater understanding on how this highly-variant branch of life evolved. For summer research, fungal genes of possibly bacterial origin were identified.

Already established in the Katz Lab is a pipeline designed to assemble genetic information made available by online databases such as OrthoMCL. Single gene trees including an array of taxa from all three domains of life were generated. Trees that contained bacterial and fungal species were selected. These were bootstrapped using RaXML, a free program online, in order to assess the confidence of the branching patterns. Trees with only bacteria and fungi were presumed possible HGT as well as trees with bacteria and an assortment of eukaryotes with at least one monophyletic fungal clade nested within the bacteria with a high level of confidence (~0.9).

Of all the genes collected through the pipeline, 185 were selected for having bacterial and fungal species. From this, approximately 100 could suggest presence of the gene in fungi because of HGT. Organization and assessment of the data collected is still in preliminary stages due to the relatively new nature of this study and the lack of established procedure.

The extent of HGT from bacteria to fungi is unknown. The identity of genes that are believed to be involved in HGT could be used to generate more accurate and detailed maps of inheritance that shed light on how and when a major eukaryotic clade incorporated bacterial genetic material. It has been suggested by other studies that HGT events have had a large effect on the pathogenicity of certain fungi as well as generally altering the history of their evolution.[2] Finding the patterns as to which fungi had HGT events and which genes were passed could explain the functionality of this phenomenon. This research will be continued into the 2013/2014 academic year at Smith College.

(Supported by the Margaret A. Walsh Grantham Fund)

Advisor: Laura Katz, Biological Sciences

[1]Renner, S.S. and Bellot, S. 2012. Horizontal Gene Transfer in Eukaryotes: Fungi-to-Plant and Plant-to-Plant Transfers of Organellar DNA. Systematic Botany and Mycology, 223-232.

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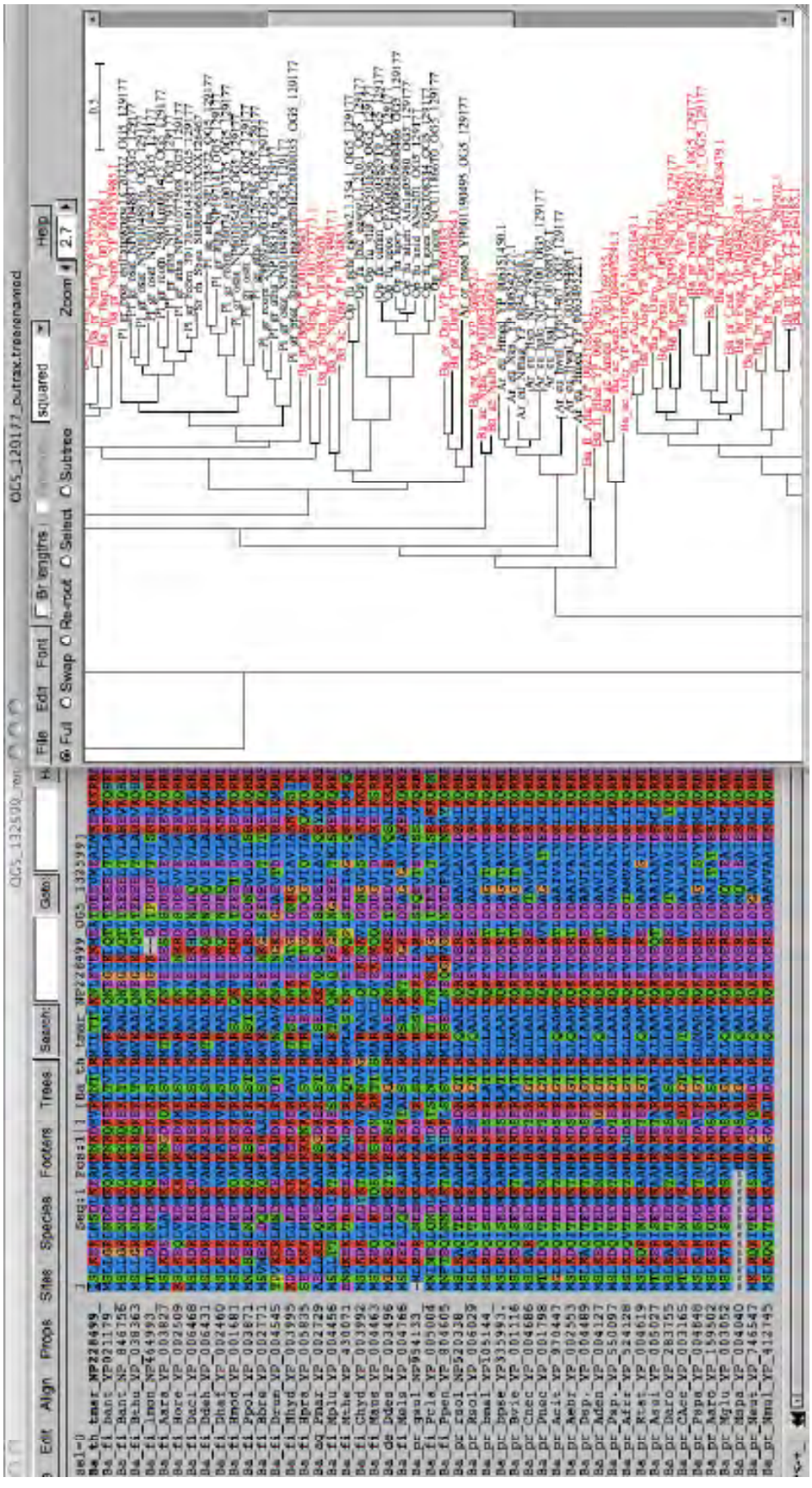

Miseq Analysis Comparing RNA sequences of *A. viteae* and *B. malayi*

Kareen Seignon/Ada Comstock Scholar and Marie Jacques Seignon/Ada Comstock Scholar

A.viteae and *B.malayi* are worms responsible for the infectious disease filariasis. Although sharing this similarity, they differ in that *A. viteae* infects animals and *B.malayi* infects humans.[1] Moreover, *B.malayi* has a symbiotic relationship with the bacterium *Wolbachia* allowing this worm to survive within the human host while *A. viteae* does not bear this symbiont.[1,2] The purpose of this research is to compare the mRNA sequences of both worms to see if they have genes that are similar in function that would explain why *A. viteae* is able to survive without *Wolbachia*.

To conduct this experiment, we did an mRNA isolation of the adult females of both worms, which we then converted into cDNA. Adaptors and index sequences were attached to the samples to prepare them for next generation sequencing. We checked the quality of the cDNA samples using a bionanalyzer. Finally, we used an Illumina Miseq to get millions of sequence reads from each sample.

The output data from the Illumina Miseq system provided useful information concerning the quantity of reads for both samples as well as the quality of those reads. An indexing quality control was given since two different samples were running with a different index each. For a total number of reads of about 23 million with 20 million identified as pass filter, index 1, associated with adult female *B. malayi,* and index 2, associated with adult female *A. viteae,* have respectively 47.7% and 51.4% identified as pass filter (Figure 1). The quality score to determine the reads that pass filter is based on the Phred scale where Q10, Q20, Q30, Q40 respectively correspond to 10%, 1%, 0.1% and 0.01% chance of the base call being wrong. For these two samples, 92.90% of the total reads have a QC greater than 30 determining the quality of our next generation sequencing was excellent (Figure 2).

Currently, we are exploring a variety of software packages that will allow us to appropriately analyze this mountain of data and to squeeze out the fraction that will spark our interest, specifically sequences of genes that are functionally similar between these two species when it comes to their survival in the blood stream of their respective hosts.

(Supported by the Smith College Provost's Office)

Advisor: Steven A. Williams, Biological Sciences

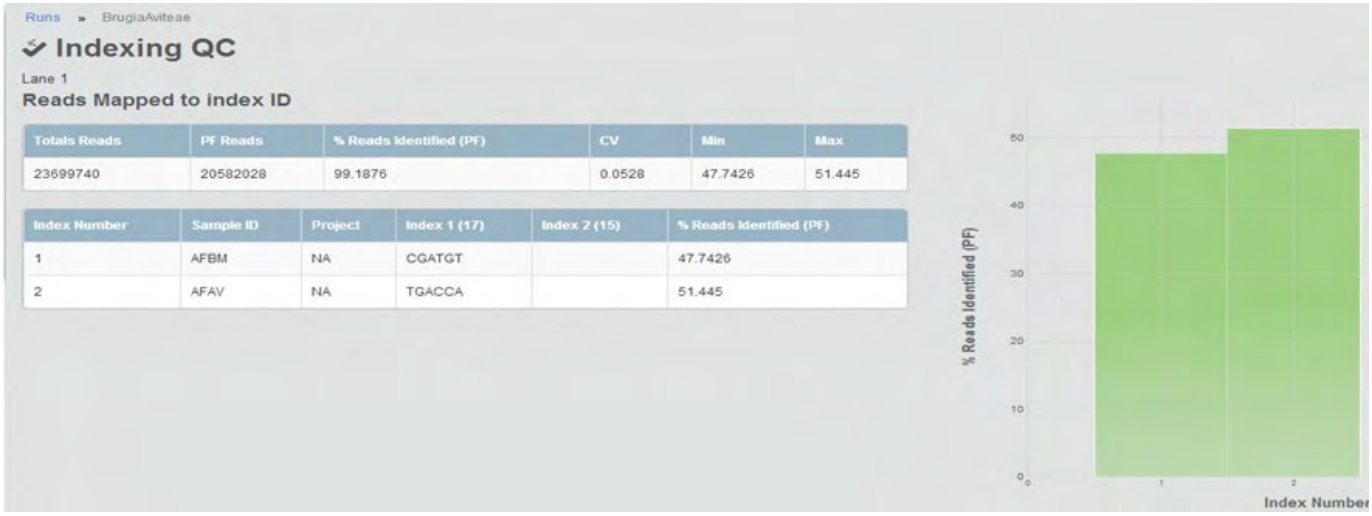

Figure 1: **Table and graph representing the indexing quality control from BaseSpace Illumina**. In the first table, the total number of reads and the percent number of reads that have passed the filter are shown. In the second table, the basic information concerning the two samples and their respective indices are shown. The graph is a representation of the percent reads identified as pass filter for each index respectively.

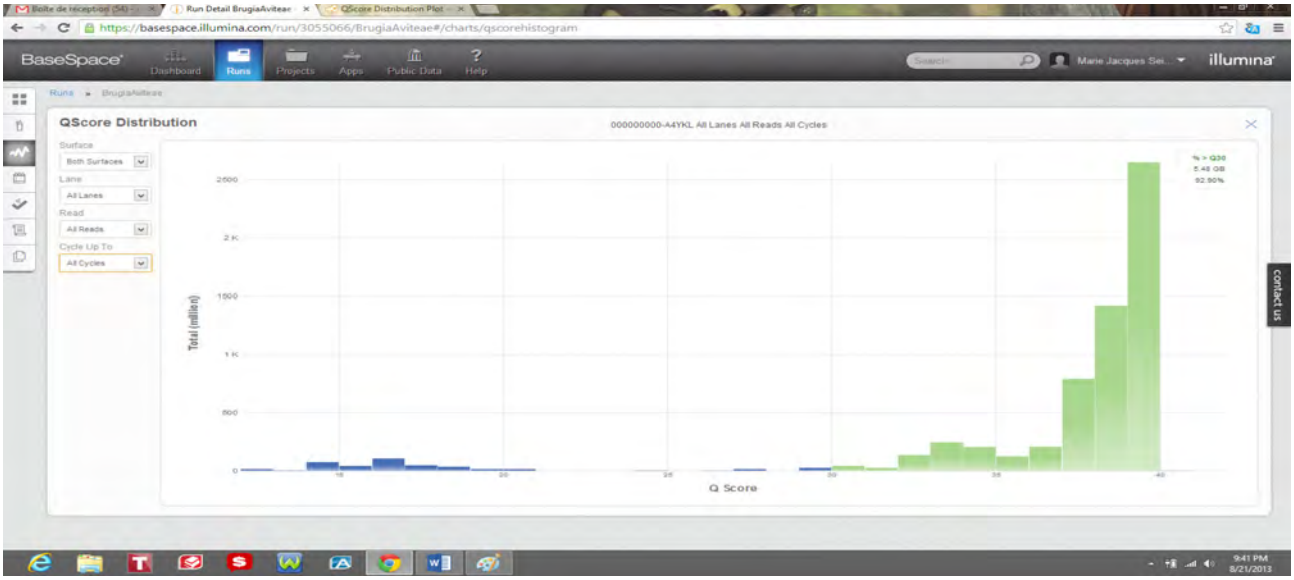

Figure 2: **Graph of the quality score (QC) distribution for all reads.** The quality score corresponds to the chance of wrong base calls by the system. QC is based on the Phred scale where Q10, Q20, Q30, Q40 respectively correspond to 10%, 1%, 0.1% and 0.01% chance the base call being wrong.

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Exploring the Promoter of the *Brugia malayi* Thioredoxin Peroxidase-2 (TPX2) Gene

Iju Shakya/2013 and Asma Amin/2015

Lymphatic filariasis (LF), one of the neglected tropical diseases, is caused by three nematodes: *Wuchereria bancrofti* (*Wb*), *Brugia malayi* (*Bm*) and *Brugia timori* (*Bt*). Currently, LF afflicts 120 million people and puts 1.3 billion people in 80 different countries at the risk of infection. LF can develop into debilitating conditions such as elephantiasis, and is also accompanied with negative social, economic and psychological effects.[1]

Upon identifying that the *Tpx2* gene was up-regulated in the infective larval stage (L3) of *Onchocerca volvulus*, Lu et al. predicted that the role of TPX2 is to protect the parasite against the host immune system.[2] Subsequently, Ghosh et al. found that *B. malayi* uses TPX as a protective measure against exogenous sources of reactive oxygen species.[3] *Bm* TPX2 is localized on the surface of the worms, which is an ideal position for this protein to detoxify toxic oxygen and protect the parasite from damage.[4] However, there is very little known about the *Tpx2* gene regulation, thereby making it challenging to fully understand this gene and also limiting ways in which we can overcome the infective L3 stage of *B. malayi*.

The objective of this project is to identify essential regulatory and core promoter domains of the *Bm Tpx2* promoter. Thus far, the *Bm Tpx2* promoter has been successfully amplified and cloned into the mCherry-1 plasmid (wild type construct). A 30 bp replacement mutation strategy was designed to make mutations in the wild type *Tpx2*-mCherry construct. We have successfully made 7 mutations using this method.

During this fellowship, we constructed a positive control plasmid by inserting a known *Brugia malayi* Heat Shock Protein 70 promoter in the mCherry plasmid. Then, we maintained five cultures of *Brugia* L3 worms and transfected these worms with the following samples: positive control (HSP70-mCherry), negative control (only mCherry), wild type (*Tpx2*-mCherry) and mutated samples. A Leica M205 FA fluorescence microscope was used to detect mCherry signal. At this initial stage of investigation, we have seen promising results, but further work is required to make any firm conclusions.

(Supported by the Blakeslee Fund in the Biological Sciences, Shakya and the Howard Hughes Medical Institute, Amin)

Advisor: Steven A. Williams, Biological Sciences

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[4]Saunders, L. J. (2000). The localization, differential expression, and potential immunological role of thioredoxin peroxidase-2 (TPX-2) in the filarial parasite Brugia malayi. Electronic Doctoral Dissertations for UMass Amherst. Paper AAI9978550. http://scholarworks.umass.edu/dissertations/AAI9978550.

Identifying Specific Zebrafish Radial Fibers 2, 3, and 4 Protein Targets in the Developing Zebrafish Brain: *Zrfs Define the Diencephalic Glial Bridge as a Heterogeneous Population of Astroglial Cells*

Risha Sinha/2014

In the developing nervous system, axons are guided across the midline by contact attractants and repellants.[1] This midline-crossing event leads to the formation of commissures, a cluster of nerve fibers that serve to connect the two sides of the central nervous system. The Barresi lab uses the zebrafish model system to study the developmental process of commissure formation at the biochemical, molecular, and cellular levels.

Previously, Barresi and colleagues have implicated astroglial cells as a supportive substrate to promote axonal growth across the forebrain midline.[2] Astroglial cells express glial fibrillary acidic protein (Gfap), which can be identified by the antibody Zebrafish radial fiber 1 (Zrf-1). There are three additional Zrf proteins (2-4), and all four ZRFs were made to understand the structuring of neural segments in embryonic zebrafish hindbrain.[3] Imagining using these antibodies in the forebrain shows differential Zrf expression in astroglia, suggesting different astroglial cell types may be recognized by these antibodies. Unfortunately the antigenic identity of these Zrf2-4 antibodies is unknown, but, based on their embryonic expression patterns, could reveal important insights upon glial development.

The goal of this project is to determine the proteins that are being recognized by the Zrf-2, 3, and 4 antibodies using biochemical techniques such as western blots, immunoprecipitation, and LC/MS (high performance liquid chromatography coupled with mass spectrometry). This summer, we were able to use these techniques to confirm that Zrf-1 targets Gfap. We are currently finishing up identifying the protein target for Zrf-3 using this technique. Identification of these proteins will provide new insight into the development of these important but significantly understudied astroglia.

As a biochemistry major and neuroscience minor, I will be continuing to develop this project into a biochemistry honors thesis. This research was presented at the International Society of Developmental Biologists Conference in Cancun, Mexico this past June by Paula Zaman ('13).

(Supported by the National Science Foundation)

Advisor: Michael Barresi, Biological Sciences

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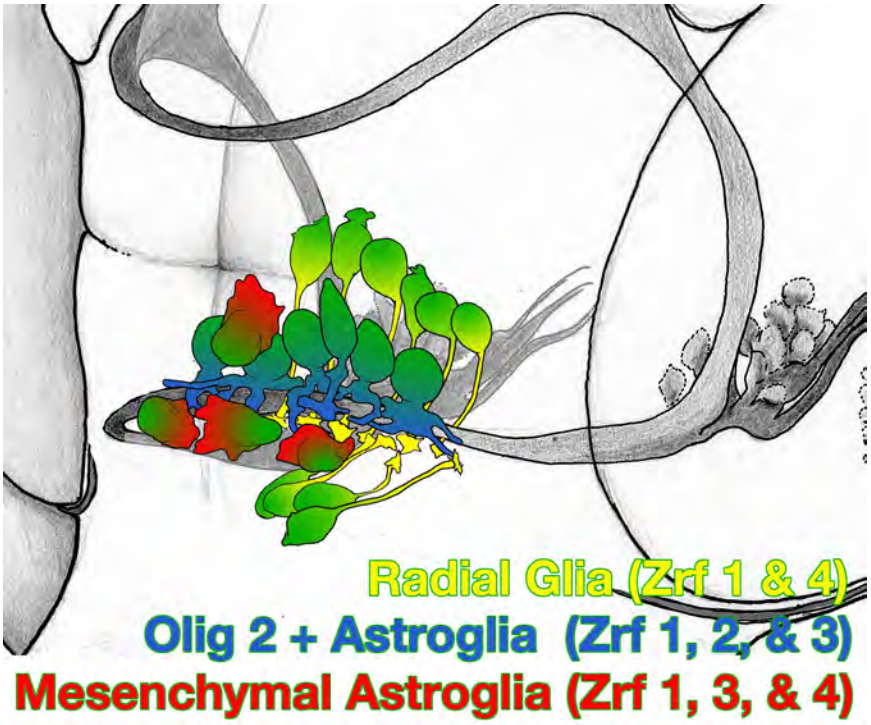

Radial Glia (Zrf 1 & 4)
Olig 2 + Astroglia (Zrf 1, 2, & 3)
Mesenchymal Astroglia (Zrf 1, 3, & 4)

Investigation of Forest Floor Microinvertebrate Communities in Contrasting Hemlock vs. Deciduous Forest Patches at the MacLeish Field Station, Whately, MA

Rebecca Taylor/2016 and Stephanie Acevedo/2015

How will forest floor mesofauna populations change in response to hemlock decline? Eastern Hemlock (*Tsuga canadensis*) is currently threated by an exotic pest from Asia: the Hemlock Woolly Adelgid (HWA; *Adelges tsugae*). Ecologists predict that Hemlock may be eradicated from New England forests in coming decades due to the impacts of this pest, as has happened in other parts of the eastern U.S., where Hemlock has been replaced by Black Birch (*Betula lenta*) and other deciduous tree species.[1] This loss is thought to have many effects on the forest understory environment and ecosystem processes, as Hemlock is considered a key "foundation species" due to its large and distinctive effects on micro-climatic and nutrient cycling conditions on the forest floor.

In Fall 2011, research plots were established at Smith College's MacLeish Field Station in Whately, MA to compare adjacent Black Birch and Hemlock-dominated forest patches. These plots included three distinct ~25 year old Black Birch-dominated forest gaps measuring ~20 x 20 m in size, that were generated by logging in the late 1980s. These gaps are surrounded by an ~100 year old Hemlock and Northern Hardwoods secondary forest. Three 10 x 15 m plots were established in the young Black Birch forest patches and 6 plots in the mature Hemlock forest adjacent to the Birch gaps. In June-August 2013, we collected forest floor samples including decomposed leaf litter and soil organic matter from the 3 Black Birch plots and 6 adjacent Hemlock plots. Ten samples including ~25 g of field-moist material were collected per plot (n=90). These samples were then placed in Berlese funnels to extract invertebrate mesofauna. Mesofaunal taxa were grouped into 3 broad groups: mites, collembolans (springtails) and pseudoscorpions. Soil samples were also evaluated to obtain moisture content for each habitat. Cover boards were surveyed every two weeks, from May 22nd to July 30th, to assess salamander populations.

We found there was no significant difference of the number of microinvertebrates per gram dry mass of organic material between the two habitats in the 90 organic matter samples from June 2013. Only mites were present in any substantial numbers in the June 2013 samples, comprising 81% of the total microinvertebrates detected; collembolans made up only 7% of the samples. On average, there were 1.0 microinvertebrates per gram (± 0.1 SE) in the Black Birch organic material compared to 1.1 microinvertebrates per gram (± 0.1 SE) in Hemlock organic material (t-test $p >> 0.05$). This non-significant result stands in contrast to our findings from Summer 2012, when significantly more mites, collembolans, and pseudoscorpions were detected in samples from the Hemlock plots. The year's inconclusive result could be due to a variety of factors. For example, it is possible that micro-invertebrate populations are highly dynamic and vary year to year, by season, or with climatic conditions. In 2013, we collected the organic material samples in early June rather than late July (2012); as such, these early summer samples had been exposed to cooler conditions and more continuous moisture than those from late summer in 2012. If micro-invertebrates are sensitive to forest floor moisture and temperature, the more moderate climatic condition prior to the 2013 sample collection might have favored a more homogenous distribution of microinvertebrates between the two habitats. In 2012, after a prolonged dry spell, micro-invertebrates may have been concentrated in the deeper organic layer material under Hemlock trees as compared to thinner organic layer under the young Black Birch canopy. Also, we modified the methods used in collecting organic material in 2013. Instead of collecting some of the leaf litter in addition to the organic soil, we only collected organic matter that had decomposed enough to be unidentifiable in 2013. It is possible that by excluding identifiable leaf litter from these samples, we excluded a major part of the mesofauna community that differs between these two habitats. To investigate this possibility, we collected an additional set of 40 forest floor samples in July 2013 that divided material by decomposed leaf litter vs. organic soil. These samples will be analyzed later in Fall 2013 to provide more insight to the distribution of microinvertebrate taxa in these forest habitats.

(Supported by B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Jesse Bellemare, Biological Sciences

[1] Orwig, D. A. and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. J. Torrey Bot. Soc. 125: 60-73.

Jesse Bellemare at work in a Black Birch-dominated plot

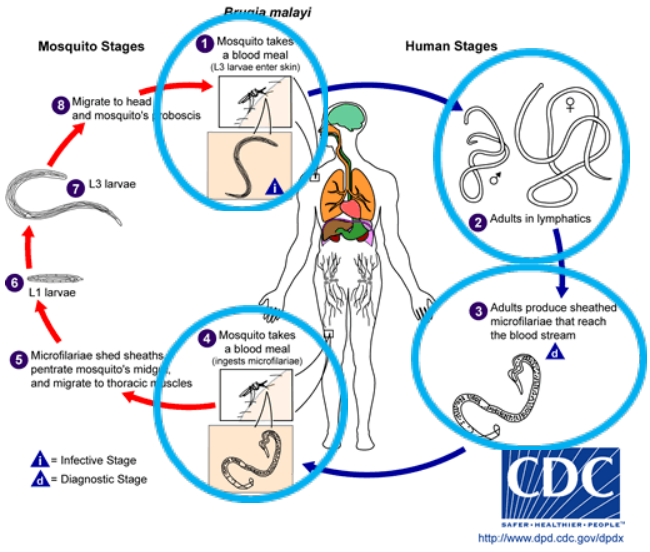
Salamander boards and leaf litter baskets

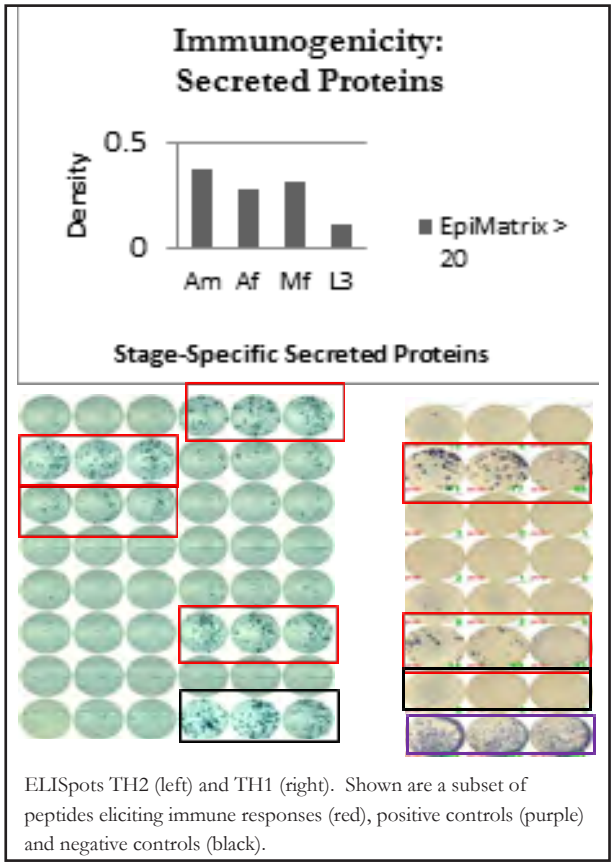
Salamander boards in a Black Birch-dominated plot

Salamander boards in a Hemlock-dominated plot

Defining T Cell Epitopes for Filarid Immunopathogenesis and Vaccine Design

Melissa Torres/GR2013





ELISpots TH2 (left) and TH1 (right). Shown are a subset of peptides eliciting immune responses (red), positive controls (purple) and negative controls (black).

The research done this summer has and continues to provide a new perspective for vaccine design efforts by promoting the development of a T cell based vaccine against lymphatic filariasis (LF). This work is essential to the public health concern of humans living in the 72 developing countries endemic for LF.

This summer, I continued the comparative data analyses of over 11,000 proteins from one of the three causative agents of lymphatic filariasis—*Brugia malayi*. This analysis compared putative immunogenicity of stage specific secreted proteins and, more specifically, those secreted during stages present inside the human host: third larval stage, adult males, adult females, and microfilarial worms (stages shown above circled in blue). Putative immunogenicity scores from the immunoinformatics software, EpiMatrix, were compared across the different stages to reveal significantly lower overall immunogenicity of proteins secreted during the third larval stage ($p < 0.05$). Results from this *in silico* study support recent findings by Wammes *et al.* (2012) indicating the importance of host immune evasion by going into "stealth mode." That is, proteins secreted during this stage are not recognized by human T cells which play instrumental roles in helping our immune system develop specific pro-inflammatory responses.

Validation studies provide proof-of-concept for the predictions made by the EpiMatrix software. Thus, I simulated infection and recorded results using ELISpots. ELISpots are used to detect the presence of immunological signaling proteins, cytokines, using cytokine specific antibodies. In the beginning of the summer, I started my work with white blood cells of patients naïve of infection and pathogen. Results from *in vitro* validation steps revealed a portion of putative T cell epitopes tested demonstrated pro-inflammatory responses promoted by T helper 2 (TH2) responses as well as T helper 1 (TH1) responses. A portion of these results, shown in the above ELISpot images, validate a subset of tested peptides to be true effector T cell epitopes. This summer of science was ended at the Center for Disease Control (Atlanta,GA) where results from work on white blood cells of patients from LF endemic areas will be analyzed this upcoming fall.

(Supported by the Blakeslee Fund in the Biological Sciences)

Advisor: Steven A. Williams, Biological Sciences

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Antihelminthic Effects of Neurolenins A and B on *Brugia pahangi* L3 Larvae

Kristine Trotta/2014

Lymphatic filariasis is a parasitic roundworm disease caused by three species of nematode (*Wuchereria bancrofti, Brugia malayi, and Brugia timori*). Previous research has suggested that *Brugia pahangi*, a cousin of *B. malayi*, is susceptible the ethanolic extract of the leaves of *Neurolaena lobata*, a Central American medicinal plant.[2,3,6] Neurolenin A and Neurolenin B (Figure 1) are natural products derived from *N. lobata* that have been identified as potentially bioactive agents against *B. pahangi*. This stage of the project serves to assess the *in vitro* efficacy of Neurolenins A and B alone and in concert against *B. pahangi* for the purpose of developing a new pharmaceutical treatment for lymphatic filariasis.

Crystalline Neurolenin A (17.8 mg) was isolated from a dichloromethane extract of 1 kg dried *N. lobata* leaves (The Arvigo Institute, LLC).[4] A stock solution of Neurolenin A was prepared by dissolution of the crystals in DMSO (0.05 mg/μL) and dilution in physiological saline (500 μg/mL).[1] A crude 1:1 mixture of Neurolenins A and B was also extracted from *N. lobata,*[4] as monitored by ^{1}H NMR spectroscopy. A stock of the Neurolenin mix was prepared by rotary evaporation to dryness (0.626g), reconstitution in DMSO (0.05 mg/μL) and dilution in physiological saline (500 μg/mL).[1] Neurolenin B could not be recovered as an isolate.

Assessment of the L3 molt of B. pahangi is a model for the human-infective stage of B. malayi. L3 B. pahangi nematodes were grown and shipped from the College of Veterinary Medicine at the University of Georgia. Upon receipt, worms were washed three times in RPMI 1640 (Gibco-Life Technologies) containing a mixture of Penicillin Streptomycin, Gentamycin, and Fungizone (Amphotericin B) to prevent bacterial and fungal contamination of the cultures.[5] Worms were plated in aliquots of 100 on 6-well plates in a complete Minimal Essential Medium (Gibco-Life Technologies) containing 10% Fetal Bovine Serum (Gibco-Life Technologies lot #1195877), Penicillin Streptomycin, Gentamycin, Ciproflaxin, and Fortaz (Ceftazidime).[5] Plates were incubated throughout the culture at 37°C.

Five days after plating, worms were treated with Vitamin C (15 μg/mL) to induce molting, and either Neurolenin A (titration: 500 μg/mL, 0.500 μg/mL, 0.250 μg/mL, 0.125 μg/mL, 0.0125 μg/mL, 0.00625 μg/mL, 0.00325 μg/mL) or the Neurolenin mix (0.6 μg/mL, 0.5 μg/mL, 0.4 μg/mL). Each culture also included a DMSO control and negative control. Mortality was assessed by death count every four to eight hours until death. Health of the cultures was determined by the ability of the control worms to molt, as reflected the number of shed cuticles on Day 11 of each culture. The average molts for negative controls were 45.67% and for DMSO controls were 54.5%, indicating that the worms were moderately healthy and that the DMSO used in the reconstitution of the compounds was not detrimental to the health of the treated worms.

Neurolenin A was an ineffective killing agent against B. pahangi. (Figure 2). The mix of Neurolenins A and B, however, was successful in achieving 100% death in vitro between 48 and 72 hours (Figure 3). In contrast, the crude ethanolic N. lobata extract previously being used to treat B. pahangi in the Williams lab was able to kill L3 by 72h post-treatment at an optimal concentration of 300 μg/mL5. In short, the Neurolenin mix is able to achieve similar results to the crude extract, but at dosages almost 1000 times lower. This may either indicate that Neurolenin B is a bioactive compound, or that Neurolenins A and B work in concert to kill B. pahangi L3. This is an optimistic step toward developing a potent, low-dose drug against LF-causing nematodes. Further work on this project will assess the effects of pure Neurolenin B on L3 B. pahangi, as well as sex-specific mortality studies on adult B. pahangi and gene expression studies on treated nematodes to determine the molecular explanation for N. lobata's efficacy against LF-causing nematodes.

(Supported by the Shultz Foundation)

Advisor: Steven A. Williams, Biological Sciences

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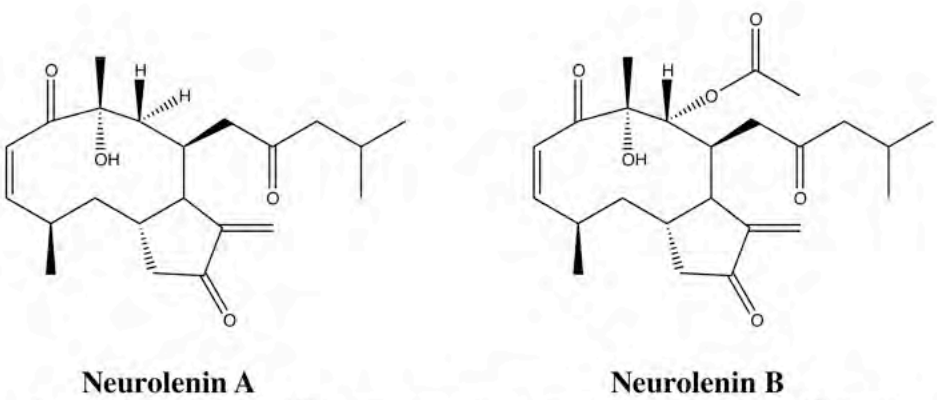

Figure 1: Neurolenins A and B are germacranolide sesquiterpene lactones found in *Neurolaena lobata* with potential bioactive properties against *Brugia pahangi*.

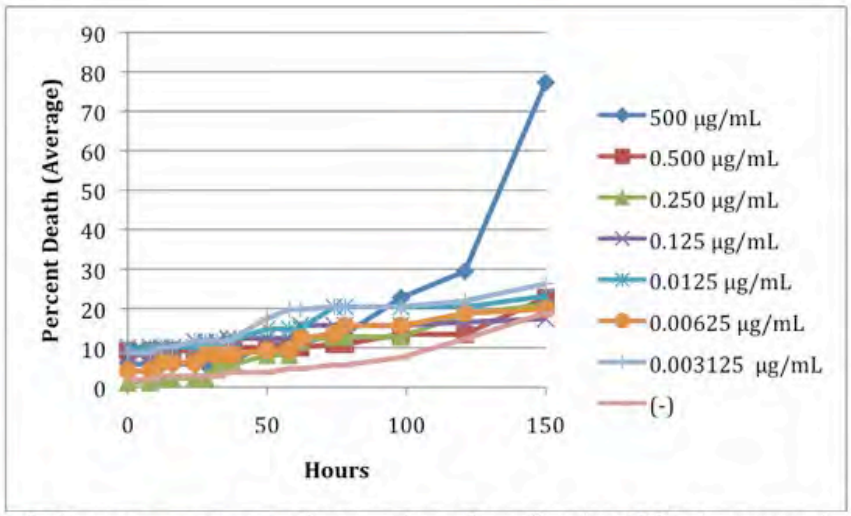

Figure 2: Average percent mortality over time of one *B. pahangi* L3 culture treated with a concentration titration of pure Neurolenin A. This compound is not potent enough on its own to cause significant or timely death in the L3 molt. (Note: '(-)' refers to a negative control.)

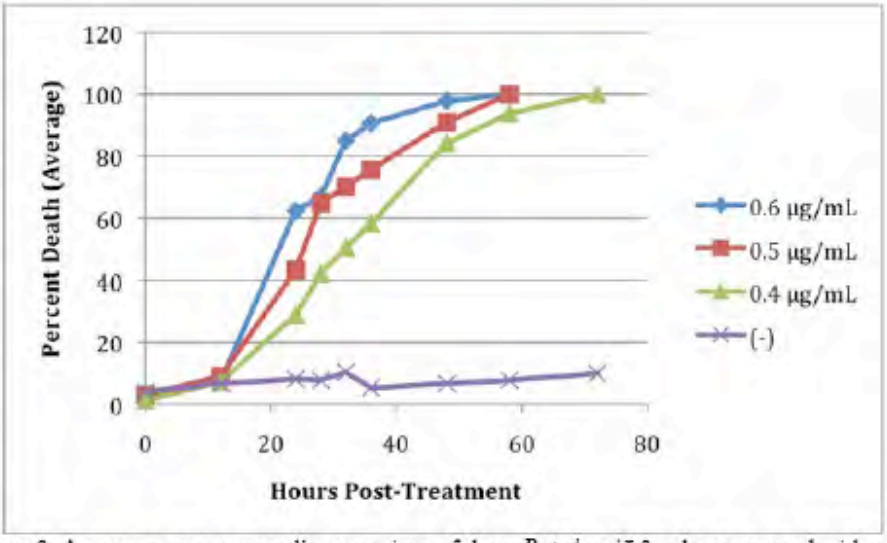

Figure 3: Average percent mortality over time of three *B. pahangi* L3 cultures treated with a 1:1 mixture of Neurolenins A and B. Worms treated with 0.6 µg/mL and 0.5 µg/mL of the mix achieved 100% death by 58 hours, while worms treated with 0.4 µg/mL of the mix achieved 100% death by 72 hours. (Note: '(-)' refers to a negative control.)

Renovation of the Smith College Systematics Beds

Katharine D. Wilson/2015

The systematics beds of Smith College are a historical, aesthetic, and educational part of the Botanic Garden. Each bed in the garden represents a different plant family, facilitating the study and comparison of the morphological and evolutionary relationships of the families. This has been a fixture of Smith biological studies since the late 19th century, but the current organization is outdated. I continued the research and planning that had been done by previous students in 2011-12 to recreate and modernize the gardens.

The newest research in plant phylogenies focuses on molecular as well as morphological data. I used APGIII phylogenies on the Angiosperm Phylogeny Website[1] to redefine which families would be represented and to locate them in evolutionary order in the garden. I organized the taxa that were below family level using *Tropicos,* a website of all of the Missouri Botanic Garden's electronic records, and *The Plant List*, a collaborative list of all known plant species.[2, 3] The taxa that were held in the old systematics beds were culled, leaving only those that were still of educational value, had a long flowering time, and were not cultivated varieties or hybrids.

Using these resources I was able to update the systematics beds family list from 30 families to 62 families. The old systematics beds were dismantled after they were culled with assistance from the Manager of Living Collections and the Assistant Curator and Gardener. The last step of the project was the design of a map on ArcMap using a preliminary draft made by previous students.[4] The map shows the placement of the beds on the plot of land as well as the new stepping stones that delineate major evolutionary boundaries.

The systematics garden renovation is now in progress. This map will be used to physically add the new beds and place the new boundaries. Because it is on ArcMap, it can be manipulated to reflect further changes. The arrangement of the beds has increased educational value because it is updated to reflect the APGIII system. This coming semester further research will focus on populating the beds with appropriate taxa as well as creating interesting interpretive labeling.

(Supported by the Schultz Foundation)

Advisors: Michael Marcotrigiano, Director, Botanic Garden and Jesse Bellemare, Biological Sciences

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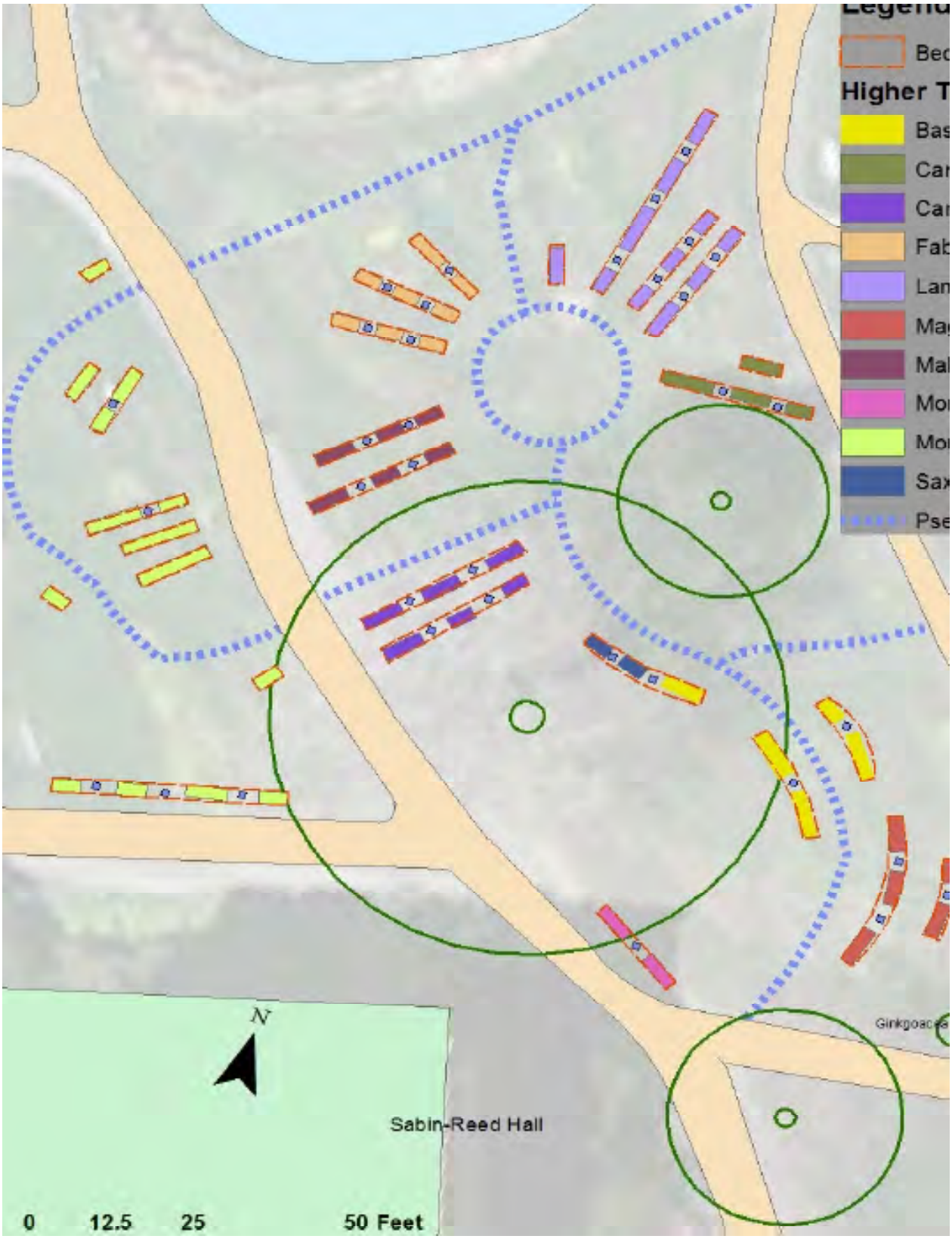
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Assessment of the Filaricidal Efficacy of *Centella asiatica* Against the Parasitic Worm *Brugia pahangi*

Marika Witkus/2014J

Lymphatic filariasis (LF) is a neglected tropical disease that affects over 100 million people, and is the leading cause of disability world-wide.[1] LF is caused by the human parasites *Wucheria bancrofti, Brugia malayi* and *Brugia timori*. These filarial worms are transmitted by mosquito vector to the human host and reside in the lymphatic system, causing swelling, lymphoedema, and elephantiasis. The worms can live for 6-8 years in the lymphatic vessels, and shed microfilariae into the blood which can be transmitted to a new hosts.[2] In 2000 the WHO launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF). Who set the goal to eradicate LF by 2020 through mass drug administration (MDA) and morbidity management.[3] The current drugs that are used to treat LF only target one life stage of the parasite, and must be administered for 4-6 years to completely disrupt transmission.[2] In order to reach the GPELF's goal and completely eradicate this disease, new treatments must be developed that target the macrofilariae life stage. Saira Huq and Jessica Wignall's work on the medicinal plant *Neurolaena lobata* paved the way for further investigation into antifilarial natural products, as they designed a procedure for assessing filaricidal activity using the parasite *Brugia pahangi*, a close relative to *Brugia malayi* and the causative agent of LF in domestic animals. Following the first semester of research for my honors thesis, my goal was to investigate the filaricidal efficacy of the medicinal herb *Centella asiatica* on various life stages of the filarial parasite *B. pahangi,* determine the bioactive constituents of *C. asiatics*, and collect RNA from adult females to use in future expression analysis.

Larval stage 3 (L_3) *B. pahangi* were cultured, according to a protocol developed by Susan Haynes, and treated with three concentration of *C. asiatica* ethanolic extract (1,400ppm, 1,600ppm and 1,900ppm) along with two controls, one treated with ethanol, and one without. A motility assay was used to assess percent mortality of the worms and motility was observed every 4-6 hours for 72 hours after the initial treatment. Each treatment was done in duplicate, and three separate cultures were performed. Following the L_3 cultures, adult males and females were cultured and treated following the same protocol. After the initial L_3 and adult cultures, a fourth culture of L_3 worms was treated with analytical grade asiaticoside and asiatic acid, two compounds signature to *C. asiatica* and found at high concentration in the dry plant material. The *B. pahangi* were treated with 60, 30, 15, 10, and 5ppm of each compound following the same culture protocol. Following these cultures, on two separate occasions, thirty adult females were treated with 1,400ppm ethanolic extract and the other thirty served as the control. 10 worms of each treatment were collected and flash frozen at 6, 12, and 18 hours after treatment.

In all three L_3 cultures treated with *C. asiatica*, 100% mortality was reached between 53 and 72 hours. In the three adult male cultures 100% mortality was reached between 42 and 72 hours, and in the adult female cultures, 100% mortality was reached between 30 and 54 hours. Worms treated with the compound asiaticoside exhibited the same behavior as the control, and the worms treated with asiatic acid reached 100% mortality in 24 hours at 60ppm and 72 hours at 30 ppm.

This research has helped to determined the antifilarial effects that *C. asiatica* has on two different life stages of *B. pahangi*, and that asiatic acid may be the active constituent in the herbal extract. I plan to continue this work for the second semester of my honors thesis to further validate the efficacy of asiatic acid as an antifilarial, and investigate some of the other triterpenoid saponins that are signature to *C. asiatica*. I plan to use the RNA collected from the treated adult females to run an expression analysis to gain insight into how the constituents of *C. asiatica* are causing premature mortality in the parasites. This work will hopefully lead to the development of a potential new drug candidate.

(Supported by the Schultz Foundation)

Advisor: Steven A. Williams, Biological Sciences

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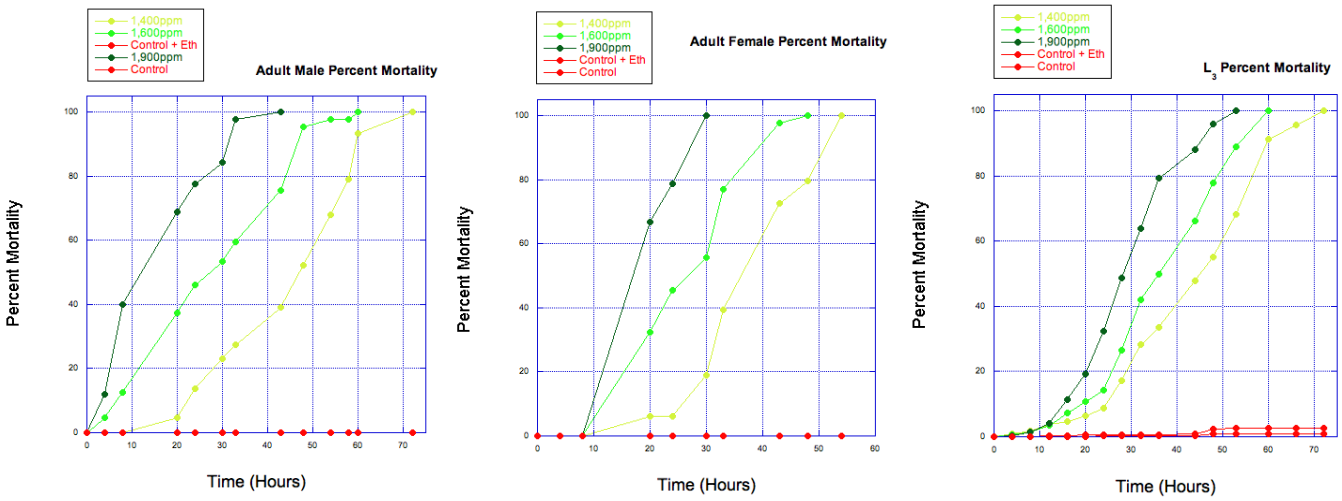

Discovering The Quadrulella Symmetrica

Cameah Wood/2015

No one knows the diversity in the world, not even to the nearest order of magnitude. We don't know for sure how many species there are, where they can be found or how fast they're disappearing. It's like having astronomy without knowing where the stars are."(Wilson) This summer one of the main focuses within my research was looking at the abundance of a microbial species of testate amoebae called the *Quadrulella Symmetrica*. Testate Amoebae dwell in freshwater, bogs, and fens, and tend to inhabit environments with a low Ph.

We collected our samples this summer from Acadia National Park, Bear Swamp, and Hawley Bog. From here we collect sphagnum moss (vegetation in which the Amoebae live). We also collect water to fill our petri dishes. We then return to the lab, and make samples in petri dishes with our water and sphagnum. From there we look at our samples using a light microscope followed by photo documentation, genome amplification, PCR, and sequencing.

As result, we can say from what was observed that the species abundance of *Quadrulella Symmetrica* has changed over time. At one time, this species was more abundant in a location at Hawley Bog, but now there is little to none, as opposed to Bear Swamp where only Quads as well as *Nebela species* are found. When looking at the genetic data, it can be seen that there is not much variation in genetics between the species. When placed in a phylogenetic tree with other species of testate amoebae, it can be seen that all the Quads fall into their own clade, suggesting that there are no cryptic species.

From here, knowing that there are not many genetic differences between individuals within the species, more attention has to be directed to the ecological factors that are having an effect on the species abundance. I plan on continuing this work through my special studies and hope to write a paper for publication on my findings.

(Supported by the National Science Foundation)

Advisor: Laura Katz, Biological Sciences

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Identification and Confirmation of *PDFR-1* Functional Conservation in *Brugia malayi*

Jenna Wurster/2014

The ultimate aim of this ongoing project is to correctly identify, isolate, and confirm circadian clock functionality of the pigment dispersing factor receptor 1 [*pdfr-1*] gene in the parasite *Brugia malayi*. Confirmation of *pdfr-1* functionality would both facilitate a better understanding of this parasite and perhaps provide a novel target for further lymphatic filariasis [LF] drug designs. LF is the result of a parasitic infection from the filarial nematodes: *Brugia malayi*, *Brugia timori* and *Wuchereria bancrofti*.[1] LF causes lymphedema and elephantiasis, and has left over 43 million people officially disabled.[2] In response, the World Health Organization has organized the Global Programme to Eliminate Lymphatic Filariasis (GPELF), the goal of which is to eradicate LF as a public-health problem by 2020.[1]

Previous work has narrowed down a candidate circadian clock gene, pigment dispersing factor receptor [*pdfr-1*], via BLAST search results of both nucleotide and protein sequences. These search results have shown that a particular *Brugia malayi* sequence has over ninety percent sequence similarity to the *Caenorhabditis elegans' pdfr-1* sequence, indicating potential homology between the two genes. With this idea in mind, experimental design has been centered around proving the unidentified *B. malayi* gene possesses the same function as *C. elegans pdfr-1*. Additional work done prior to summer 2013 includes long PCR amplification of the *pdfr-1* regulatory regions from a cosmid vector containing the gene of interest in entirety, PCR amplification of the unidentified gene from a *B. malayi* cDNA library, acquisition of *C. elegans pdfr-1* mutant strains, and design of a transgenic construct using the promoter and regulatory regions of *C. elegans* ligated to the coding regions of the proposed *B. malayi* gene.

The work conducted during summer 2013 has consisted primarily of optimizing a cloning experiment that will efficiently yield successful *C.elegans-B.malayi pdfr-1* transgenic constructs. This entails first using a *pfu*-polymerase enzyme to "blunt" the end of the long-PCR generated fragment in preparation for ligation to the coding region PCR fragments. Due to inherent incompatibility between the two enzymes used to generate the PCR fragments, this polishing, or blunting, is an essential first step in generating the desired constructs. The next major fraction of work done this summer was the optimization of a ligation reaction between these PCR generated fragments that would create a construct of the correct size (oriented in the correct direction), such that it could be successfully ligated into a bacterial vector. Successful ligation reactions resulted from the use of both a 16-hour reaction using T4 DNA Ligase (New England Biolabs®) and from a 15-minute reaction using Quick Ligase (New England Biolabs®), resulting in the need for further identification of the optimal enzyme and incubation time to use. It is still unclear whether the PCR fragment ligation reaction and the insert-vector ligation are optimal with the same ligation conditions, or varying ligation conditions.

While the cloning of this transgenic construct is still in need of further optimization, successful recombinant clones were obtained over the course of this summer. Further work must use the sequencing and PCR diagnostic primers that were designed for *pdfr-1* insert to screen these recombinant clones. The primers are designed such that, if the insert is in the correct orientation and is of the correct size, PCR amplification will generate small identifier fragments. Then, samples that generate the correct identifier fragments will be sequenced to confirm the construct presence and orientation, ultimately allowing for the use of successful constructs in further experiments.

(Supported by the Schultz Foundation)

Advisor: Steven A. Williams, Biological Sciences

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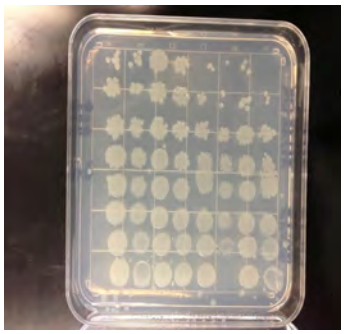
The Role of RpoS and Temperature in Oxidative Stress Response of *E. coli*

Priscilla Yong/2015



One of the many stresses encountered by *Escherichia coli*, oxidative stress is the production of reactive oxygen species such as hydrogen peroxide and a critical host defense mechanism.[1] The sigma factor RpoS regulates the general stress response and is up-regulated in lower temperature, resulting in high expression of RpoS-dependent stress resistance genes at room temperature (23˚C) as compared to host temperature (37˚C). I was interested in the bacterial response to oxidative stress governed by RpoS in the host environment, hoping to gain insight on treatment strategies.

E. coli K-12 commensal MC4100 RpoS wild type and mutant strains were grown in M9 glycerol minimal media at 23˚C. Bacteria grown at 23˚C were shifted to 37˚C to mimic the movement of bacteria into a human host with some cells retained at 23˚C as control. Cells removed after 0, 0.5, 1, and 4 hours were treated with 50mM hydrogen peroxide, and the other half was kept as a control. Samples were incubated for 10 minutes, diluted in a microtiter plate and plated onto agar plates where they were tested for cell viability.

RpoS-dependent mechanism was shown to be the most prominent defense mechanism against oxidative stress in *E. coli*. Primary data indicated that wild type bacteria showed a tenfold higher resistance to hydrogen peroxide stress than the RpoS mutant. Surprisingly, some growth was observed in the mutant, suggesting that there may be RpoS-independent stress resistance mechanisms.

Lower temperature of 23˚C was found to trigger stronger resistance, whether the defense is RpoS-dependent or not. Both the wild type and the mutant showed higher cell viability when grown in 23˚C as opposed to 37˚C. It was hypothesized that the residual RpoS from 23˚C would result in higher stress resistance early on, followed by decreased resistance at 4 hours into 37˚C due to low expression and degradation of RpoS. However, this trend was not observed consistently across data sets.

Inconsistencies in trends, possibly due to small sample size, made it difficult to draw solid conclusions. However, results confirm that RpoS is a central stress resistance regulator and indicate that RpoS-independent, temperature-dependent oxidative stress responses may protect the bacterium in both host and external environments. Optimizing the assay and testing pathogenic strains may be the next step. I have had the opportunity to present my findings to the Sarah Moore lab and my work will continue into the academic year as special studies project.

(Supported by the National Institutes of Health)

Advisor: Christine A. White-Ziegler, Biological Sciences

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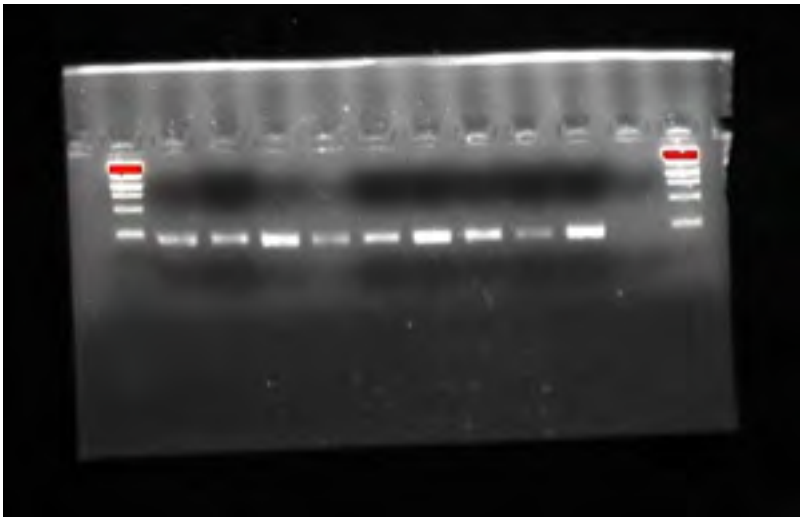
Finding a way to use Denaturing Gradient Gel Electrophoresis on a community of Testate Amoebae

Jennifer Yoo/2016



My lab is focused on looking at certain protists at the molecular level and to see what they can reveal to us and to science. Specifically, I focused on trying to find a way to implement DGGE, Denaturing Gradient Gel Electrophoresis, on a community of testate amoebae, which is a polyphyletic group of unicellular protists characterized by the physical appearances of a test and pseudopodia to name a few. DGGE is a tool that is essentially used to see the diversity of species within a community as one band reveals one haplotype and so this tool could help reveal many unknown things that could potentially make further discoveries.

To do this, I used the tools of PCR, Gel Electrophoresis, DNA Sequencing software such as SeqMan, mAlign, and BLAST. To summarize the process, I would pick about 50 cells of Hyalosphenia Papillio, Hyalosphenia Elegans, and Nebela Tincta into a 1.5 mL tube to be phenol chloroformed, which is a process to isolate the DNA. I would then take the DNA and run a PCR using the primers that I designed by matching base pairs with the specific amoebae species. Throughout the summer I have been successful (as seen in the gel picture above) and the sequences have come back positive. However, at the very end of summer, I had run into a slight problem. I realized that the sequences contained the intron of the fragment of DNA that I wanted and so I had to redesign the primer so that it would exclude the intron and therefore make the fragment smaller, which is ideal for DGGE. After this process, I realized that I had omitted the GC clamp in the primer so then I had to redesign the primer once again.

The purpose of this experiment is to revolutionize the way scientists obtain molecular data for these testate amoebae. Rather than picking each amoebae and running a PCR and gel individually, DGGE presents an opportunity to analyze a whole community of amoebae rather than just a single cell. If I am successful in accomplishing this task, I will fundamentally be opening up a whole new tool for scientists to use to analyze amoebae. I hope to continue working on this project throughout the next school year and I hope to achieve my goal of designing a way to utilize DGGE for these protists.

(Supported by the National Science Foundation)

Advisor: Laura Katz, Biological Sciences

Bioinformatic Analysis of Genome Evolution of Living Ciliates, *Chilodonella uncinata*

Xinzhu Zhou/2014

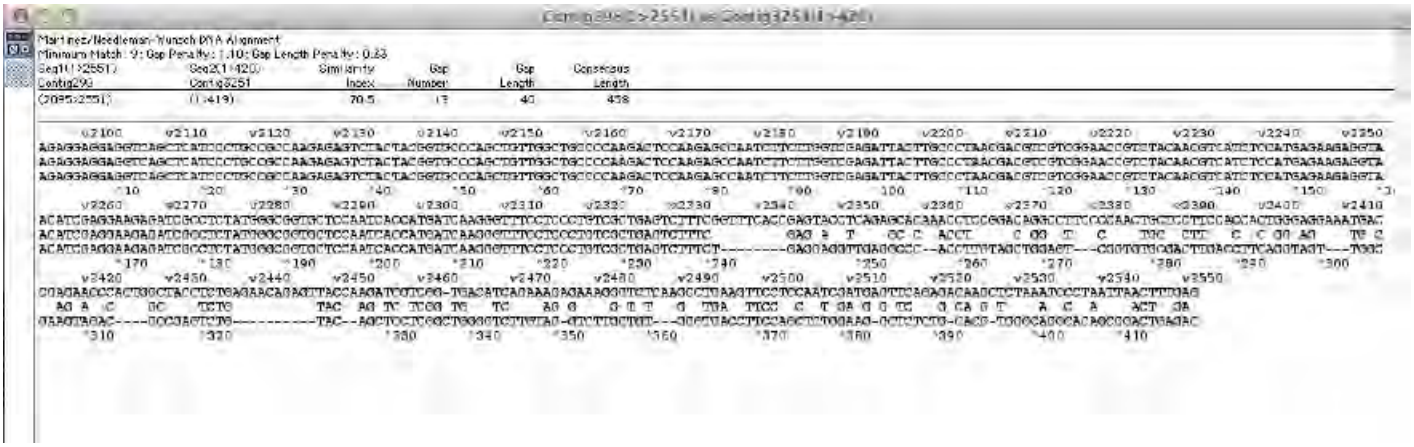


Figure 1. One pair nucleic acid alignment of two contigs of elongation factor 2 using MegAlign (Lasergene, DNASTAR). The divergence between the two contigs might be biological difference or alternative processing, a form of chromosomal rearrangement[1].

The two nuclei of Ciliates, the macronucleus (MAC) and the micronucleus (MIC), contain two separate genomes that could undergo large-scale chromosome fragmentation, duplication, deletion, recombination, alternative processing, and gene scrambling. Given the massive possibilities of sequences arrangement for protein coding in macronucleus, my SURF project in the Katz lab studied the genome evolution of Ciliate lineages by examining chromosomal rearrangement patterns within the two genomes in MAC and MIC and explored its influence on protein family evolution.

With the help of Jessica Grant, research assistant of the Katz lab, and NGen, a bioinformatics software that generates contigs from DNA sequence reads from our transcriptome data, I was able to obtain contigs of functional genes, such as actin, alpha/beta tubulin, vWFA, elongation factor, heat shock protein, and ribosomal protein, that might have played a role in the evolution of *Chilodonella uncinata*. Using alignment software modules from the Lasergene core suite, I aligned the nucleic acid and amino acid sequence of the selected genes, analyzing whether the divergence between any of the two contigs is real biological signal that suggests the existence of paralogs in the gene. Because of the fact that paralogs are indicative of gene duplication, which could lead to speciation, we were very interested in finding paralogs in the contig alignment of *Chilodonella* genes.

We have seen some genes exhibiting patterns of paralogs, suggesting biological divergence (e.g., elongation factor 2 in Figure 1). However, no definitive results are available at this stage; we are still gauging the best matching contig percent cut off in NGen. Since I am going to continue working on this project in my senior Honor Thesis, further bioinformatic analysis will yield clearer patterns of paralogs in protein coding genes of *Chilodonella uncinata*.

(Supported by Blakeslee Fund in the Biological Sciences)

Advisor: Laura Katz, Biological Sciences

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Longer-Term Changes in Soil Nitrogen Cycling as a Result of Eastern Hemlock Removal

Jenna Zukswert/2013 and Alana McGillis/2015

Eastern hemlock (*Tsuga canadensis*) trees in New England are threatened by the recent spread of the exotic hemlock woolly adelgid (*Adelges tsugae*) and pre-emptive logging motivated by this infestation.[1] Because eastern hemlock is a foundation species, meaning that it strongly influences environmental conditions, such as soil nutrient cycling, where it is found, hemlock removal is bound to have large consequences for the forest and its inhabitants.[1] This study began in 2011 to compare soil nitrogen cycling in a mature hemlock-dominated plot and a plot dominated by young black birch (*Betula lenta*) produced when hemlocks were logged 25 years ago at Smith's MacLeish Field Station. In 2012, we added a nearby mature deciduous forest plot. We measured the net production of ammonium (NH_4^+) and nitrate (NO_3^-), nitrogenous compounds used by plants, by performing soil incubations. We took "initial" soil cores from each plot and immediately measured NH_4^+ and NO_3^- concentrations in the laboratory using ion chromatography. On the same day, we collected cores near the initial cores, but placed them back into the ground in PVC sleeves and let them incubate for 3+ weeks before measuring NH_4^+ and NO_3^-. NH_4^+ and NO_3^- concentrations in initial and incubated cores were used to determine net N mineralization (N_{min}, net production of both NH_4^+ and NO_3^-) and net nitrification (net production of NO_3^-) rates. We expected black birch soil N cycling rates to more closely resemble those in the mature deciduous plot than in the hemlock plot due to greater similar in leaf litter chemistry, which influences N cycling.[2]

In 2011, black birch and hemlock soils had similar net N_{min} rates, and these rates remained low in the black birch plot throughout the study, more closely resembling hemlock than mature deciduous soils. This suggests that soil N cycling may not yet reflect differences in tree species composition. Surprisingly, the production of NH_4^+ peaked in Summer 2012 in the hemlock plot. Several hemlock trees died, possibly due to the adelgid, explaining the observed "pulse" in net N_{min} rates, also seen in a recent study at Harvard Forest,[3] which we predict will be a short-term response to hemlock mortality. Our results suggest that ecosystem "function" (soil N cycling) may change decades later than "structure" (tree species composition) following disturbance. These results were included in a manuscript we prepared this summer and recently submitted for peer review.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences, Center for the Environment, Ecological Design & Sustainability, Zuskwert and the Schultz Foundation, McGillis)

Advisors: Jesse Bellemare, Biological Sciences and Amy L. Rhodes, Geosciences

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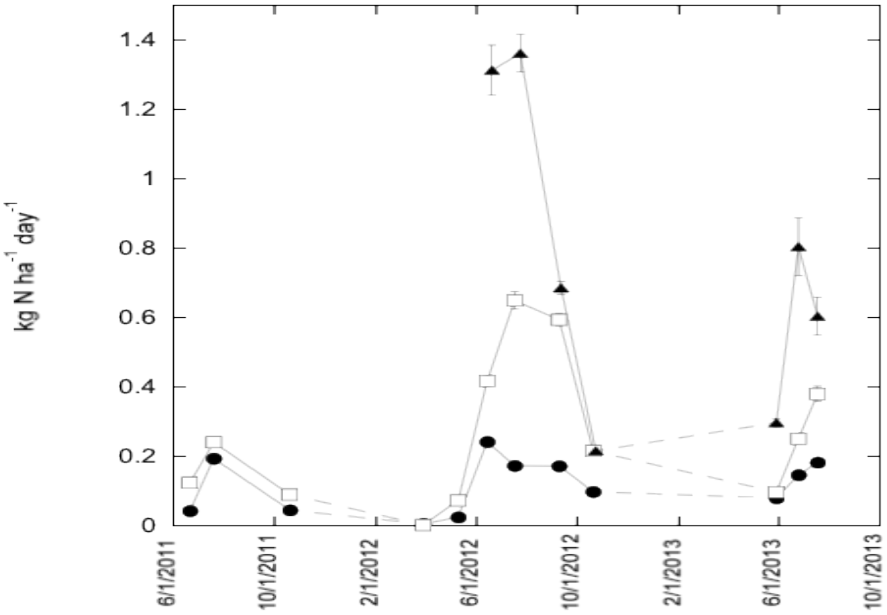

Net N mineralization rates (and standard deviation) in our Hemlock (white square), Black Birch (black circle), and mature deciduous (black triangle) plots from 2011 through 2013. The rate plotted at the midpoint of the incubation period. Dashed lines indicate periods where no incubations were occurring.

Synthesis and Purificationof Spiroiminodihydantoin Lesion-containing DNA Duplexes for Structural 2D NMR Studies

Emilia Argüello/2016

Oxidative damage in the nucleobases of DNA can be caused by DNA-damaging agents, such high valent metals, [1, 2] and it can result in premature cell aging, mutagenesis, and carcinogenesis.[3] Guanine, in particular, has a low reduction potential, [4] and is easily oxidized to 7,8-dihydro,8-oxo-2'-deoxguanosine (8-oxoG). Moreover, 8-oxoG, which has an even lower reduction potential, [2] is susceptible to undergo further oxidation when exposed to the high-valent metal Cr (VI). One of the products is an enantiomeric pair of a hyper-oxidized guanine known as the spiroiminodihydantoin (Sp) lesion, [2, 5, 7] which is highly mutagenic[2, 6] and can compromise the configuration and stability of the DNA double helix.[7] The primary focus of this project was to synthesize enough of a lesion-containing 11-mer oligonucleotide duplex to prepare a sample suitable for structural analysis using two dimensional nuclear magnetic resonance (NMR) spectroscopy. The spectroscopic portion of this study was carried out in association with Prof. Cristina Suarez's research group.

The single stranded, Sp-containig adducts were made by oxidation of 5'-(CCATC-**8oxoG**-CTACC)-3' strands through a reaction with the sodium hexacloroiridate(IV) hexahydrate. The samples containing 12μM 11-mer 8-oxoG oligonucleotides were reacted with 100μM $Cl_6IrNa_2 \bullet 6H_2o$ at 65°C in a buffer composed of 100mM sodium chloride and 10mM sodium phosphate (pH 7), for a total of 30 minutes. The reaction was quenched with 20mM EDTA, and the iridium salt was removed with cellulose membrane centrifugal devices. The two Sp enantiomers, which become diastereomers when attached to DNA's deoxyribose, were separated and purified by HPLC (Figure 1). The collected products were desalted using cellulose membrane filters. The filtered, purified diastereomeric Sp-containing single strands were annealed to their 5'-(GGTAG**C**GATGG)-3' complementary strand at 90°C for 3 min with slow cooling to room temperature in a 10% D_2O medium and 90% aqueous medium composed of water, 100mM sodium chloride, and 10mM sodium phosphate.

Several two dimensional NOESY NMR experiments using 3mm diameter tubes have been attempted up to date. The first of them, involving relatively dilute 0.26mM samples containing 65 nmol of the duplexes, did not display any characteristic DNA cross-peaks. The concentration of the first diastereomeric sample was raised to 0.5mM, now having 125 nmol of the duplex. The NOESY NMR experiment carried out with this more concentrated sample is still in process, yet results seem promising. Visible cross peaks in the aromatic and imino regions of the DNA double strand have been located, and some have already been characterized. The sample containing the other Sp diastereomer still remains at 0.26 mM until the current experiment is concluded and understood.

During the course of this project, enough of both diastereomeric lesions were prepared for the first significant NMR experiment, and there are even additional nanomoles of each diastereomer available to prepare future samples suitable for upcoming NMR runs. Future research, including the use of shigemi NMR tubes and the study of lesion-containing duplexes where Sp is paired with guanine, is expected to continue under the special studies program during the fall of 2013.

(Supported by the Schiffer Fund)

Advisor: Elizabeth R. Jamieson, Chemistry

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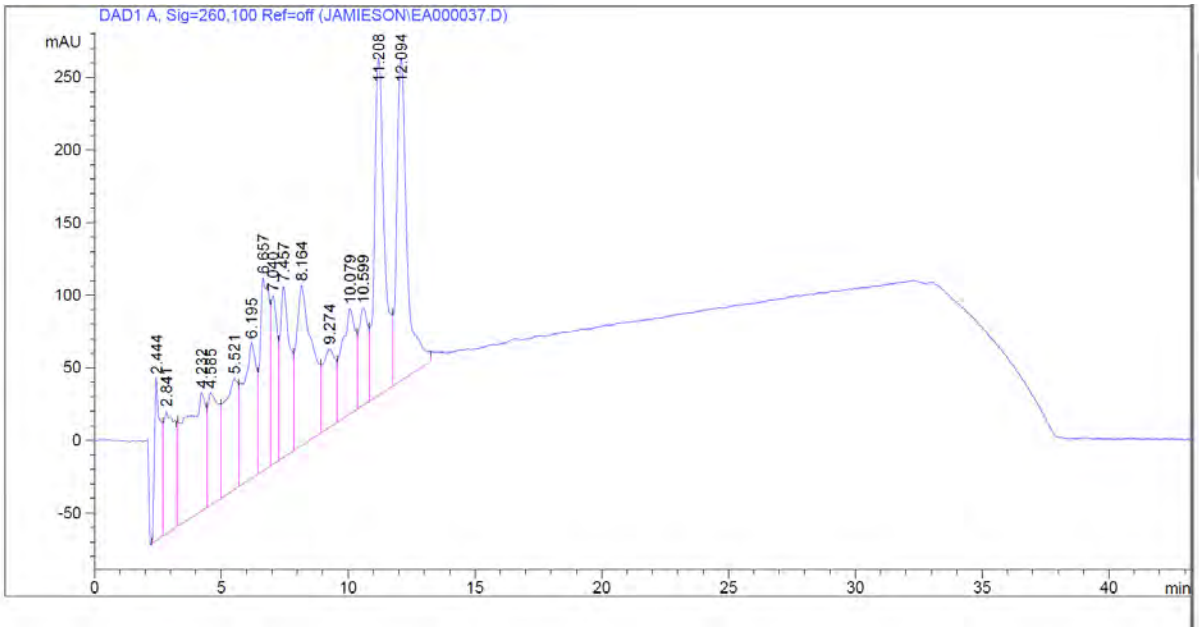

Figure 1: HPLC chromatogram of the purification and separation of the two Sp lesion diastereomers. The tallest peaks at 11.2 min. and 12.1 min. indicate the appearance of both diastereomers, detected by the HPLC UV-Visible detector at a wavelength of 260nm.

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An Investigation of the Reactivity of Carbonyl Compounds with CH_3Li

Theresa Baffour/2014

Houk et al. published a study giving detailed mechanistic information about the reactions of alkyllithiums with carbonyl compounds. Their study was focused on formaldehyde and monomers of methyl lithium.[1]

This study mimicked the approach of Houk et al. but included more carbonyl compounds. The objective of the study, therefore, was to investigate the reactivity of other carboxylic acid derivatives with methyl lithium. In addition, this research tried to explain the reasons for the trend in reactivity.

The data obtained from this research were results from calculations done using Gaussian 09 program with basis set M06/6-31 as well as Amsterdam Density Functional (ADF2013) program. The investigation involved calculating the energies of intermediate structures and the energies at the transition state. From these the activation energy of each reaction was calculated. The activation energy gives information about the difficulty in forming the activated complex.

Also the distances between the carbonyl carbon and the methyl anion in the transition structure were investigated and recorded and used to help explain differences in activation energies and the general trend in reactivity of carboxylic acid derivative compounds and methyl lithium.

The results showed that the activation energy increased generally from acid chlorides to amides. Also, the distances between the carbonyl carbon and methyl anion decreased generally from acid chloride to amide, showing that the transition structure lies very early in the reaction coordinate of acid chlorides, while it occurs later in amides. Therefore, it was shown that there was generally a decrease in reactivity of carbonyl compounds with methyl lithium going from acid chlorides to amides.

The results show that to an extent that reactivity generally decreased as the group attached to the carbonyl group changes from a chlorine atom to an amide group. This may be because of the charge of the carbonyl charge, which is influenced by how electronegative the group attached is. There is a general decrease in carbonyl carbon charge from acid chloride to amide, but there were some anomalies in the thioester and the ester.

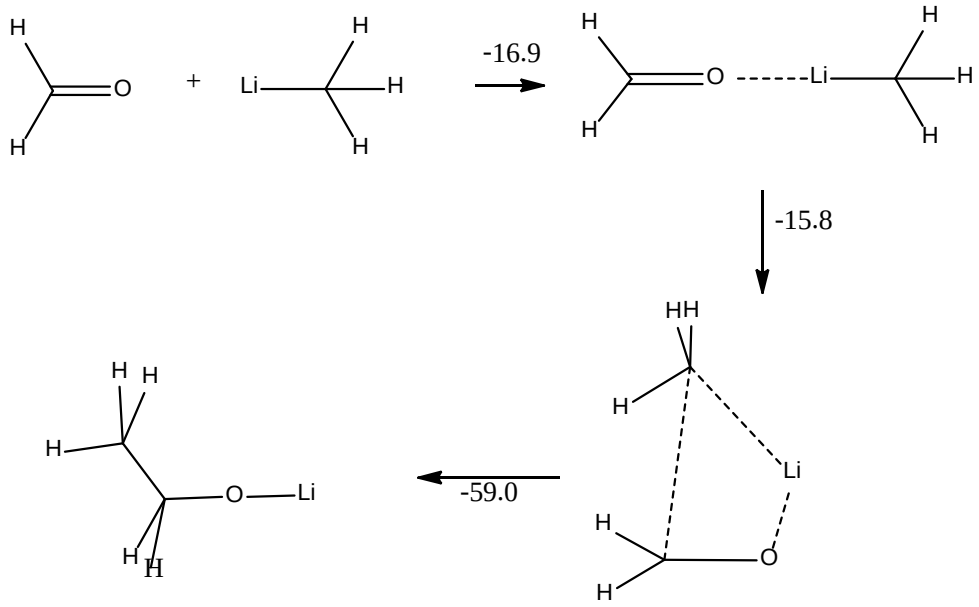
This showed that charges did not successfully explain the reactivity trends; therefore other factors were investigated. Factors, such as electrostatic interaction, orbital interaction, and Pauli repulsion, helped explain the reactivity trend of carboxylic acid derivatives and methyl lithium.

(Supported by: Committee on Faculty Compensation and Development (CFCD))

Advisor: Robert G. Linck, Chemistry

[1]Houk, K.N.; Kaufmann, E; Schleyer, P v. R.; Wu, Y. D. *J. Am. Chem. Soc.* **1985**, 107, 5560-5562

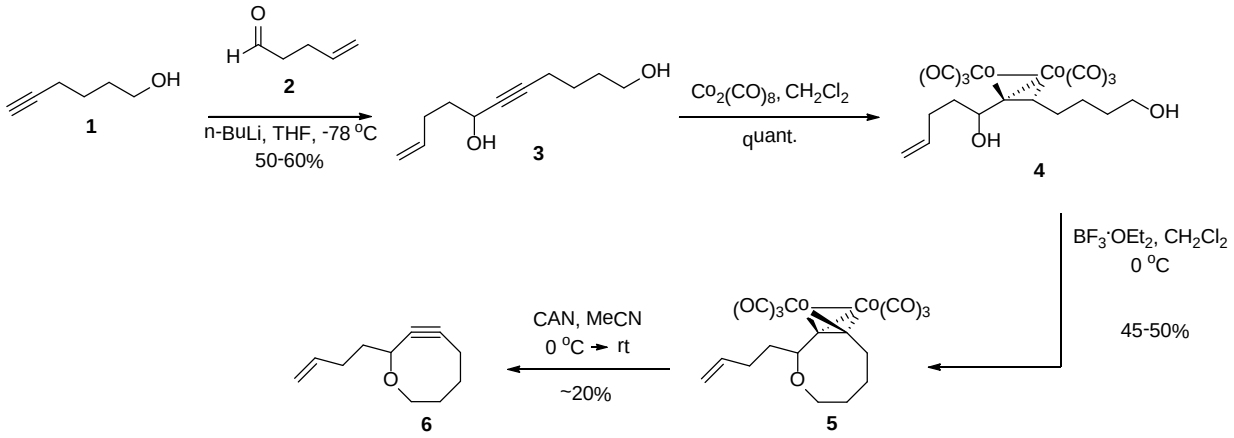
Image 1: Formaldehyde-CH_3Li Addition Pathway [a]



[a] Energies in kcal/mol. Data at M06/6-31+G(d,p) basis set.

Development of an Efficient Cobalt-Mediated Synthesis of Cyclic Alkynes

Katie Barbor/2015



In recent years, organic chemists have looked to chemical processes found in nature for inspiration, an approach often using what is known as click chemistry. Perhaps one of the better known click chemistry reactions, the Huisgen 1,3 – dipolar cycloaddition of azides and alkynes has been the focus of recent work by Carolyn Bertozzi, who uses cyclooctyne substrates in bioorthogonal applications, specifically live-cell imaging. Because the sp-hybridized carbons in a cyclooctyne have bond angles of about 160°, instead of the more stable 180°, these substrates react readily without the use of a cytotoxic copper catalyst.[1]

To prepare cyclooctyne substrate **6**, Shea lab uses an intramolecular Nicholas reaction. The Nicholas reaction involves cobalt-complexing a linear propargylic alcohol, followed by the introduction of either a protic or Lewis acid to generate a propargylic cation. The propargylic cation is then attacked by an intramolecular nucleophile, usually a nitrogen or oxygen, yielding a cobalt-complexed cyclic alkyne.[3] Although substrate **5** has been produced with great success in Shea lab, decomplexing to compound **6** has never proceeded smoothly, and the synthetic route has involved at minimum 7 or 8 steps.[3]

This summer, I repeated the original synthesis of compound **6**. While I believe I successfully decomplexed compound **5**, I never successfully isolated compound **6**. In an attempt to stream-line the synthesis, I decided to eliminate the use of protecting groups. The original synthesis involved protecting alcohol **1** and methylating the product of the reaction with substrate **2** before cyclization, creating a propargylic methoxy leaving group for the Nicholas reaction.[3] I was successfully able to demonstrate that the synthesis could be shortened by eliminating the use of protecting groups and proceeding with diol **3**, yielding the same compound as the previous synthetic strategy in only 4 steps. As part of a special studies in Shea lab, I hope to increase the yields and optimize reaction conditions for the shortened synthesis, as well as continue the development of a more successful method for decomplexation.

(Supported by the Alumnae Gift Fund in Chemistry)

Advisor: Kevin Shea, Chemistry

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2D NMR Spectroscopy of Mutagenic DNA Duplexes

Elizabeth Bayne/2014 and Lindsay Roth/2015

Transition-metal complexes such as chromium and iridium can create insidious lesions in DNA through the oxidation of the nucleobase guanine. When left unrepaired, these lesions can oxidize further and form the Spiroiminodihydantoin (Sp) lesion, a mutation that can lead to cancer.[1] Our research was focused on the use of two-dimensional Nuclear Magnetic Resonance (2D NMR) spectroscopy to elucidate the structural damage caused by the oxidation of a single nucleobase (G6) in an 11-mer DNA duplex synthesized by the Jamieson lab.

NMR is a spectroscopic technique that uses magnetic fields to manipulate the orientation of nuclear magnetic dipoles and generate molecular structural information. In our research, the main type of 2D NMR experiment used was Nuclear Overhauser Enhancement Spectroscopy (NOESY). A NOESY spectrum shows direct through-space interactions of two nuclei that are five angstroms or less away from one another, creating crosspeaks in a predictable pattern that allows us to assign them to protons on the DNA duplex. Being a large biomolecule, DNA requires the use of 2D NMR because many of its proton peaks overlap in a conventional 1D proton spectrum.

Our DNA NMR samples were prepared in a 90:10 H_2O:D_2O solvent system, which necessitated suppression of the overpowering water signal. The WATERGATE program was found to be the best water suppression scheme for our experiments to adequately observe the DNA resonance signals. Additionally, multiple parameters were optimized in order to obtain clearer spectra, such as resolution, delay times, and temperature.

This summer, confident resonance assignments were completed for a control (non-oxidized) DNA duplex. The non-exchangeable and exchangeable proton crosspeaks were divided into three and eight distinct regions, respectively. Assignments within those regions were confirmed with literature values.[2] The Sp lesion has two possible diastereomers, which were separated using HPLC. A NOESY spectrum of the oligomer containing one of the diastereomers was obtained and tentatively assigned based on literature assignments.[3] It is suspected that this spectrum represents the oligomer with the Sp-*S* confirmation.

In the future, NOESY spectra of the Sp-*S* and Sp-*R* DNA duplexes will be assigned. Further areas of study include base-pair exchange experiments using 1D proton spectra[1] as well as creating a 3D structural model of the Sp-containing duplex. By comparing the structure of the Sp lesion duplex to the control, more information can be gleaned about the Sp lesion and how it can be prevented or repaired.

(Supported by the Alumnae Gift Fund in Chemistry, Bayne and the Schultz Foundation, Roth)

Advisor: Cristina Suarez, Chemistry

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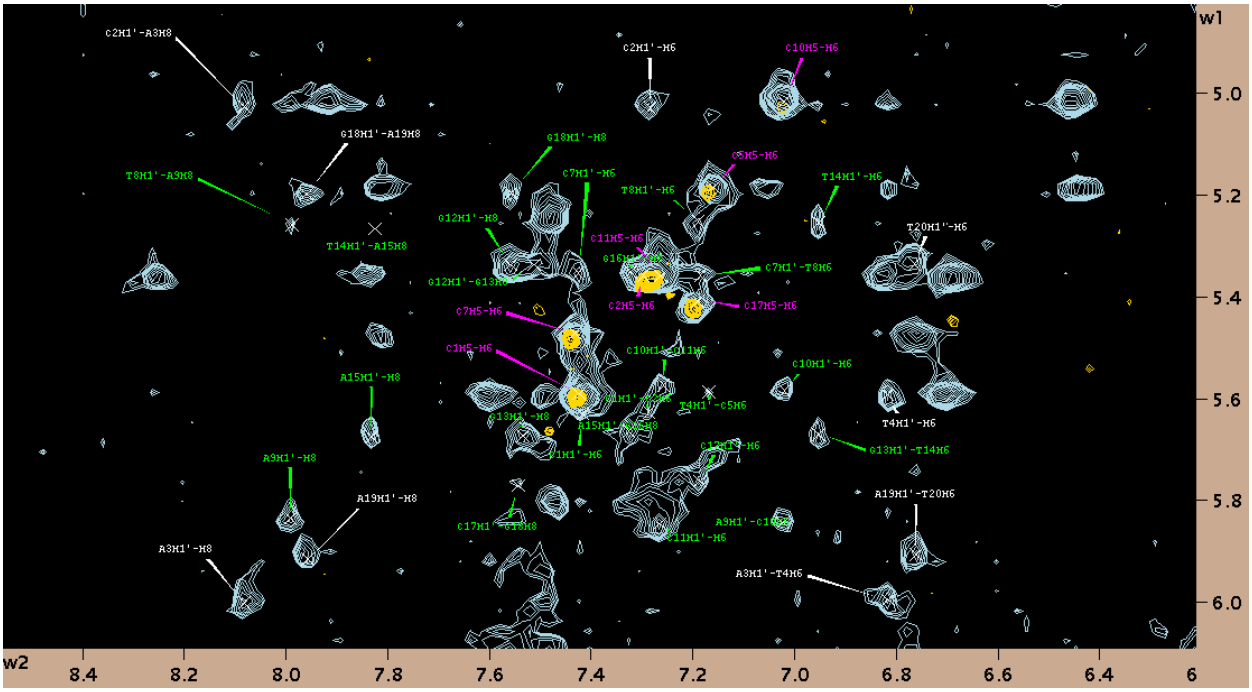
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C10H5-H6
G18H1'-A19H8
G18H1'-H8
C5H5-H6
T8H1'-A9H8
C7H1'-H6
T8H1'-H6
T14H1'-H6
G12H1'-H8
C11H5-H6
T20H1'-H6
T14H1'-A15H8
C7H1'-T8H6
C2H5-H6
C17H5-H6
C7H5-H6
C1H5-H6
C10H1'-H6
A15H1'-H8
T4H1'-C5H6
T4H1'-H6
G13H1'-T14H6
A9H1'-H8
A19H1'-H8
A19H1'-T20H6
A3H1'-H8
A3H1'-T4H6

Chemical and Topographical Understanding of Silicon Nanoscale Surface Features

Maddy Beasley/2014 and Rebecca Gerdes/2015

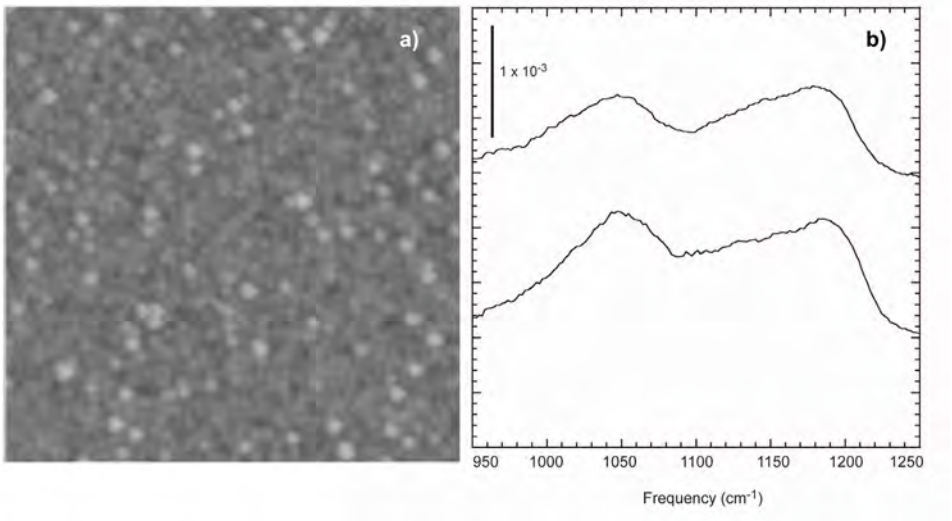
Silicon is the basis of transistors, which most microelectronics require to function, and it is an ideal model of the chemistry and topography of surfaces. The growth of biofilms (biofouling) can affect many various surfaces[1] and the flexibility of a silicon surface makes it a good model for studying biofouling. Additionally, since silicon is so often used in microelectronics, it is well characterized and understood. However, there is still a lot to learn about understanding the characteristics of the silicon surfaces. Our current work, continued from past work in the Queeney lab, focuses on understanding the chemistry and topography of rough oxidized silicon surfaces.

Samples of Si(100) were etched in deoxygenated water for 24 hours to produce surfaces that were hydrogen-terminated and covered in hillocks approximately 100nm diameter.[2] Half of the rough surfaces were then oxidized via a modified RCA SC-2 clean (ultrapure water: hydrogen peroxide: hydrochloric acid in 4:1:1 ratio), resulting in an oxidized (SiO_2) surface. Comparing the Si-O surface to the Si-H surface provides us with information about how varying the termination of the surface affects the chemistry and topography of the surface. The samples were analyzed using a combination of Atomic Force Microscopy (AFM) and Fourier Transform infrared absorption (FTIR) spectroscopy. The use of both instruments is key, as AFM provides topographical images and FTIR provides information about chemical structure. Both the H-terminated and oxidized silicon samples were analyzed in order to compare the two surfaces. Figure 1 shows both an AFM image (a) and an FTIR spectrum collected at both normal (top) and glancing (bottom) angles (b) of a rough oxidized surface. The AFM image gives us a good idea of the hillock shape and 100nm diameter of the topographical features, while the FTIR spectrum shows the Si-O bond stretch region, giving us information about the chemical composition of the surface.

This research will be continued through an honors thesis by M. Beasley in 2013-2014 focusing on further control of the chemistry of Si(100) by attempting to selectively functionalize different regions of the surface with oxide and alkane regions.

(Supported by a Henry Dreyfus Teacher-Scholar Award from the Camille and Henry Dreyfus Foundation)

Advisor: Kate Queeney, Chemistry



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Extruded Large Unilamellar Liposome Composition Study on Monensin Mediated Na^+ Transport using ^{23}Na NMR Spectroscopy

Sigal Eini/2016 and Mariam Bhuiyan/2014

Liposomes are artificially prepared vesicles composed of phospholipid bilayers that act as models of cell membranes.[1] Clinical applications of liposomes include drug delivery, diagnostic imaging, and cancer therapy.[2] There are a variety of liposomes classified according to size and preparation. In this study, large unilamellar vesicles, approximately 200 nm in size, as indicated by a particle analyzer, were used to observe sodium cation transport with the use of an antibiotic, monensin. Ionophores, such as monensin, disrupt trans-membrane ion concentration gradients to act as carriers for the movement of ions.[3] The use of NMR spectroscopy, with the addition of a lock solvent and a shift reagent, allows the detection of differences in cation transport amongst intra-liposomal and extra-liposomal environments. A lock solvent offsets the effect of the natural drift of the NMR's magnetic field and a shift reagent perturbs the signal of the extra-liposomal space by introducing a complex with a massive electron cloud.[4] The goal of this project was to understand how the composition of the phospholipid bilayer of liposomes affects monensin-mediated sodium ion transport. Transport properties were tested and compared using different preparation protocols and common constituents of a cell membrane including cholesterol (CH) and a variety of phospholipids such as phosphatidylcholine (PC), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE).

The different liposomes that were prepared varied in composition as follows: PC, PC/cholesterol (2:1), PC/PE (2:1), and PC/PG (10:1). Freeze-thaw extrusion cycles were used to prepare these liposomes that encapsulate 200mM NaCl.[5] The PC/PE liposomes were prepared by a combination of reverse phase evaporation and freeze-thaw extrusion cycles.[6] NMR tubes were prepared by combining NaCl liposomes (500 uL, 200 mM, 0.1 mmol), deuterated water (100 uL), dysprosium chloride(12.5 uL, 200 mM, 2.5 umol) and sodium tripolyphosphate (12.5 uL, 400 mM, 5 umol) where the deuterated water acts as a lock solvent and the dysprosium tripolyphosphate $[Dy(PPP)_2]^{7-}$ complex acts as a shift reagent. Aliquots of 2 -15 uL of 20mM monensin were added to the liposomes and the transport was observed using ^{23}Na NMR spectroscopy.

Using the Bruker TopSpin 3.0 program, the linewidths were recorded, and the k observed values for each concentration of monensin were calculated by multiplying π with the change in linewidths (Figure 1). The k observed values were averaged, and these k average values were plotted against their respective concentrations of monensin. The slopes of the graphs, which represent the rates of transport, were calculated to be (1.91 +/- .07) x 10^5 for PC liposomes, (1.49 +/- .04) x 10^5 for PC/CH (2:1) liposomes, (2.22 +/- .03) x 10^5 for PC/PE liposomes and (3.60 +/- 0.06) x 10^5 for PC/PG (10:1) liposomes, where the error in the rate of transport was derived from each of the standard deviations of the means of k observed values (Figure 2).

By evaluating the different rates of transport for the varying compositions, it can be seen that the PC/CH (2:1) liposomes have the slowest rate of monensin-mediated Na^+ transport. This may be explained by the fact that cholesterol increases the rigidity of the membrane by occupying the spaces between the unsaturated hydrocarbon tails of the PC. This makes it more difficult for monensin to move between the phospholipid membranes, therefore decreasing the rate of transport. On the other hand, both the PC/PE (2:1) liposomes and the PC/PG (10:1) liposomes have faster rates of transport than the regular PC liposomes. This may be due to the fact that the addition of phospholipids such as PE and PG increase the fluidity of the membrane due to their unique structures, where the unsaturated hydrocarbon tails occupy different locations in the membrane. This creates a more spacious membrane compared to regular PC liposomes, thus increasing the rate of transport. For future studies, the effect of varying the preparation protocol as well as the ratios of different phospholipids would be investigated to determine how it affects the rate of monensin mediated sodium ion transport.

(Supported by the Schultz Foundation, Eini and the Schiffer Fund, Bhuiyan)

Advisor: Cristina Suarez, Chemistry

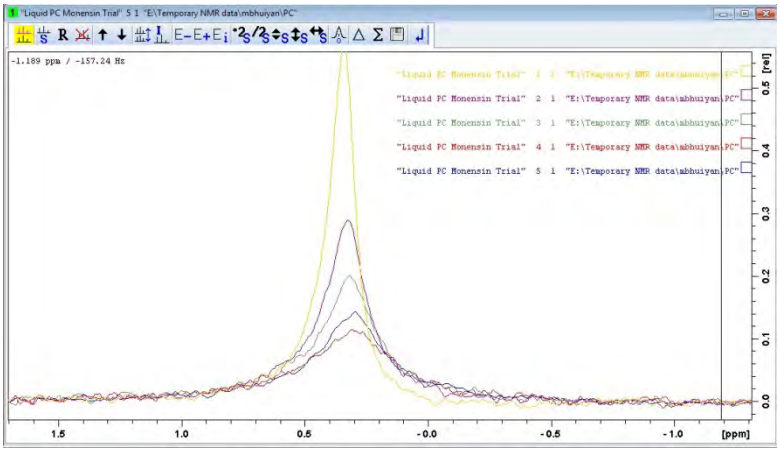

Figure 1 illustrates how the linewidth of the intra- PC liposomal space changes with the addition of different volumes (2 to 15 uL) of 20 mM monensin as shown using ^{23}Na-NMR in Bruker TopSpin 3.0

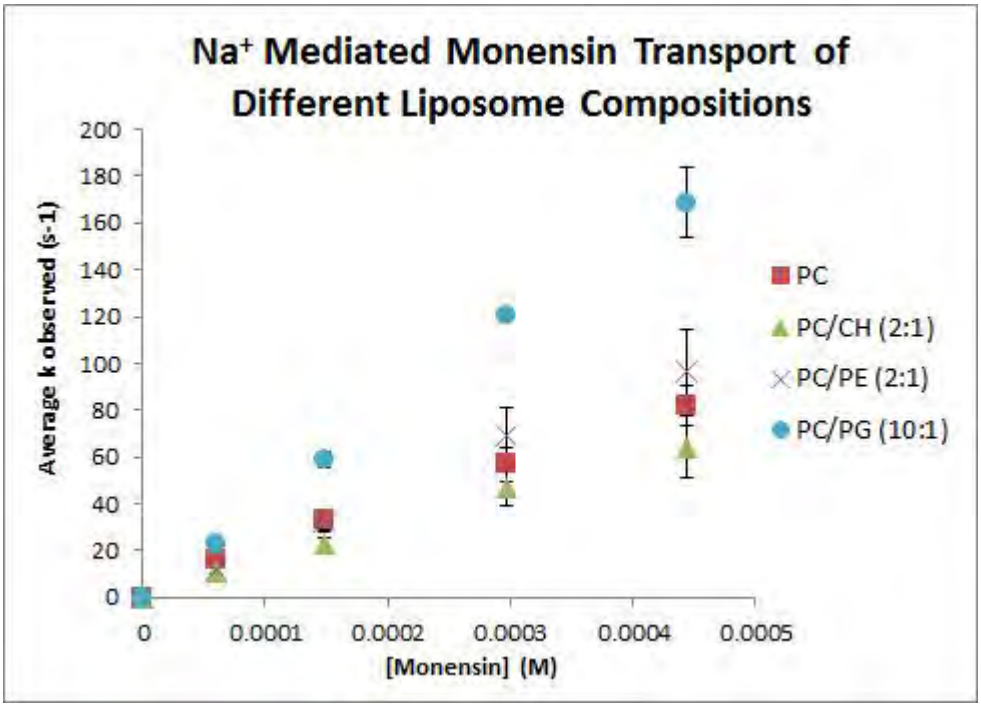

Figure 2 illustrates the differences in the rate of transport for each of the liposomes with varying compositions. The PC, PC/CH (2:1), PC/PE [REV] (2:1), and the PC/PG (10:1) liposomes had respective rates of transport of (1.91 +/- .07) x 10^5, (1.49 +/- .04) x 10^5, (2.22 +/- .03) x 10^5, (3.60 +/- 0.06) x 10^5 where the error in the rate of transport was derived from each of the standard deviations of the means (as indicated by the error bars).

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The Mapping of A Reaction Coordinate Diagram

April Birnie/2015

We started this summer by attempting to research hard-soft acid-base theory. We set out with the intention to determine if this theory could predict reactions of α,β-unsaturated compounds. In order to do this we used a computer program that models chemical reactions called Gaussian 09™. The Gaussian program allowed us to optimize the structures of both ground-states and transition-states. The energies that are outputted can then be connected in a reaction coordinate diagram.

The original plan was to map out the reaction coordinate for two α,β-aldehyde systems with attack by a methoxide or thiomethoxide system, both complexed to a lithium ion. Within these two systems two locations on the α,β-aldehyde were considered for attack: the carbonyl and the beta position. However, once we set out to find the transition states between products and reactants we found that there were multiple stable ground states. Upon further investigation it was determined that for the oxygen system there were two dihedrals (α,β-aldehyde dihedral and the lithium, carbonyl, alpha-carbon) that the molecule could rotate around, thus producing four different versions (see diagram). For the thiol system, however, because the optimized thiol is bent there are three different dihedrals (the two listed in the oxygen system above and the orientation of the methyl group), thus producing eight different conformations (not pictured). Instead of focusing on both systems at one time the oxygen system was investigated further.

It was found that there was a stable intermediate between the cis-trans conformer and the trans-trans conformer. This stable intermediate, the 'bent conformer', is also an intermediate in the carbonyl attack. This led to queries if there were other stable intermediates between conformers or if there was only one. This stable intermediate also made the rate determining step of this reaction the change of the α,β-aldehyde dihedral. It takes roughly 11.7 kcal for the conformation of the α,β-aldehyde to go from cis to trans but only 8.7 kcal for the un-complexed α,β-aldehyde to go from cis to trans, further suggesting that the rate determining step of the reaction was the change in this conformation.

Further investigation of the oxygen system, and sulfur system needs to be done to understand what is happening in the two systems and how they are, or are not related.

(Supported by the Schultz Foundation)

Adviser: Robert G. Linck, Chemistry

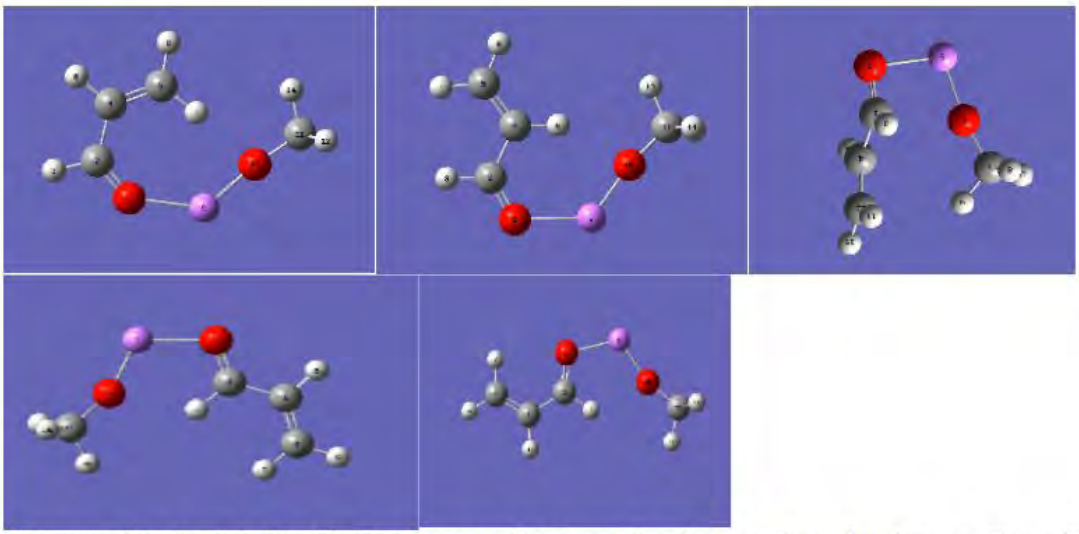


Figure 1. (Clockwise) Cis-Cis Conformer, Cis-Trans Conformer, Bent Conformer, Trans-Trans Conformer, Trans-Cis Conformer.

DNA-Small Molecule Catalyst Conjugates for Site-Selective Chemistry

Drew Colman/2015

Chemical reactions are often carried out under highly controlled conditions; thus, these reactions cannot proceed in complex environments, such as in living systems. There is currently no way to target a small molecule among many with the same functional groups in a mixture. Our project goal is to use DNA as a macromolecular targeting domain. DNA aptamers can fold into three-dimensional structures, which allows them to target very specific substrates. Our lab has previously linked a DNA aptamer to a small-molecule catalyst (imidazole). This structure is a DNA-small molecule catalyst conjugate (DCat, see figure); we hypothesize that DCats can select for and bind molecules with functional groups that exist in living organisms to the small-molecule catalyst. This summer, I attempted to prove that DCats can perform ester hydrolysis more effectively than a free small-molecule catalyst.

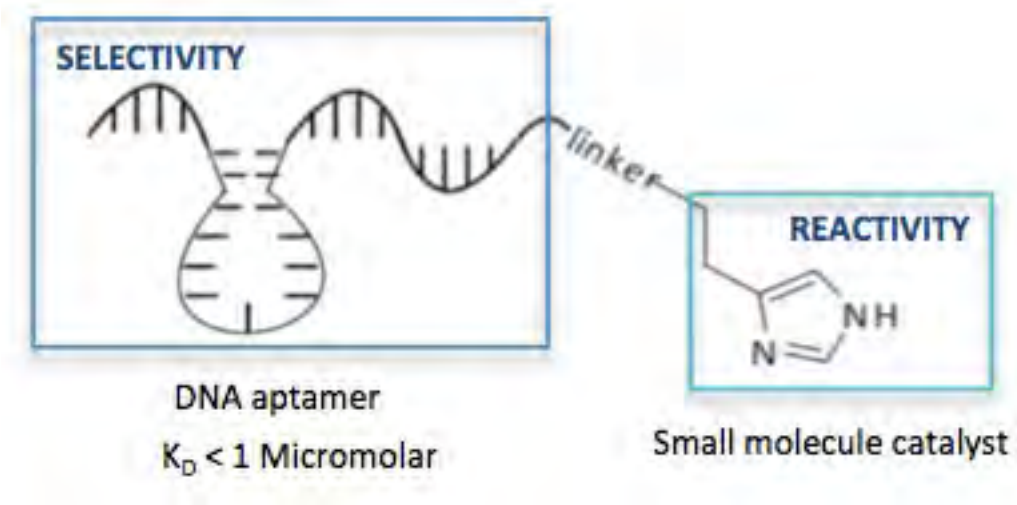
I synthesized esters (substrates) that are susceptible to hydrolysis catalyzed by imidazole. Each substrate is composed of two initial compounds: a fluorescently active molecule and a molecule for which one of our DNA sequences has a binding affinity. The substrates are fluorogenic—they are only fluorescent after hydrolyis occurs. We created an assay that monitored the fluorescence, and therefore hydrolysis, of these substrates in the presence of a DCat, imidazole, or no catalyst. Finally, I synthesized new DCats according to our findings.

Initially, the assay illuminated little, but eventually DCats appeared to hydrolyze the substrates at a faster rate than imidazole alone. Some DCats (with longer linkers between the DNA and catalyst) catalyzed the hydrolysis faster and more effectively than others, which led to the synthesis of new DCats with longer linkers (see figure).

The results indicate that DCats are potentially useful for ester hydrolysis. We have determined that DCats can be more reactive than a small molecule catalyst alone. However, we have yet to determine if a DCat can select for one substrate over another with the same susceptible functional groups. If our assay proves that DCats can perform site-selective chemistry, this could open the door to a wide range of reactions, not merely ester hydrolysis. Once we demonstrate DCat-mediated site-selective chemistry, a number of biomedical applications are possible, including hydrolysis in bacterial quorum sensing. I helped present a poster at the American Chemical Society, Connecticut Valley Section in the spring, and hope to do this again while at Smith. I plan to continue this project my senior year, potentially as an honors thesis.

(Supported by the Schultz Foundation)

Advisor: David Gorin, Chemistry



Synthesis of Cyclo[-L-Phe-D-N-Me-Ala]$_4$ via Solid Phase Peptide Synthesis

Virginia Diaz-Arteaga/2014

Organic nanotubes, based on cyclic polypeptides, have been extensively studied for their potential application in molecular transport.[1] *Ghadiri et al.* found that cyclic polypeptides, when protonated, crystallize to form stacked, nanotubular structures extending for hundreds of nanometers and having tube internal diameters of 7-8 Å. The internal diameters are similar to those of transmembrane ion channels and pores.[1,2] The stacking of the nanotubular structures can be controlled through selective methylation of the nitrogen backbone to afford dimers, trimers etc. The objective of this project is to synthesize an eight-residue cyclic peptide *cyclo*[-L-Phe-D-N-me-Ala]$_4$, which has shown to self assemble into discrete membrane-soluble cylindrical dimers.[3]

Extensive literature research suggested that the best method for the synthesis of the target cyclic peptide was through the synthesis of its linear precursor H(-L-Phe-D-N-Me-Ala-)$_4$ – an eight residue linear peptide – via solid phase peptide synthesis (SPPS) followed by in-solution cyclization. Conventionally, organic syntheses are carried out in solution. However, SPPS is performed using amino acids attached to a solid anchor, called a resin (the technique was developed by Bruce Merrifield who later won the Nobel Prize for his work). In SPPS, the C-terminal amino acid of a peptide is first attached to an insoluble solid resin support via its α-carboxylic acid group. A solution containing an amino acid with a protected α-amino group and a coupling reagent is added, and a peptide bond is formed between the α-amino group of the amino acid attached to the solid support and the carboxylic acid group of the amine-protected amino acid in solution. After the reaction has occurred, the solution is removed, and the resin is washed to remove any reaction products or unreacted starting materials. The protecting group on the α-amino group is then removed, and after again washing the resin repeatedly, the next amino acid of the polypeptide is added and the peptide-forming reaction is repeated. With each sequential addition of an amino acid, the chain polypeptide chain grows.[4] The coupling and de-protection steps are repeated until the desired amino acid sequence has been reached. The final step in SPPS is referred to as cleavage; it involves cleaving the desired linear peptide from the resin.[4]

The synthesis of precursor H(-L-Phe-D-N-Me-Ala-)$_4$ was carried out using fluorenylmethyloxycarbonyl chloride (Fmoc) de-protection strategy, and starting with a Wang resin, preloaded with Fmoc-L-Phe. The linker attached to the polystyrene core of the resin is a 4-hydroxybenzyl alcohol moiety. The amino acids were purchased with their α-amino groups already protected with an Fmoc group. Using standard SPPS protocols, the crude linear octapeptide precursor was successfully synthesized. The yield for each peptide bond formation ranged from 94-98%, giving an estimated 74% overall yield. After cleave from the resin, the crude peptide was isolated in 47% yield. The final steps for the synthesis of the cyclic polypeptide include purification, characterization, and cyclization. I plan to continue my work on cyclic peptide synthesis during fall 2013, as the subject for my undergraduate honors work.

(Supported by the Alumnae Gift Fund in Chemistry)

Advisor(s): David Bickar and Cristina Suarez, Chemistry

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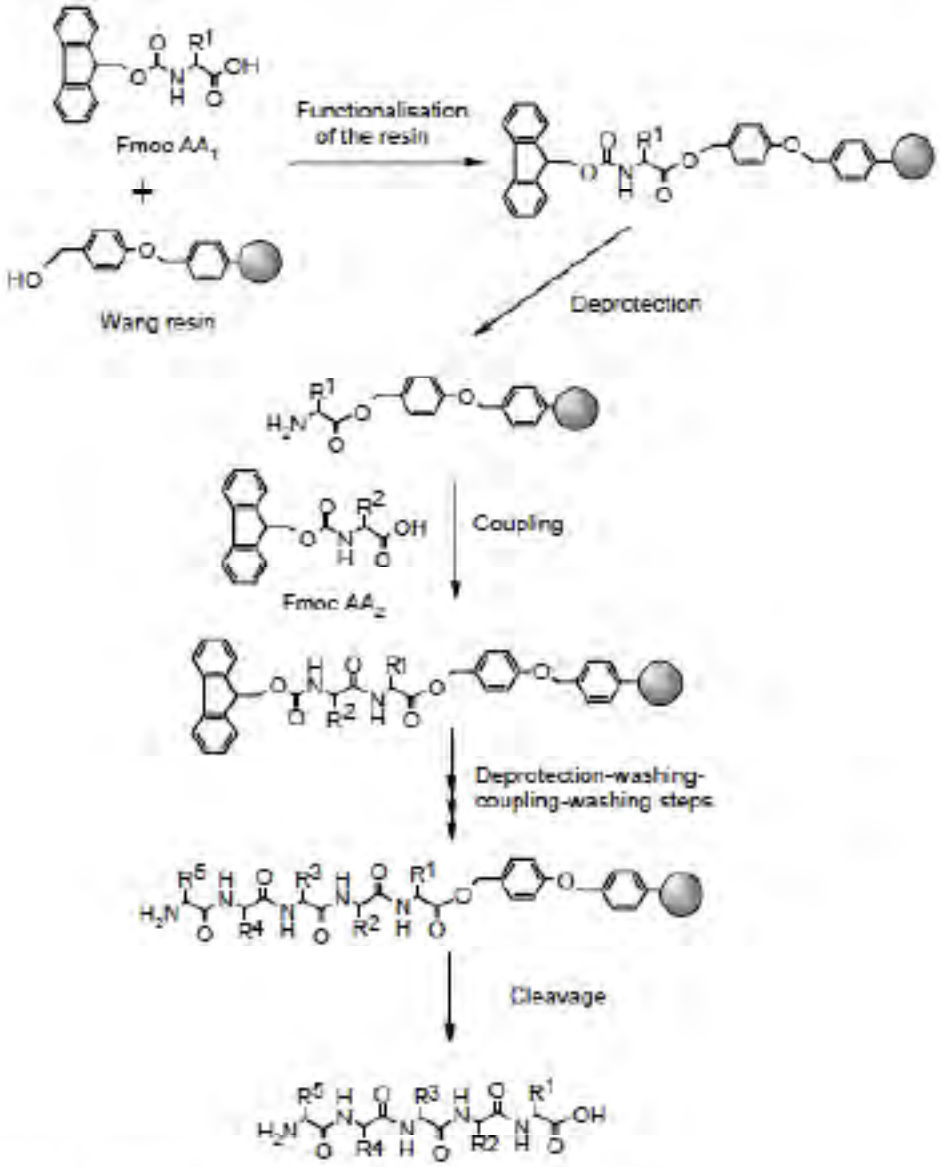
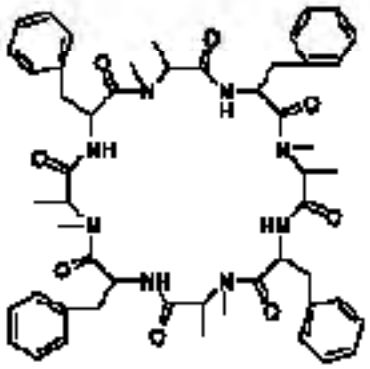
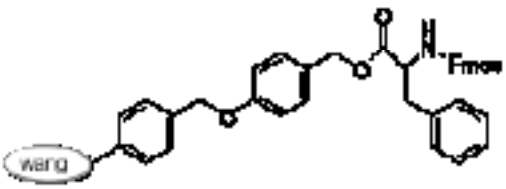


Figure 1. Synthesis of a pentapeptide



cyclo-[L-Phe-D-N-Me-Ala]$_4$

Figure 2. Desired eight-residue cyclic peptide



Pre-loaded Phe-fmoc Wang Resin

Figure 3. Wang Resin

Biomolecule Adsorption on Hydrophilic and Hydrophobic Si (100) Samples:

Taleen Dilanyan/2016

Research in the Queeney Lab is focused on the nucleation of biofilms on silicon-based surfaces that have different topography and wettability. Two cleaning methods can be used to prepare hydrophilic samples, P-cleaning and SC-2 cleaning. P-cleaning is a self-heating reaction made of hydrogen peroxide: sulfuric acid in a 1:4 ratio. SC-2 clean is made of deionized water: hydrogen peroxide: hydrochloric acid in a 4:1:1 ratio, heated in a 70° water bath. Previous studies[1] showed that SC-2 cleaning is less disruptive to the topography of surfaces that have been intentionally roughened, making this chemistry more desirable than p-clean for use with rough surfaces, even though p-clean creates a higher density of surface silanol (Si-OH) groups. Since the bulk of our previous work on hydrophilic surfaces was carried out on p-cleaned surfaces, we sought to compare the effects of surface cleaning on subsequent biomolecule adsorption, focusing on the adsorption of poly-L-lysine (PLL). Ellipsometry was used for measuring the thickness of the adsorbed films, and contact angle goniometry was used to measure the wettability of the hydrophilic and hydrophobic silicon surfaces.

Previously collected data[2] were compared to current data to examine the consistency of these two methods and to modify procedures as needed. The collected data show that p-cleaned surfaces were adsorbed with approximately 1.1 Å thicker layer of Poly-L-Lysine than SC-2 cleaned surfaces (Figure 1). As for the average thickness of PLL biomolecule adsorbed on flat silicon surfaces silanized with octyldimethylchlorosilane (hydrophobic), it was 18 Å. The adsorption was conducted using a 9:1 ratio of PH 11 buffer: PLL stock solution which is neutral. This neutral solution allowed the PLL biomolecules to be more closely compacted on the surfaces and created a thicker layer of Poly-L-Lysine.

These data help understand the effect of silicon surfaces' topography and wettability on biofilm adsorption. Further research is needed to be conducted on the adsorption of biomolecules on hydrophobic flat surfaces, hydrophobic and hydrophilic rough surfaces, and eventually, multi-functionalized rough surfaces. This research will be continued in the fall of 2013 as a special studies project.

(Supported by a Henry Dreyfus Teacher-Scholar Award from the Camille and Henry Dreyfus Foundation)

Advisor: Kate Queeney, Chemistry

[1] Say, C. Queeney, Kate T. (2009). Chemical Functionalization of Water-etched Si(100). MRS Proceedings, 1228.

[2] Jasmine Wallas lab notebook 2009-2011.

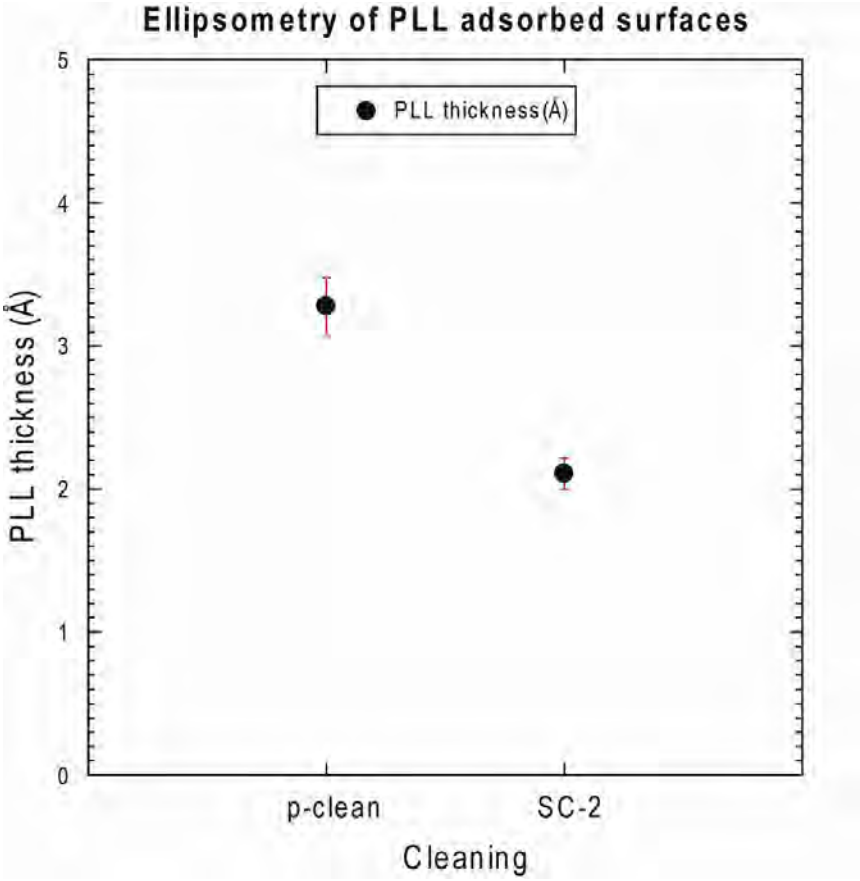


Figure 1: A comparison of the average Poly-L-Lysine biomolecule thickness (Å) adsorbed in SC-2 and p-cleaned surfaces. The standard error bars were calculated by dividing the standard deviation of the measurements on the square root of the number of measurements (N) and multiplied by 2.

Synthesis and Purification of 2,6-diethylcyclohexanol for Application as Potential Anesthetic

Alexandra Gatsios/2014

Propofol (Fig. 1) is a commonly used intravenous anesthetic that works by binding to $GABA_A$ receptors in the central nervous system. Structure-activity relationship (SAR) analyses has shown that the aliphatic groups adjacent to the hydroxyl group are essential for propofol's activity.[1] In the interest of developing novel anesthetics, Adam Hall's Laboratory tested isomeric mixtures of numerous cyclohexanol derivatives containing aliphatic groups in the same 2,6 positions on $GABA_A$ receptor currents and obtained positive results.[1] Due to the chirality of most biological receptors, it is possible that $GABA_A$ is stereoselective and a particular di-substituted cyclohexanol isomer is responsible for the majority of the activity. Thus, the goal of this project was to follow up on the work done by Kelly Smith, in which she synthesized and separated the diastereomers of 2,6-dimethylcyclohexanol, by isolating the diastereomers of 2,6-diethylcyclohexanol (Fig. 3).

For her research, Kelly purchased 2,6-dimethylcyclohexanone as a mixture of isomers and obtained pure diastereomers after column chromatography. Unlike 2,6-dimethylcyclohexanone, 2,6-diethylcyclohexanone was not commercially available. The desired 2,6-dimethylcyclohexanone was synthesized via a dianion which was generated using strong bases, potassium hydride and n-butyllithium (Fig. 2).[2] The dianion was then ethylated using ethyltriflate. The (E) and (Z) isomers were separated via flash column chromatography and then the (Z) isomer was reduced using lithium aluminum hydride. Column chromatography was again utilized to separate the cis,cis isomer (**1**) from the trans,trans isomer (**2**). Separation of the cis,trans and trans,cis isomers (**3** and **4**) is extremely difficult, because they are enantiomers and was not attempted.

^1H and ^{13}C NMR spectra confirmed a successful synthesis and then separation of the (Z) and (E) 2,6-diethylcyclohexanone isomers. However, not enough of the (E) isomer was obtained for further experimentation. The (Z) isomer was collected and reduced to a mixture of **1** and **2** and then separated. Synthesis and purification of **2** was confirmed by ^1H and ^{13}C NMR spectra, matching the peaks reported by Hall et al. While the ^1H NMR spectrum appeared to confirm the isolation of **1**, the peaks from the ^{13}C NMR spectrum showed some discrepancies from those reported in the literature.

The next step of this project includes synthesizing a larger quantity of the 2,6-diethylcyclohexanol diastereomers as well as synthesizing and separating the isomers of 2,6-diisopropylcyclohexanol, which, of the di-substituted cyclohexanol mixtures tested, appeared to be the most potent. Hopefully, isolation of the single diastereomers will enhance the abilities of these potential anesthetic 2,6-di-substituted cyclohexanol molecules.

(Supported by the American Chemical Society Summer Undergraduate Research Fellowship)

Advisor: Kevin M. Shea, Chemistry

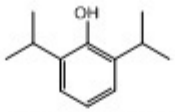


Figure 1. Propofol

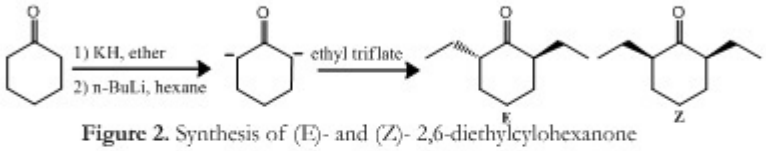


Figure 2. Synthesis of (E)- and (Z)- 2,6-diethylcylohexanone

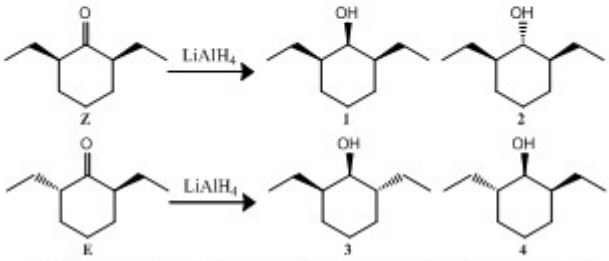


Figure 3. Reduction of (E)- and (Z)- 2,6-diethylcylohexanone

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Investigating Blood Dopamine Levels in Response to Environmental Stimuli

Leen B. Hayek/2016

Dopamine is a neurotransmitter found in the blood stream as well as the nervous system. Blood dopamine exists in the sulphonated form and its role there is unknown. However, it is established that blood dopamine concentrations vary and are unrelated to the dopamine concentration in the central nervous system.

The main obstacle facing this study is dopamine's instability; since dopamine is a very unstable molecule, dopamine levels are difficult to measure accurately. This will be this study's primary goal: to devise a method that will allow rapid detection of dopamine levels, or one that prevents the molecule from decomposing before it is assayed. The ultimate objective of this study is to determine whether there is a correlation between blood dopamine levels and environmental stimuli as this would provide insight into the role of blood dopamine.

The method devised for detecting dopamine levels in the blood is capillary electrophoresis (CE) with amperometric detection. Initially, the method involved modifying the CE instrument to include an "L" shaped gold electrode that is partially plated with silver. The sample would pass through the electrode and the CE instrument would record the difference in current. This part of the research focused on synthesizing the electrode. The method for synthesis involved stripping gold compact discs (CD) from the protective layer using nitric acid, washing in ethanol, printing the electrode design onto the CDs using a 1-octadecanethiol solution in 2-propanol instead of ink in order to form a protective layer over the gold in the shape of the electrode. The unprotected gold is etched by submerging it in a mixture of potassium salts. The CDs are exposed to short wave ultraviolet light (UV) thus removing the protective layer. The electrodes are cut out of the CD structure using pliers and are then partially plated with silver epoxy. The evolution of the electrode quality produced is displayed above.

Once the testing of the electrodes commenced the results showed that the electrodes could not handle the high voltage of the CE instrument thus causing the gold to chip off the polycarbonate base of the CDs. As a result, another method was devised involving using very thin metal wires as electrodes and wrapping them around the capillary. This method will be investigated in the coming semester as special studies.

(Supported by the Schultz Foundation)

Advisor: David Bickar, Chemistry

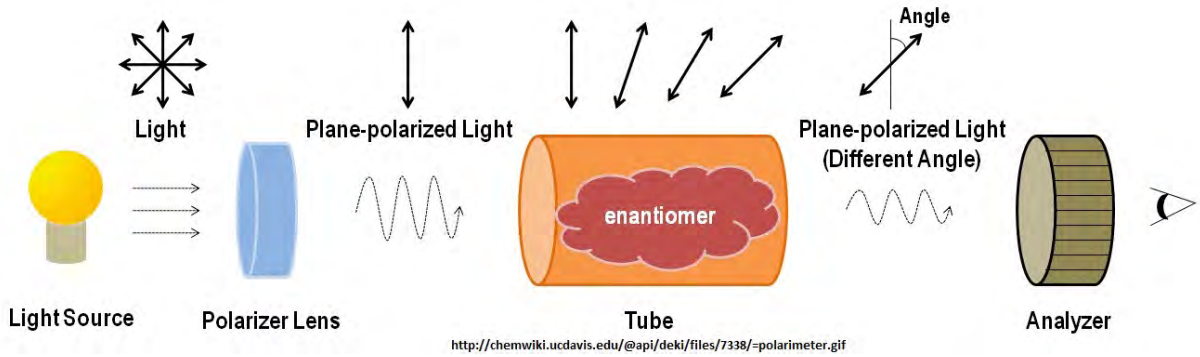
Epimerization in the Bicarbonate-Catalyzed Methylation of Carboxylic Acids

Yuan Ji/2014

Methylation reactions of carboxylic acids are commonly used in organic synthesis to modify the properties of small molecules such as drug targets and polymers. One recent example from the literature is Myers' synthesis of the antibiotic tetracycline, in which trimethylsilyl diazomethane was used to methylate the tetracycline precursor.[1] Although useful, current methylating agents such as diazomethane are hazardous to work with due to their acute toxicity and sensitivity to light, moisture, etc. The other alternative, Fisher esterification, requires acidic conditions.

For the past two years in the Gorin lab, we have been developing a safer means of methylation using dimethyl carbonate (DMC), a green, inexpensive, and non-toxic methylating reagent.[2] Our current reaction conditions involve catalytic inorganic base (0.4 equiv.), excess DMC (20 equiv.), and DMSO as solvent (0.2 M) at 90 °C. Because our reaction conditions are mildly basic and tolerated by a variety of substrates, we hypothesized that for substrates with inherent stereochemistry, the stereocenters will be retained.

To test our hypothesis, I methylated (S)-(+)-ibuprofen and Boc-protected L-isoleucine and L-phenylalanine under our optimized reaction conditions with good yield (87% for ibuprofen, 83% for isoleucine, and 67% for phenylalanine). Since isoleucine has two stereocenters and racemizing one stereocenter results in a pair of diastereomers, the extent of epimerization was determined by proton NMR (d.r. >25:1). Since ibuprofen and phenylalanine each have one stereocenter, epimerization leads to enantiomers, which were detected using polarimetry. A sample of known concentration was placed in the polarimeter, which measures optical rotation. The enantiomeric ratio was calculated from the optical rotation of the sample and a literature value of the pure enantiomer. For ibuprofen, 3% epimerization was observed; for phenylalanine, 7% epimerization was observed.



The low rates of epimerization for these three substrates support our hypothesis that stereochemistry is mostly retained under our methylation reaction conditions. These results are significant, given the importance of methylation in organic synthesis and the importance of preserving stereocenters

(Supported by Funding from an American Chemical Society, Petroleum Research Fund Award)

Advisor: David Gorin, Chemistry

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Directing Chemical Reactions with DNA-Catalyst Conjugates

Shimu Liu/2015

Chemical reactions in complex biological or environmental systems are gradually attracting more attention. The challenges of doing such chemical transformations are selectivity. As Figure 1B[1] shows, besides the target molecule B, both A and C have the active alkyne functional groups that can react with R_2-N_3 and yield a mixture of products. In Professor Gorin's lab, we are aiming to provide solutions for these problems by using DNA-small catalyst conjugates (DCats). A DCat is a DNA aptamer attached to a catalyst with small molecular weight (Figure 1A[1]). We expect the aptamer to increase the selectivity since it can bind to a specific target molecule, and after aptamer-target binding, the small molecule catalyst can react with the target molecule. By applying DCat in the reaction of Figure 1B, we should be able to selectively perform the transformation of molecule B even with the presence of A and C.

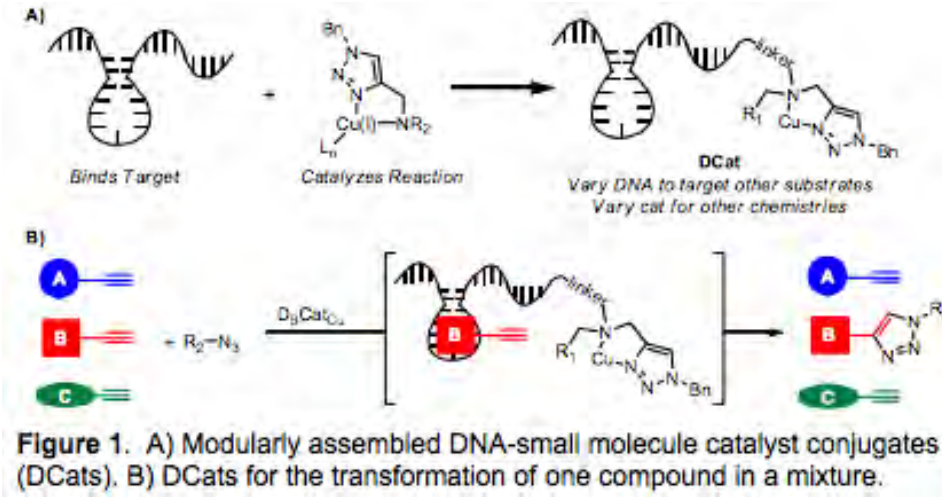
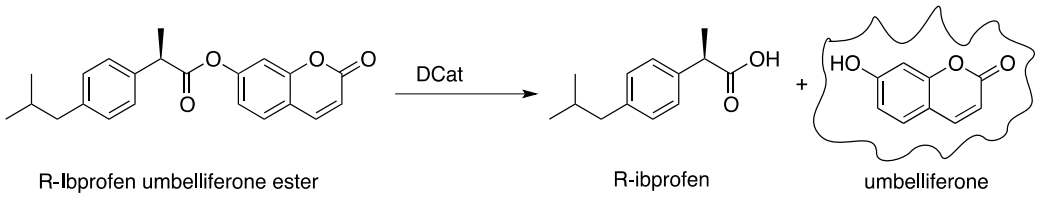


Figure 1. A) Modularly assembled DNA-small molecule catalyst conjugates (DCats). B) DCats for the transformation of one compound in a mixture.

This summer, my major focus was the proof of concept of DCats. In order to show that DCats are actually effective in catalyzing desired reactions, my partner Drew and I designed and optimized a fluorogenic assay, which can display visible changes of the reactions after catalyst is added. Since the previously synthesized DCats we worked with were designed to target ester hydrolysis reaction, we made the ester substrates containing this fluorescent piece called umbelliferone. (Scheme 1) With the help of a microplate reader, we were able to monitor the change of the fluorescent signal after addition of DCat and learn about the rate and yield of the reaction.



Scheme 1. Hydrolysis reaction of one umbelliferone ester substrate with DCat

The problem we encountered with this assay was the low solubility of the substrate in water, which decreases the interaction between the DCat and the substrate. After a series of experiments, it was found that raising the temperature to 65°C and increasing the percentage of organic solvent (DMSO or DMF) in the assay both helped to get the fluorescent signal higher, which essentially means more umbelliferone was generated.

However, since we're hoping to apply DCats in biological context, both high temperature and high concentration of DMSO should be avoided. In the future, we are planning to investigate about the pH environment of the assay, as well as redesign the substrates and the DCats. Since there's not a good way to optimize the assay by changing the reaction condition, creating more water-soluble ester substrates and more active DCats will be the future goal of this project.

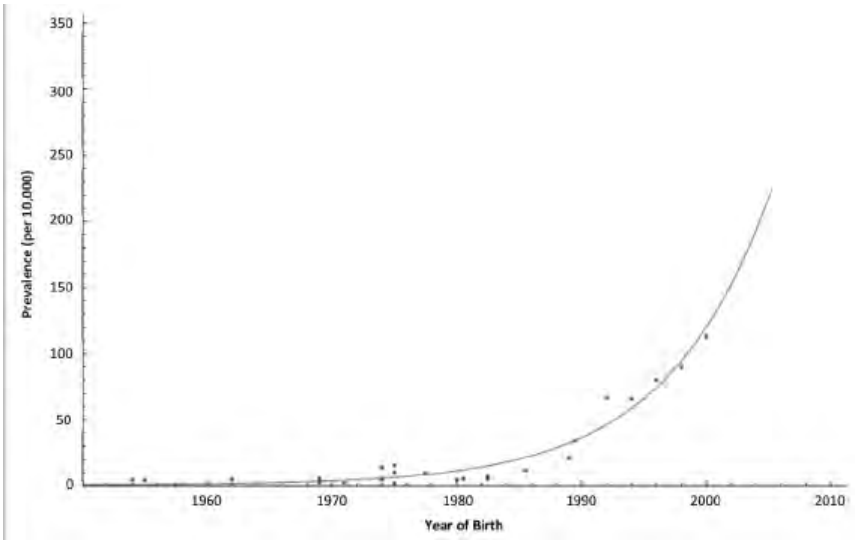
(Supported by the Research Corporation for Science Advancement, Cottrell College Science Award)

Advisor: David Gorin, Chemistry

[1] Figure courtesy of David J. Gorin. From personal correspondence.

Clinical Analysis of the Correlation Between the Increasing Prevalence of Regressive Autism and the Incidence of Viral Co-Infection

Mojdeh Mostafavi/2015



Autism, a developmental disorder affecting 1 in 88 children in the United States today, has increased dramatically since ~1970. Epidemiological models suggest that if the prevalence continues to increase at the current rate, by 2015 one in 14 of the children born that year will be autistic. The increasing prevalence indicates that in addition to its genetic determinants there is an environmental component to the disease. Although there are different causes for the several diseases classified together under autism-spectrum disorder, we propose that one type of autism, sometimes called severe/regressive autism, may have a unique etiology. In some children, a viral co-infection, occurring at a critical age (around 12 months old), causes the already limited immune response to be further suppressed. The compromised immune response permits one of the viruses to enter the brain, causing a physiological response that eventually leads to autism. There are several lines of evidence that support this hypothesis; the abrupt onset of symptoms, starting between 12 and 24 months of age, is at a time when a child's immunity is at its lowest and viral infections are common. The gender ratio for children with severe/regressive autism, about 8 boys to every girl, matches the ratio observed in some common childhood viral infections and may reflect the differences between males and females in their immune response to these viruses. Finally, several of the viruses we intend to test exhibit neurotrophic behavior and are known to infect the central nervous system of immunocompromised individuals.

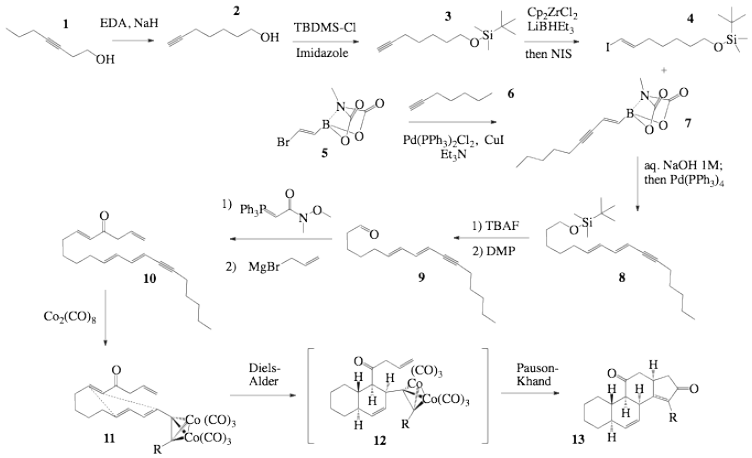
By analyzing blood samples from children under the age of four recently diagnosed with severe/regressive autism, we hope to determine the frequency of co-infection by these viruses and the immune response they elicit. Analyses will include serological assays for antibody responses to these viruses, for viral genetic material, and for nagalase. We hope to determine if viruses are the critical environmental factor responsible for producing severe/regressive autism. If successful, this work will allow better diagnostic tools for autism to be developed and perhaps even provide ways to prevent or limit the severity of the disease. The work done this summer was primarily in preparation for this study, specifically in starting the Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC) approval process as well as numerous grant proposals.

(Supported by the Alumnae Gift Fund in Chemistry)

Advisor: David Bickar, Chemistry

Development of a Tandem Diels-Alder/Pauson-Khand Strategy for the Synthesis of Tetracycles

Natalie Vaninov/2014



Two of the most powerful ring-forming reactions in organic synthesis are the Diels-Alder and Pauson-Khand reactions, which we intend to use in a tandem sequence to synthesize steroid backbone 13. The Diels-Alder reaction is a [4+2] cycloaddition driven by constructive overlap of the π-orbitals and small energy gap between a diene and dienophile to form a cyclohexene. This cycloaddition is powerful because the reactivity can be manipulated by perturbation of the π-systems, achieved by addition of electron-withdrawing groups to the dienophile and/or electron-donating groups to the diene.

The cobalt complex is an electron donating group known to activate the diene, facilitating the Diels-Alder reaction to form bicyclic intermediate 12. We predict this species would then participate in the Pauson-Khand reaction, a dicobaltoctacarbonyl mediated [2+2+1] cyclization coupling an alkene, alkyne, and a molecule of carbon monoxide to yield a cyclopentenone, to form our desired product 13.

Research until now has focused upon synthesizing tetraenyne 10, the precursor for this tandem sequence. While there is strong evidence that this product was formed previously by Elsa Hinds, there was not enough product to complete full spectral analysis. This summer, I have attempted to duplicate the results and build upon the synthetic scheme developed by Catie Blunt and Elsa Hinds in 2011. The 8-step sequence features an isomerization, alcohol protection, hydrozirconation/iodination, Suzuki-coupling with Sonogashira product 7, followed by alcohol deprotection, oxidation, Wittig reaction, and finally, a Grignard reaction to yield our desired tetraenyne 10.

The isomerization and protection proceeded without fail, but the hydrozirconation/iodination, Sonogashira, and Suzuki reactions have been quite problematic in the past. It was suggested at the ACS/CVS conference in April 2013 that a different electrophilic source of iodine should be investigated, such as N-iodosuccinimide. This proved to work much better than the previously used iodine, likely due to purity problems associate with the reagent. The Sonogashira reaction was attempted several times, never running until completion despite attempts to purify reagents, use a more oxygen-free environment, and heat the reaction. As part of a special studies project, I will continue this work in the fall on the Suzuki-coupling and continue to improve upon the Sonogashira-coupling of 5 and 6 by experimenting with various stoichiometric ratios of reagents as well as different tertiary amine bases to optimize the catalytic cycle.

(Supported by the Schultz Foundation)

Advisor: Kevin M. Shea, Chemistry

Extraction and Analysis of Neurolenins

Signe Dahlberg-Wright/2014

At the root of modern pharmaceuticals lies natural product chemistry—the study of chemical compounds found in nature. Our interest in natural products stems from the medicinal benefits of the plant *Neurolaena lobata*,[1] which has long been used in the Caribbean and Central America for the treatment of several diseases,[2] and its pharmacologically active components, neurolenins, have been the subject of anti-cancer studies. The neurolenin compounds (**A** and **B**, see figure 1)[1] are a type of sesquiterpene lactones—a class of plant terpenoids known for their medicinial effects[1]—that we believe owe their activity to the presence of enone and olefin functional groups, which are the reactive components of the molecule.

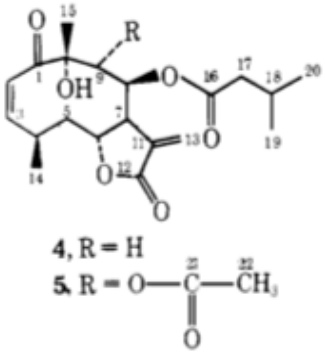


Figure 1: Neurolenin A (4) and B (5)[1]

This summer, we successfully extracted the neurolenin compounds from a source of dried *N. lobata* leaves using a Soxhlet extraction apparatus; purified them with charcoal to eliminate other inactive and irrelevant compounds;[1] and, using column chromatography, separated both isomers for anti-parasitic toxicity testing by Kristine Trotta in the Steven Williams lab. Once a sufficient supply of these isomers is secured, we will inquire into their mechanism by attempting to selectively deactivate both potential active sites (e.g. by olefin hydrogenation, etc.). These new neurolenin derivates will then be sent to the Williams lab to determine if they have changes in anti-parasitic qualities compared with the original neurolenin compounds.

(Supported by the Schultz Foundation)

Advisor: Kevin M. Shea, Chemistry

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Investigating the Helix Stability of DNA Containing an Sp Lesion

Bo Ram Yoo/2014

Oxidative damage in DNA has been linked to various diseases and conditions such as cancer, rheumatoid arthritis, aging, and cardiovascular disease.[1] Since guanine has the lowest reduction potential out of the four bases, it is easily oxidized into 7,8-dihydro-8-oxoguanine (8-oxoG). 8-oxoG has an even lower reduction potential and is further oxidized by oxidants such as Cr(V) or Ir(IV), producing a lesion such as the Sp lesion. The Sp lesion is highly mutagenic and can lead to transversions (i.e. C-G to T-A).[1]

The objective of my summer research was to synthesize the Sp lesion and to separate the two stereoisomers (R and S) using High Performance Liquid Chromatography (HPLC). In order to synthesize the Sp lesion, a 15-mer 8-oxoG containing oligonucleotide (5'-ACTGATC-8oxoG-CCGCACT-3') was treated with 100μM iridium (20 mM stock) along with 10mM sodium phosphate buffer (0.1M stock) and 100 mM sodium chloride (1M stock). The reaction was heated at 65°C for 30 minutes and then quenched with 20 mM EDTA (0.5 M stock). After quenching, most of the iridium was removed by desalting with the Millipore Amicon Ultra centrifugal device. The synthesized Sp lesion was then purified by HPLC using the Dionex anion exchange column, which gradually exchanges a low salt buffer (90% H_2O, 10% acetonitrile) for a high salt buffer (90% 1.5M ammonium acetate, 10% acetonitrile) in order to separate the two stereoisomers. Over the course of 35 minutes, the 60% to 40% low to high salt buffer separation gradient changed to 0 to 100%. The two peaks, present at about 17 minutes (Fig. 1), were collected separately as they eluted at slightly different times and were desalted to remove the ammonium acetate.

Because running the HPLC took up a bulk of the time, I was not able to obtain thermodynamic data for the lesion as I had originally planned. However, I will be continuing my research during the academic school year in order to collect data using Differential Scanning Calorimetry (DSC) and assess the thermodynamic stability of the Sp lesion. After combining the top strand containing one of the two stereoisomers (Sp1 or Sp2) with the bottom strand, I will be able to observe the thermodynamic stability of the lesion when paired with the different bases.

(Supported by the National Institutes of Health)

Advisor: Elizabeth R. Jamieson, Chemistry

[1] Chinyengetere, F., and Jamieson, E.R. Impact of the Oxidized Guanine Lesion Spiroiminodihydantoin on the Conformation and Thermodynamic Stability of a 15-mer DNA Duplex. *Biochemistry*, 2008, *47*, 2584-2591.

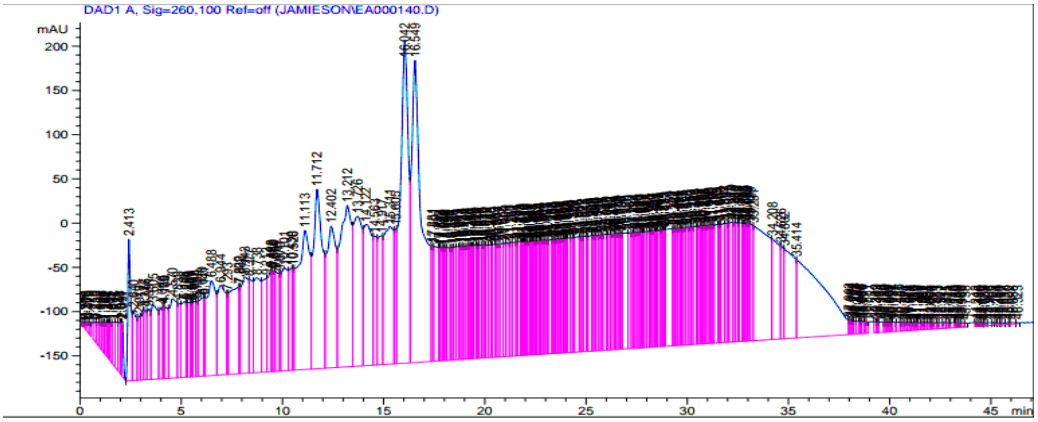
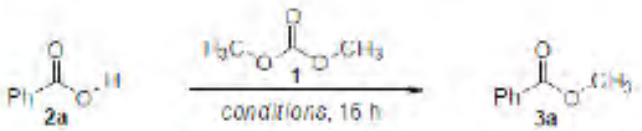


Figure 1. Elution of the two stereoisomers by using HPLC shown by the two peaks at 16.042 and 16.549 minute

Optimization of a Green, Catalytic Methylation Reaction

Jillian Zoglio/2014

Table 1. Optimization of Base-Catalyzed Esterification



entry[a]	catalyst[b]	solvent[c]	T (°C)	yield 3a[d]
1	DBU		90	5%
2	DBU	CH_3CN	75	trace
3	DBU	DMF	90	20%
4	DBU	DMSO	90	58%
5	DABCO	DMSO	90	94%
6	KOH	DMSO	90	88%
7	K_2CO_3	DMSO	90	98%
8	K_2CO_3	DMSO	75	20%
9	$KHCO_3$	DMSO	90	96%
10	KH_2PO_4	DMSO	90	trace
11	Na_2CO_3	DMSO	90	87%
12	Cs_2CO_3	DMSO	90	99%

a) Ratio of 1:2a = 20:1 b) 0.2 equiv. c) [2a] = 0.2 M
d) Determined by GCMS against an internal standard

Chemists methylate carboxylic acids and alcohols in many organic syntheses in order to form methyl ethers or methyl esters.[1,2] Reagents that are currently used for methylation include trimethylsilyl diazomethane, methyl iodide, trimethyloxonium tetrafluoroborate, and dimethyl sulfate. Other than being unstable and expensive, these methylating reagents have something else in common: they pose an incredible safety hazard. Acutely, methylating reagents are highly toxic and can cause death upon inhalation.[3] With chronic exposure, they act as carcinogens.[4] Therefore, even if a chemist were to take precaution, they would still be at risk from chronic long term exposure.

The goal of this project is to optimize the conditions of the methylation reaction so that it produces a comparable yield using the non-toxic methylating reagent, dimethyl carbonate (DMC) and catalytic amounts of a mild base. Currently, the most advanced methods in the literature are not economic nor green. State of the art methylation conditions using DMC require stoichiometric amounts of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), which is an expensive organic base.[5] In order to determine the success of a reaction, the conversion of the simplest carboxylic acid (benzoic acid) to its methyl ester (methyl benzoate) was quantified by GC-MS with dimethyl pimelate as the internal standard. Using the aforementioned reaction conditions as a starting point, a general catalytic methylation reaction was developed.

When the amount of DBU was decreased from stoichiometric to catalytic quantities, the reaction yield decreased dramatically despite further heating of the solution (entry 1). While addition of solvents facilitated product formation, DMSO produced the best turnover (entries 2-4). The effects of other organic bases were explored (entry 5). The base DABCO gave a 94% yield. In addition, inorganic bases were investigated. The base K_2CO_3 was found to catalyze the formation of the product with the greatest efficiency (entry 7). The base KH_2PO_4 did not facilitate product formation, suggesting that a certain bascitity must be reached in order to deprotonate the nucleophile (entry 10). There was no evidence of a countercation effect (entries 11, 12). Potassium carbonate was selected for larger scale methylation because it is an inexpensive, mild, and effective base.

(Supported by the Alumnae Gift Fund in Chemistry)

Advisor: David Gorin, Chemistry

[1]Peng, F.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 18860-18867.

[2]Petronijevic, F. R.; Wipf, P. *J. Am. Chem. Soc.* **2011**, *133*, 7704-7707.

[3] a) *Dimethyl Sulphate.* MSDS No. D5279 [Online]; Sigma-Aldrich, 05/04/2009, www.sigma-aldrich.com, (accessed 03/20/2013). b) *Iodomethane.* MSDS No. 289566 [Online]; Sigma-Aldrich, 02/25/2011, www.sigma-aldrich.com, (accessed 03/20/2013)

[4] Kemsley, J. N. "Firm Fined For Chemist's Death." *Chemical and Engineering News* **2011**, *89*, 15.

[5] Carafa, M.; Mesto, E.; Quaranta, E. "DBU-Promoted Nucleophilic Activation of Carbonic Acid Diesters." *European Journal of Organic Chemistry* **2011**, 2458-2465.

Knowledge Transfer Algorithms in Markov Decision Processes

Pratistha Bhattarai/2015

We have implemented a variety of existing algorithms to create agents capable of transferring knowledge from one domain to another. These domains are represented by Markov Decision Processes (MDPs), a computational framework for decision making. In such a model, at any given time step, an agent is in a state where it can choose one of many available actions. After choosing any one action, the agent transitions into different possible next states with different probabilities. Depending on what this next state is, the agent is granted a reward specific to that state. The agent tries to maximize its cumulative reward and in trying to do so, comes up with a policy that dictates the action to be taken in each state. Since for large state spaces, finding this policy can be computationally expensive, we programmed an agent capable of abstracting a problem and then solving it and transferring this policy to matching new problems.

By solving a more general version of a problem, our agent not only achieves computational efficiency but also becomes capable of solving other similar problems. For example, an agent capable of stacking cups is also capable of stacking plates. By drawing analogies between a known MDP and an unknown one, it can "automatically" solve the latter.

We have used different kinds of MDPs for the purpose of our research: tic-tac-toe, with the opponent built into the MDP model, crossing-river MDP, where the agent has to move objects across the river while satisfying a number of constraints, grid-world, where the agent moves around in a grid, and blocks world, where the agent moves blocks off and on top of one another to achieve the desired configuration.

Our agent uses a sequence of algorithms to solve MDPs by drawing analogies. First, it uses a pre-existing Homomorphism Algorithm to take a set of MDPs and generate equivalent abstract MDPs with fewer states and actions. It uses the Value Iteration Algorithm to find the optimum policy for the MDPs. Using this set of models, our agent maneuvers in a new, unknown world, observing at each step the state it visits, the action it takes and the reward it accumulates. It generates samples and by running our algorithm on them, infers the nature of the world by matching it to a known model. We designed a Bayesian network (a probabilistic model with directed conditional dependencies) to draw analogies between the old MDP and the new world. From this network, the agent uses the Forward Backward algorithm to calculate the likelihood that a trajectory of states and actions it experienced are consistent with an existing model.

Our agent predicts the homomorphic mapping between an unknown MDP and a known abstract MDP. It does this by maintaining a belief of the probabilities with which the states it visited could map to the different states of a known abstract MDP. For example, while trying to stack cups, an agent could compare certain features of the world to the world of stacking plates. For example, it could believe that a stack of cups – a,b,c, in that order – to be analogous to the stack of plates – q,r,s – with a certain probability.

With further calculations, the agent can predict which known abstract MDP best fits the unknown MDP. For example, it could conclude that stacking plates is more analogous to stacking cups than to washing dishes. It could then transfer the learnt policy for stacking cups to stacking plates without having to apply the value function to it. Learning about a world via sampling and analogies is very useful since in many real-life situations an agent is not given complete information about its environment and it can efficiently come up with an optimum policy only by transferring knowledge from relevant past experiences.

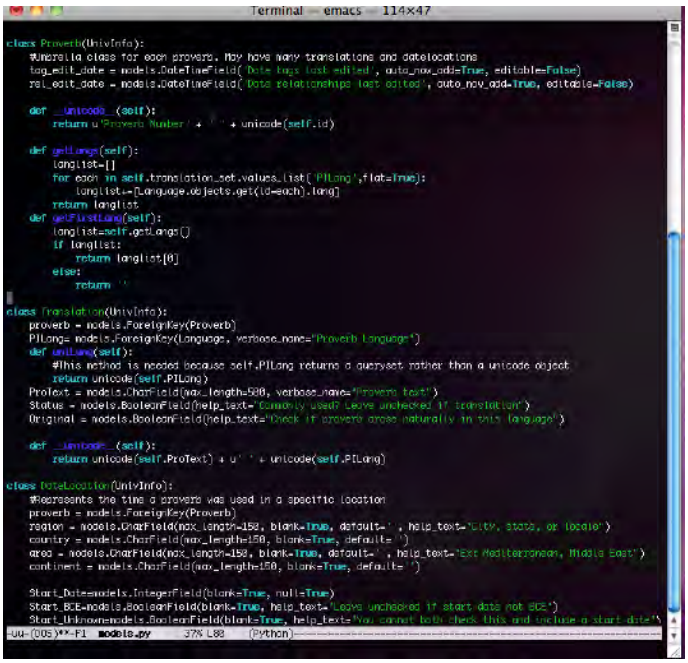
(Supported by the Schultz Foundation)

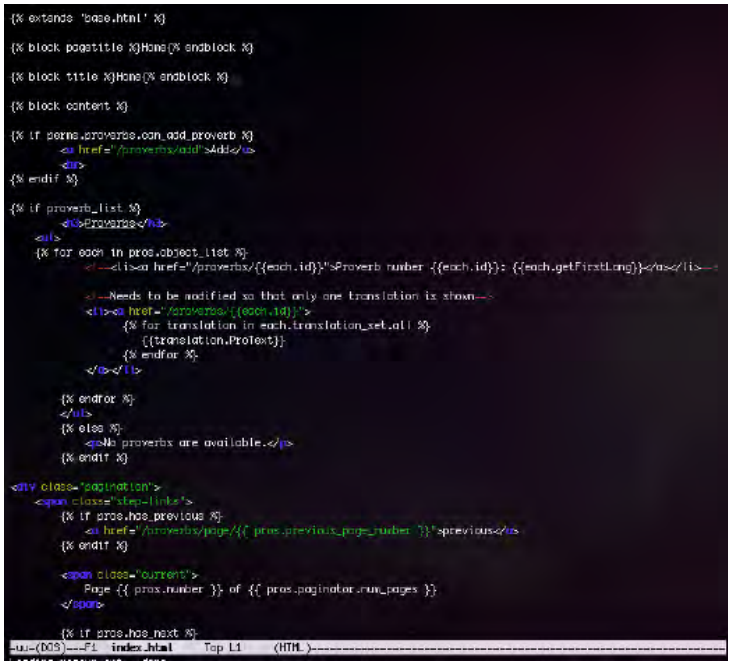
Advisor: Alicia P. Wolfe, Computer Science

SMITH COLLEGE

The Global Proverbs Project

Grace Lee/2015





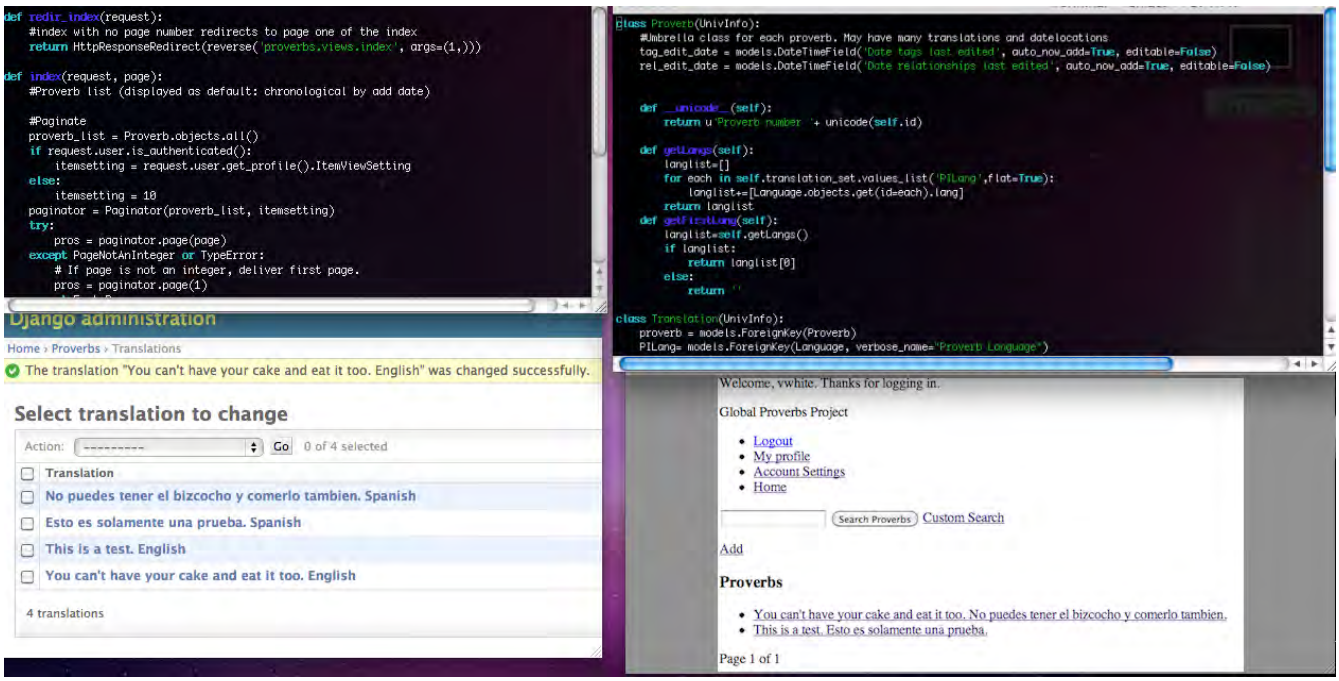
As part of a larger project called Global Proverbs, my research was focused on the maintenance and development of a multilingual database built with the Django web framework and MySQL. Global Proverbs is an interdisciplinary project that involves the use of natural language processing, data mining technologies, and art production in order to explore the displacement of meaning that occurs when cultural artifacts such as proverbs are taken out of their cultural context. The Global Proverbs database, which was developed to address the lack of an online collection of proverbs with varied categorization patterns, will be used as source material for an art piece in addition to serving as an online resource for paremiologists and the general public. I built upon the work of previous students who completed the initial development of the site by adding a supplementary database of external resources, in addition to examining the structure of the current website and finding bugs and potential defects. Throughout this code review process our efforts were primarily focused on trying to correct various usability issues by clarifying ambiguity in certain form fields, displaying the proper translation of a proverb in the index rather than its identification number, and altering certain built-in frameworks to allow for easier navigation. The Django framework, which employs the model-view-controller pattern, includes a web templating system and data models mapped to a relational database. This proved to be an especially effective framework for a database-driven website such as Global Proverbs: for example, representing each proverb as a data model (defined by a Python class) allowed each proverb to be easily annotated with cultural, temporal, geographic, and thematic information – areas in which currently available databases were inadequate. Such utilization of current database and web technology in developing this more functional proverbs database will likely be useful in facilitating future paremiological research.

(Supported by the Schultz Foundation)

Advisor: Eitan Mendelowitz, Computer Science

The Global Proverbs Database Project

Vatasha White/2015



The global proverb database is a research project that focuses on gathering and creating a public database of proverbs. The database uses Django, which is a high-level python framework that encourages rapid development and clean, pragmatic design.[1] My primary focus this summer was learning how to understand and manipulate Django. This would allow me to work on the database code that was put together by another student.

Some of the most important modules in the application are the views.py and models.py. Inside of the models.py module all of the data for the database is defined. All of the data inside of models.py is written using classes so that each chunk of data is an object. Each object can then have methods, which are the functions that are defined in the class. The views module then helps format the way the data should be displayed; this can be thought of as different pages for each class. This summer I focused on trying to indentify bugs within the database and then correct them. Some of the bugs I encountered were displaying the text of the proverb on different pages and redirecting the comments page after a comment is submitted. I was able to fix problem one partially; now the text of the proverb is displayed on some pages. This causes some inconsistency and also created other bugs within the database. To resolve this issue, a function needs to be created that uses the user's information as an argument. Once the language is determined, the function can then return the appropriate translation. Another challenge was being able to understand the functionality of the built in comments app. This application is used in the code when a comment is added to one of the proverbs. Once a comment is submitted, the user is automatically directed to a "thank you for your comment" page. Within the comments application, manipulating the code so that this redirection does not happen has not been solved yet. We hope to continue to find ways to get the database up and running effectively so that it can be visible to the public.

(Supported by the Schultz Foundation)

Advisor: Eitan Mendelowitz, Computer Science

[1] "Django at a Glance | Django Documentation | Django." *Django at a Glance | Django Documentation | Django*. N.p., n.d. Web. 15 Aug. 2013.

Protein Engineering for Triple Negative Breast Cancer Detection

Fatima Bassir/2015

Triple Negative Breast Cancer is a type of breast cancer that is bereft of estrogen, progesterone and HER2 receptors and hence does not respond to hormonal therapies. This type of breast cancer accounts for about 16% of all breast cancers yet there are no means of effective diagnosis for the disease and as in most cancers early detection results in higher survival rates. Hence the goal of my research is to engineer a protein that will bind to mesothelin receptors, a known receptor for this type cancer, and can be used in the diagnosis of triple-negative breast cancer through molecular imaging.

As a starting point I have used MUC16 ,a molecule that binds to mesothelin, from which I will engineer a new protein with mesothelin binding ability. However this new protein should not signal the same disease progression pathway as MUC16 does. In order to study the binding of this molecule, I have used a technique called yeast surface display. Alongside this, I also prepared a plasmid for soluble MUC16 secretion, which is needed for a partner project. For this, I engineered a plasmid vector to carry the MUC16 gene and amplified the engineered plasmid in DH5, an Escherichia coli strain of bacteria, as seen in Fig 1.0.

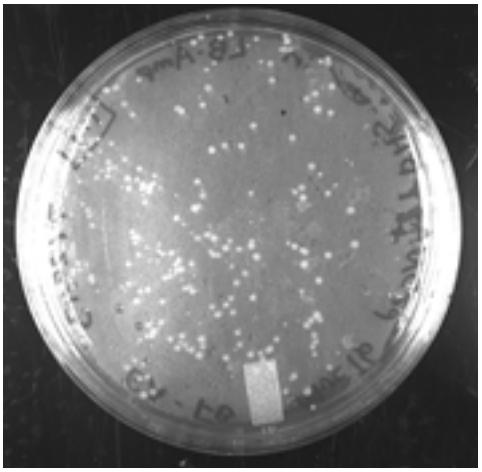


Figure 1.0 - muc16_pCT inDH5alpha

I then extracted these plasmids and transformed them in EBY100 yeast cells, which then produce MUC16 on their surface.

At the end of the SURF program I was able to engineer the vector plasmid and amplify it in DH5. I verified that the correct vector was engineered through checking for correct gene lengths with electrophoresis; the plasmid is yet to be sequenced to ensure it has the correct gene sequence. No fluorescence was detected when the flow cytometer was used to determine whether the protein was being displayed on the yeast's surface. I suspect that the glycosylation present in the molecule has a role to play in the difficulty in producing the protein. In order to continue this research, I would like to do a special study this coming semester. Through group meetings with a neighboring lab, I have had the opportunity to present my research to professors and fellow student researchers.

(Supported by the Schultz Foundation)

Advisor: Sarah Moore, Engineering

Developing a Pedagogical Approach to Engineering

Anna Beatty/2016

In this project, I worked on an online platform that utilizes an innovative theory of imaginative education to bring middle school students and engineering together. This summer, the research team focused on developing the first two units that will appear on our website, ThroughMyWindow. We brainstormed storylines that would engage students emotionally and intellectually and worked in groups to conduct knowledge building and improve our ideas. The team completed the Artificial Intelligence (AI) unit, designing interactive, narrative and visual components to foster learning.

The unit begins with a story in the form of a graphic novel, in which the main character, Rio, has his brain stolen by an evil laboratory. To get him a new brain, the user needs to travel through three rooms of an engineer's mansion and learn about AI. The first room asks the user to explore the question of "what is intelligence" as it applies to humans and animals. The second introduces the idea of intelligent machines, how they work, and what kinds of tasks are difficult and easy for them. The third introduces Alan Turing, the father of AI, and relates his life and ideas to the present through neural nets. Today's most promising AI technology, neural nets, developed from Turing's ideas about thinking machines. At the end, the user comes to the realization that technology as it exists today is not able to replicate the human brain. Throughout the unit, an online journal gives students an opportunity to write and share their ideas in response to guided, plot-driven prompts. Students ask, discuss, and attempt to answer important questions about the meaning of intelligence and the capabilities and limitations of technology.

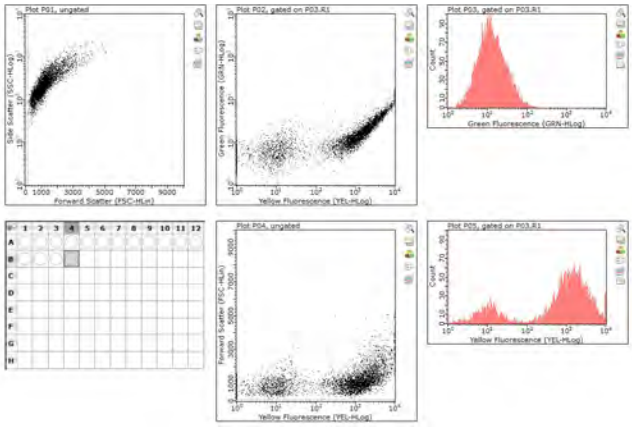
The AI unit provides a model for our next unit, which will focus on engineering design. Users will go back in time to learn about the engineering design cycle through the story of the electrical revolution and the clash of two great inventors of the age, Tesla and Edison. In the fall, we will continue to develop the design cycle unit and begin work on an engineering ethics unit. We will also test the results of our idea-driven, story-centered approach to discover if we succeeded in fostering deep learning. Based on the result, we will continue to improve our methods and hope that our work could lead the way for thoughtful STEM education.

(Supported by the Schultz Foundation)

Advisor: Glenn Ellis, Engineering

Engineering Proteins to Block Cancer Cell Signals

Katia George/2015



Mesothelin is a tumor differentiation antigen with high expression in epithelial cancer cells such as ovarian, breast, and various types of pancreatic cancer cells, and low expression in normal cells.[1] When mesothelin binds with the tumor antigen MUC-16, it can lead to metastasis of tumor cells. Antibodies have been engineered to bind to mesothelin, successfully blocking the interaction between MUC-16 and mesothelin.[2] However, antibodies are large and are often ineffective in reaching the centers of dense tumors. This summer, I began to engineer a protein, which is smaller and likely more effective than an antibody, in order to bind to MUC-16 and prevent the interaction that causes metastasis of certain cancer cells.

My intention was to create something that will bind to MUC-16, and it is already known that mesothelin does this, so I decided to use mesothelin as a starting point and then make mutations to this. Thus, the first step of my research was identifying a binding domain of the mesothelin protein. I then ordered the DNA sequence and primers for this domain from *New England BioLabs*. After PCR amplifying the sequence, I digested it with restriction enzymes and ligated it to a previously created plasmid. The ligated plasmid was then amplified in a competent bacteria cell, and then transformed to an electrocompetent yeast cell, where the mesothelin protein was expressed on the surface of the cell. The protein on the surface of the cell was then labeled with fluorescent antibodies and binding was detected using flow cytometry. The figure above is the data collected shows that the mesothelin protein was successfully expressed on the surface of the yeast cell.

Throughout the summer I was able to present my findings and troubleshoot with other students and faculty in the building during weekly meetings. The next step for my project will be to begin making mutations on the protein, also called directed evolution, as a means of creating new proteins that will possibly fulfill the intended function. This fall I plan on continuing my research as a special studies project with Professor Sarah Moore.

(Supported by the Schultz Foundation)

Advisor: Sarah Moore, Engineering

[1] Ma, Jichun. "Recognition of Mesothelin by the Therapeutic Antibody MORAb-009: Structural and Mechanistic Insights." *Journal of Biological Chemistry*. n. page. Print.

[2] Ho, Mitchell. "A novel high affinity human monoclonal antibody to mesothelin." *International Journal of Cancer*. n. page. Print.

Wind Power Integration

Astrid Harradan/2015

Wind energy can be seen as one of the most viable renewable energy options, but the questions of predictability and integration remain prominent in the minds of house owners and consumers.

We used Jiminy Peak as a case study to better understand the concept of wind power integration. Jiminy Peak is a ski resort located in Hancock MA. Its owners installed a turbine to aid the environmentally friendly efforts of their business. Through articles, interviews and news clips we found that Jiminy has a completely seamless system that allows them to run their business uninterrupted while profiting immensely from their single wind turbine. Jiminy receives energy credit from the energy they supply to the grid and this is used when they need power from the grid. A similar set up can be used by home owners.

A further look into both predictability and integration brought to light misconceptions on our part. We looked at wind variability as a barrier in terms of day to day issues but the software offers seamless supplementation from the grid. The real barrier lies in the months that experience less wind. A look into consumer usage through the months shows that the months with the highest amount of wind does not correlate with the months of highest usage. Location and size of the turbine can also hinder such a system on the homeowner level.

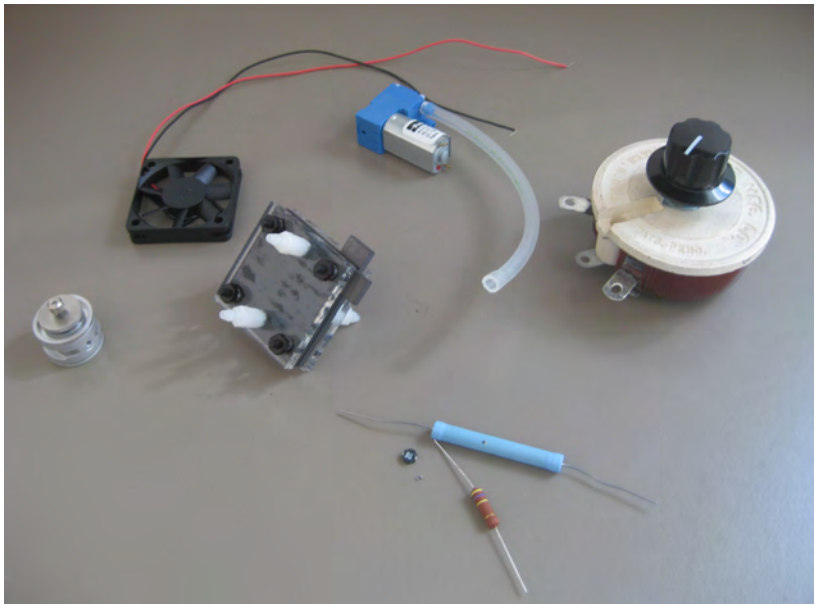
Further study would lie in exploring a way to scale down a system such as the one used at Jiminy to a single home or to scale up a system to a small community.

(Supported by the National Science Foundation)

Advisor: Judith Cardell, Computer Science/Engineering

Fuel Cell System Design for Lightweight Applications

Lauren Hilton/2014, Raisa Rubin/2014 and Wiame El Bouhali/2014



Most applications for lightweight portable electronic systems in our everyday lives are powered by batteries. Alkaline and lithium-ion batteries dominate because they are cheap; however, they are caustic and non-renewable. As environmental problems become more critical, the need to reduce waste and find a fuel that is domestically produced and readily available becomes crucial. Hydrogen fuel cells are clean and efficient energy producers that rely on oxygen and hydrogen to produce electricity and water. The challenge with fuel cells currently is that they are typically large and bulky systems intended for larger scale applications. Our challenge for the summer was to simplify the fuel cell system design and create one that is small and lightweight.

The anode inlet, cathode inlet and power output were all analyzed and potential difficulties determined. At the anode, air from the atmosphere needs to be supplied to the fuel cell at a rate of 27 Nl/hr. The chosen device for directing air must also be able to withstand the backpressure created by the fuel cell[1]. The cathode, on the other hand, uses highly pressurized hydrogen and the pressure needs to decrease to half the pressure supplied at the anode at the inlet from the higher pressure tank. The tank needs to be made of a high pressure rated material that is also lightweight. As these reactants flow through the fuel cell they create power. As this power comes out of the fuel cell it is not usable for our application. Somehow we needed to double the output voltage and half our input current for our use. After looking at the requirements for each component of the fuel cell, we reviewed specifications for parts and ordered the best ones for testing.

This project gave us the opportunity to learn about all of the individual components of a fuel cell and how they work as a whole. Further testing and prototyping will be necessary for many components, such as the DC/DC converter, pressure testing for the hydrogen tank, and testing of the pressure regulator. This project will be continued as a thesis.

(Supported by the Schultz Foundation)

Advisor: Denise McKahn, Engineering

[1] Spiegel, Colleen. *Designing and Building Fuel Cells*. New York: McGraw-Hill, 2007. Print.

Household Wind Integration: Load Analysis

Chelsea Hinds-Charles/2015

The field of power systems is expanding to new heights as it begins to focus on the individual consumers' usage to better understand the aggregate solution. With wind power integration being the current form of creating electric energy on an aggregate scale, this same method of integrating wind can service the individual consumers. However, arriving to that point of efficiently integrating wind into the typical consumer's energy consumption lifestyle with the most resulting benefits is to first understand the loads and their effects on the demand and response of electricity during the peak electricity cost hours.

The study focused on the time segment from 10am to 4pm in a model of the typical Smith College student's room and a model of a kitchenette during lunch time. Some of the integration variables that were in the running to be most effective were dependent on high cost time periods, wind availability, and load influence. The named wind integration variables all played a role in creating wind powered electricity. To maintain within the scope of this study of the demand and response of electricity created by the loads applied by a typical student, four home electricity monitoring devices were used. The four monitoring devices used were Watts Up, Kilowatt, Check-It Solutions and Elite Classic. The use of four devices increased the accuracy when analyzing the effects of the loads on the electricity produced to service the area in question. As the measured watts of the electric loads applied instantaneously and constantly throughout the experiment were collected and analyzed, the wind conditions were observed to note any connecting results between maximum wind availability and load application. The research provided that the most beneficial time periods which accommodates usage and available for a typical student would be between 11:30am to 12:45pm and 2:45pm to 4pm.

(Supported by the National Science Foundation)

Advisor: Judith Cardell, Engineering/Computer Science

Fig 1: Display of the setup of devices connected to the loads.

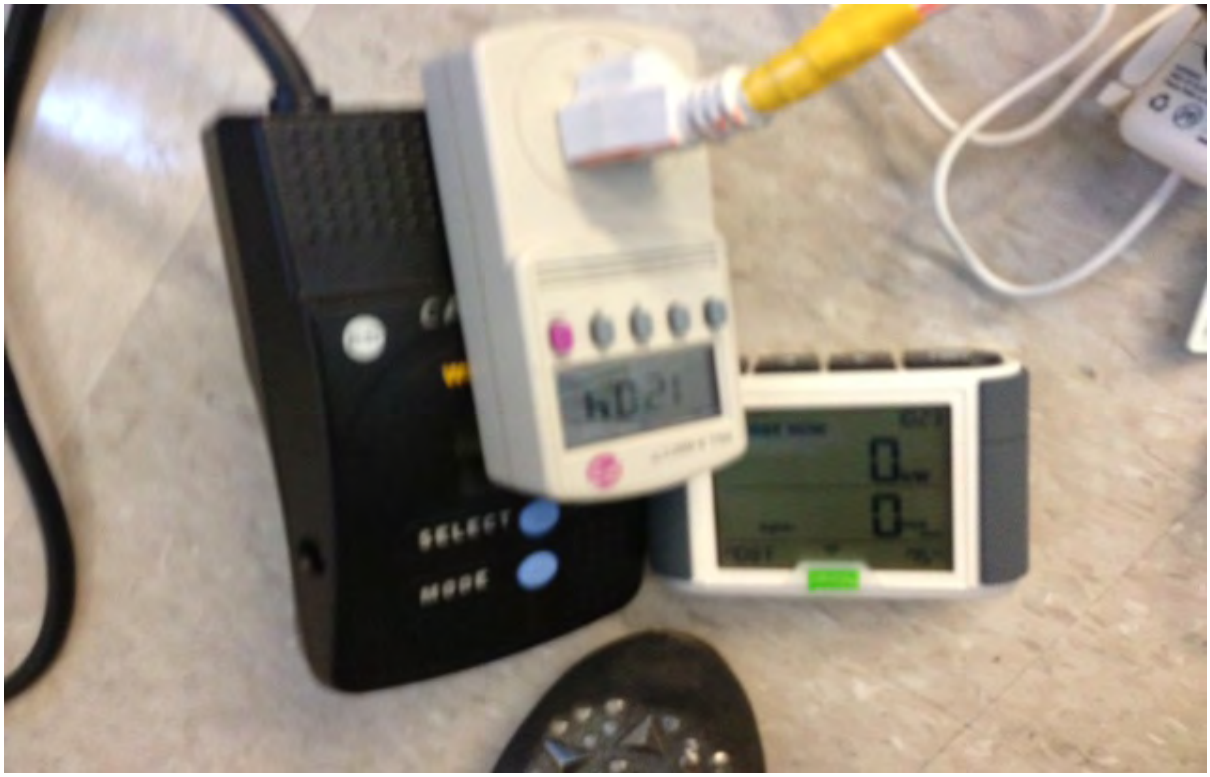


Fig 2: Display of the set up of devices observed as loads are applied.

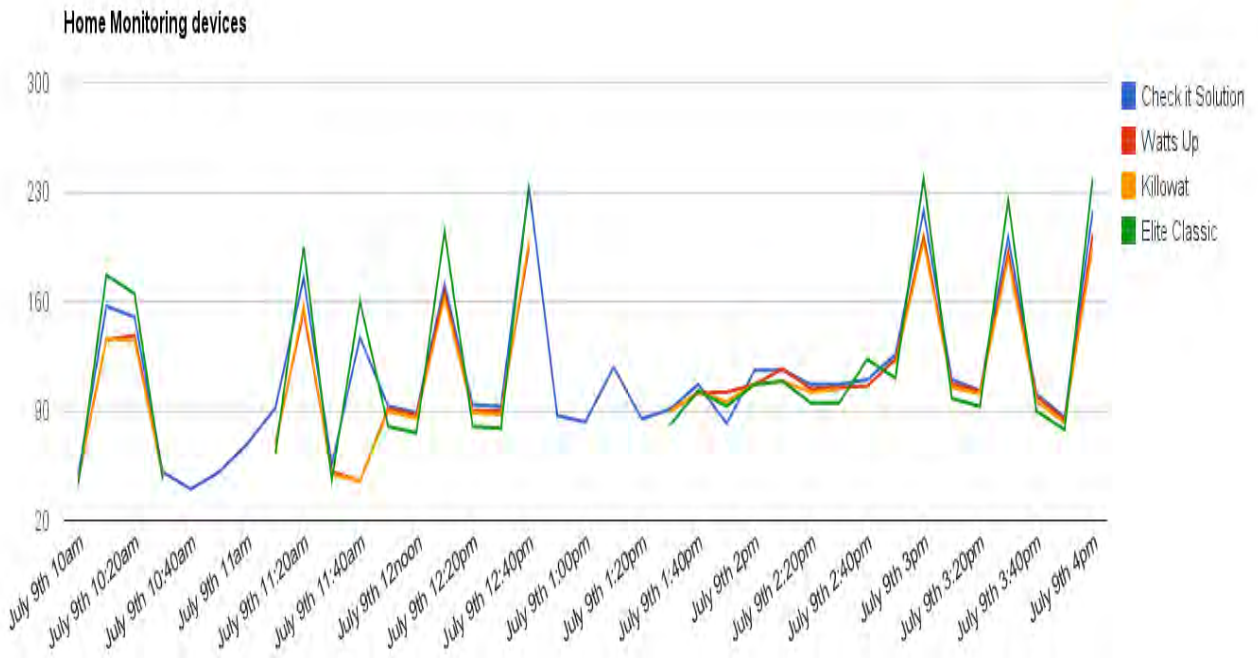


Fig 3: The graph above shows the results of the load effects on the different devices (measured in watts) during the high energy cost time period from 10am to 4pm.

Sensitivity Analysis of the Approximation of Bilevel Generation Expansion Equilibria

Adrienne Horne/2014

The objective of this project was to introduce myself to optimization and equilibrium problems used in power generation planning and become comfortable with modeling in GAMS. These new skills would enable me to analyze the effectiveness of an existing singlelevel equilibrium generation expansion model developed when taking into account a larger number of generation companies and asymmetric parameters.

By first becoming familiar with convex optimization and the GAMS programming language, it was possible to develop a simple market equilibrium problem, optimization problem, and complimentarity problem on paper, which could then be transferred into GAMS code. Thus mastering the major concepts of bilevel equilibrium modeling, the more complicated market equilibrium bilevel approximation problem could then be coded in GAMS. This program was then streamlined and extended the existing approximation to take into account multiple players and asymmetric parameters.

At this point, there exists a functioning GAMS program that allows for the modeling of multiple players and asymmetric parameters. I plan to continue my work with this program into the fall as a special studies project so that I may perform a complete sensitivity analysis with parameters of the number of generation companies, demand and wind data, cost data, and strategic spotmarket behavior. A poster of the aforementioned objectives and future plans was presented at the Wind Integration Simulations Partnership for International Research and Education workshop at the Technical University of Denmark in June of 2013. After completing this further analysis, it will be possible to assess the effectiveness of the approximation and complete a paper on these findings which may be submitted for publication in a scholarly journal.

This opportunity allowed me to learn more about tools for power generation planning and modeling and to explore my future career options within engineering science and research. With very limited prior experience with optimization, economic models, and GAMS, this summer I was able to increase my knowledge in these areas to a point where I feel comfortable using my new skills to pursue an independent special studies project in the fall.

(Supported by the National Science Foundation)

Advisor: Judith Cardell, Computer Science/Engineering

Supervisors: Sonja Wogrin and Andrés Ramos

Design and Develop Online Learning Environment

Xi Jiang/2014

How can we help students engage more in getting to know about engineering? Smith College and Springfield Technical Community College (STCC) propose a Full-Scale Development Project for engaging children and teens in engineering. The project will apply innovative approaches to learning about engineering that are emerging from the learning sciences. We are testing the hypothesis that the use of narrative & imagination along with the principles of knowledge building will help student learn more about engineering and let students become more interested in engineering. My research happens during the implementation phase of this long-term development project and my work will continue into the new academic year as a special studies.

This summer I worked with another student under the guidance of Professor Alan Rudnitsky and Professor Glenn Ellis. Our group engaged in both design and development of online environments for the purposes to increase students' interest in and knowledge about engineering for middle school students. During these ten weeks, we designed the framework for online environment and the flowchart of online learning process based on the story line of the Graphic Novel "Where am I?" by Daniel Dennett. We detailed every corner of each scene and every possible operation in the flowchart. We also collected different forms of information enlightening students to think more about interesting facts about engineering through the design of artificial intelligent brain for Rio, the leading character in the novel.

(Supported by the Schultz Foundation)

Advisor: Glenn W. Ellis and Alan Rudnitsky, Engineering

Demand Response and Wind Ramping Analysis

Jinjin Lu/2014

Part I

Historically, wholesale and retail electricity markets have been designed and maintained as separate markets, without any mechanisms for interactions between participants in the distinct marketplaces. Growing interest in developing responsive demand brings into question the logic of this separation, as retail customers could begin to play a role in maintaining a stable power grid and market price. With this in mind, the California ISO has proposed a price-based signal – the grid state indicator – that would be initiated by the CAISO, updated by distribution system operators, and sent to responsive demand. Thus the signal will allow retail customers to respond to the state of the wholesale market and high voltage grid. In this project, an electricity price signal model consistent with the proposed CAISO electricity grid state indicator is developed, along with an electricity load model. A customer-agent model applying Q-learning, a form of reinforcement learning, is designed to predict electricity load reduction based on price-responsive demand. Results based on data from the New York ISO assess potential savings, as well as load reduction to smooth out demand, from implementing the proposed grid state signal.

Part II

The variability of wind power poses a big challenge to power system operators in large-scale wind energy integration to maintain the power grid reliability. The fluctuation of the individual wind turbine can be compensated by the installation of multiple turbines over a large wind farm. The smoothing behavior needs to be analyzed to capture the ramping character (i.e., the change in MW output over time) of a wind farm, in order to ameliorate increasing wind integration into the power grid. The research is designed to find the ramping character of aggregated wind power over New England area, emphasizing typical rates, magnitudes, and duration of the ramping events, while identifying extreme situations.

Wind energy integration increases power system variability and uncertainty. To mitigate such effect, demand response can be used to fill the gap. The potential rampability of responsive load will be explored to increase the opportunities to balance wind ramping events. The electric appliances, equipments, and electricity consumption behavior with feasible ramping character will be identified as capable of providing ramping products. Interacted with wind ramping events, the ramping products will hopefully provide better overall response.

(Supported by the Schultz Foundation)

Advisor: Judith Cardell, Computer Science/Engineering

Artificial Intelligence Learning Journey

Zhenzhen Tan/2015

How can we introduce middle school students to the fascinating world of engineering? In my research, I focused on designing an interactive online game based on an engaging graphic novel.[1] In this game, students will help Rio, whose brain is stolen by a group of evil scientists, find an artificial brain substitute. The whole design process is aiming at triggering interests of students in engineering as well as giving them opportunities to explore artificial intelligence.

Our group designed the landing page to be outside an abandoned mansion of Doctor X, who is an expert in artificial intelligence. There are three rooms in the mansion which are designed to incorporate different aspects of artificial intelligence including the basic concept of what is intelligence, classical artificial intelligence, neural network, language processing and Turing test. In this game, students can track their progress through writing their own learning journey. The biggest challenge in my design was how to convey information in an amazing way so that the students are intrigued to explore the seemingly foreign and complicated topic. I researched intensely into videos, online applications and other scholarly work to extract a simple model for each activity and tie all the stops on the learning journey to the story line of the graphic novel. The user experience is the key in design so I came up with ways to give smart feedback to students and to make games, for instance, 20 Questions Game more educational. Moreover, the game is also designed to evaluate the performance of students at the end of the game and endorse qualified students a chattercat which is a live model of artificial intelligence to help students find a brain for Rio.

We have completed design for two rooms in the game so far and I will continue working on this project in coming fall semester. By working on this project, I have learned a lot about artificial intelligence. I also accomplished my goal as to develop skills in communication, design and editing.

(Supported by the Schultz Foundation)

Advisor: Glenn W. Ellis, Engineering

[1] *Talk to Me* Sonia K. Ellis

Android Programming for Smart Electricity Monitoring

Yamin Tun/2014

One of the big challenges of maintaining our campus sustainability is to control the electricity usage and save energy in a collective way. In the spring semester of 2013, Rumbidzai Vushe and I developed a hardware device that can display the electricity usage data of Smith campus buildings in public areas of the building. However, as I realized the design limitations of the device such as the necessity of Ethernet and the limited mobility of the device, I came up with another idea. Nowadays, smartphones allow individual users to customize their device by installing and running applications according to their personal needs. If the information is displayed on a mobile application, the user can be informed more instantly with no need of separate hardware. With this purpose, this electricity-monitoring application was developed to make the electricity usage measurements of campus buildings accessible to smartphone users on campus and alert them if the electricity consumed exceeds the usual needs.

This Android application was developed in Eclipse IDE. The major purpose of the application was defined as showing electricity usage information on the screen. The application was designated to extract the electricity usage data from a html sample page by using the device network. An additional library named JSOUP was used for parsing html data. The Android software development kit (SDK), Eclipse and Android Virtual Devices (AVD) were installed. According to the design, the Android project was created with source code, resource files and Android manifest file. Throughout the iterative design process, the project was run in debug mode using AVD emulator and Android debugging and logging tools. As the final step, the application was tested on a hardware android device.

Though it is relatively straightforward to create an android application, several design factors were considered to write a 'good' application. For example, since Android powers several different brands of phones, tablets and other devices with a wide range of screen sizes, the application has to accommodate different screen sizes. Three design principles suggested by android developer website were considered in developing this application: beautiful visual, simplicity and personal touch. The whole application is organized in different layouts. Each layout of the application is kept simple with concise language and intuitive GUI user control, which can lead to another layout. The green-colored theme was chosen to represent the purpose of the application which is to 'go green' by monitoring electricity use. The electricity use in an on-campus building is displayed in kilowatt on the screen. The current electricity usage is compared with the historical electricity consumption data of the building to provide the information on three modes of use: high, medium and low. The mode will be indicated in three different colors to give a sense of whether the electricity use is more than the average need of the building. According to the mode, a further suggestion will be provided to reduce the usage.

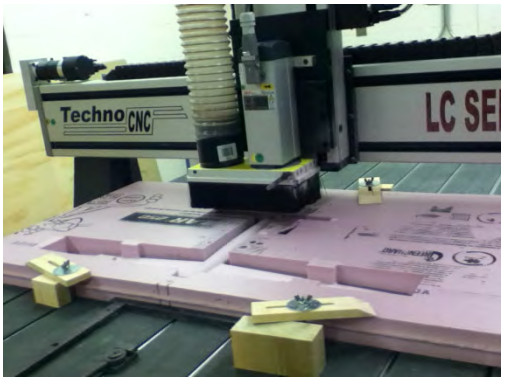
Since the general users will rely mostly on the colors as the feedback, the reasonable thresholds for the electricity usage modes were determined using the historical data from BuildingDashboard website created by Lucid Design Group. With the use of this application, the users can have a better sense of how much electricity they are consuming and take further actions to reserve energy and costs.

(Supported by the National Science Foundation)

Advisor: Judith Cardell, Engineering

Designing Unmanned Aircraft Model with Pressure Sensor

Lingyi Wu/2014



The objective of my project is to manufacture a functional airplane model and to continue working on the pressure sensor board that was already designed and manufactured from past research. The connection of the pressure sensor board needs to be tested and programs need to be created and uploaded so that the pressure sensor can take the data correctly. Taking pressure data is especially important to model airplane because wind speed can be obtained from the measurement of static and dynamic pressure, which are a good indication of the atmospheric environment. Stability and controllability of the airplane model can be analyzed from the measurement as well.

The connection of the circuit board was checked using a multimeter. The data sheet of A to D converter was perused and spin program to make the pressure sensor taking data was loaded on the circuit board. A canard-wing airplane model was modified in Solidworks 3D software and manufactured in the machine shop using 3D CNC router from pink styrofoam. A pair of steel landing gear was designed and attached to a wooden block which is then glued to the bottom of the model. The control surfaces were designed to be at the main wing. The placement of the landing gear, the battery and the radio was determined based on the weight balance and the general aerodynamic stability of the model.

Every connection on the board was tested and checked to be correct. The interface between the microprocessor and the A to D converter was found to be SPI protocol. Different codes and programs were created and tested and after receiving multiple wrong messages, the pressure sensor can eventually take data correctly. The manufacturing of the airplane model came out to be perfect and a lot of precautions were taken during the routing process like securing the foam block with wooden clamps and marking the block so that it will stay at the same position when machining the other side of the model.

This research is crucial in that it has laid a solid foundation for future research which might turn into a special studies or thesis in the following year with the airplane model with landing gear already been manufactured and the circuit board with pressure sensor proved to be working correctly. The aerodynamic stability and the characteristic of the airflow over the wing profile will be analyzed once the circuit board is attached to the airplane model and indoor flight test has been done.

(Supported by the Schultz Foundation)

Advisor: Paul Voss, Engineering

The Development of 21st Century Skills in the Knowledge Building Environment

Yezhezi Zhang/2016

The world is currently experiencing a shift from industrial economy to knowledge economy in which an increasing amount of activities are around the production of knowledge. Compared to the dramatic transformation in economics, the reform of education structures in the society, however, is left behind (Seltzer & Bentley, 1999). The simple knowledge transmission without deeper understanding and up-to-date assessment does not accord with expectations from either learning scientists or the engineering profession (ABET, National Science Board, etc.). Moreover, the current education does less than enough in engaging students in long-term knowledge advancement.

To change the situation and help students actively engage in innovation work, schools should be aiming at educating students who could participate in sophisticated thinking and flexible problem solving with strong collaboration, communication, and leadership skills, which are all captured by the 21st century skills, an analytic framework consisting of a set of educational standards. (Binkley et al., 2012) Transforming schools into knowledge building organizations - which encourages students to pursue knowledge and eventually creates knowledge in an knowledge building environment - is what has be proposed by Bereiter (2002) and what is implemented in a class that I studied this summer.

My research addressed a key concern identified by top researchers by focusing on analysis of 21st century skills in current learning environments. With the guidance of my advisor, I analyzed students' online discussions in Knowledge Forum, problem-framing homework, reflections and portfolios, and instructor's notes from the engineering courses in the past 2 years. T-test and other statistical techniques were also used to support my findings. The research found out that Knowledge Building feature is an effective learning environment that could develop students' 21st century skills and support knowledge creation. It also found out that the Knowledge Building environment developed both efficiency and innovation in knowledge transfer and helped students become adaptive experts who could better fit in and contribute to the society.

At the end of the research, I drafted a paper to advocate reforming engineering education by turning the classroom into knowledge building environment. The paper starts with the description of current issues in engineering education plus a review of education theory (mainly Knowledge Building). Then it elaborates the designed learning environment with an emphasis on the Knowledge Building feature and demonstrates the effectiveness. I will continue working on the project and hopefully submit the paper to Journal of Engineering Education by October.

(Supported by the Schultz Foundation)

Advisor: Glenn W. Ellis, Engineering

Binkley, M., Erstad, O., Herman, J., Raizen, S., Ripley, M., Miller-Ricci, M., et al. (2012). Defining Twenty-First Century Skills. *Assessment and teaching of 21st century skills* (p. 17~66). Dordrecht: Springer.

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Public Policy Research with NOAA's MPA Center

Catherine Aguilar/2015

My internship with the National Oceanic and Atmospheric Administration (NOAA) Marine Protected Area (MPA) Center gave me insight about the public policy aspect of environmental science. This internship provided me with the opportunity to take part in national-level conservation and education efforts for Marine Protected Areas.

Throughout the internship, I worked on a variety of projects that focused on three areas: communications, policy, and professional development. The communications projects included designing the MPA Center's newsletter banner using Apple Keynote; editing the MPA Center's newsletter layout using Microsoft Word Publisher; posting on the MPA Center's Facebook page; and compiling a "Did You Know" list that highlighted interesting facts about a number of MPA's to raise public interest in these protected areas. My policy projects consisted of gathering data on three types of protected areas—sanctuaries, national parks, and wildlife refuges—and organizing the data in an Excel spreadsheet; data that would later be used by the MPA Center when writing its monthly newsletter and website's articles. I also assisted with research and policy needs as requested, such as creating a calendar of holidays related to marine animals/ecosystems and listing the holiday's relation to marine protected areas. In addition, I completed several professional development projects. I assisted with checking-in attendees, and setting up NOAA's booth at Capitol Hill Oceans Week (June 3-7)—an event filled with many seminars and discussions ranging from ocean economy and its linkage to ocean exploration to the need for marine conservation; attended several NOAA seminars, one of which covered fishing as a social and cultural touchstone for American communities; and attended staff meetings.

Overall, this internship's project tasks emphasized communication and professional development skills. In addition to learning about Marine Protected Areas and their policy and management issues, I also gained experience with a number of software programs including Apple Keynote, Microsoft Word Publisher, Microsoft Word Document, and Microsoft Excel. Furthermore, the skills essential to completing the assigned projects included concise writing, creative designing, and basic research skills. Through these communication and policy projects, I was able to contribute to NOAA's MPA Center by increasing the Center's Facebook post views from the general public and assisting with numerous research and data collection/organization projects that will enhance the MPA Center's public education and outreach efforts.

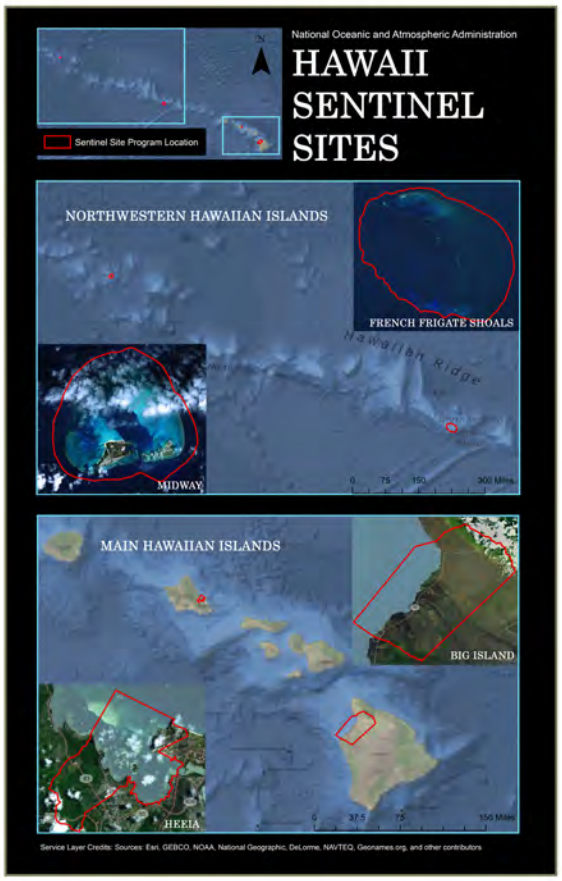
(Supported by Smith College PRAXIS Summer Internship Funding)

Advisor: Anne Wibiralske, Environmental Science & Policy Program and Lauren Wenzel, National Oceanic and Atmospheric Administration (NOAA), National Marine Protected Areas Center, Silver Spring, Maryland

NOAA'S Sentinel Site Program, Hawaii Cooperative: Sea-level Change and Coastal Inundation - from Observation to Stewardship

Anna Campbell/2015

The Sentinel Site Program (SSP) is an initiative launched in 2011 by the National Oceanic and Atmospheric Administration (NOAA)'s National Ocean Service that aims to consolidate the resources of NOAA, federal and state governments, and the nonprofit and private sectors in a specific location to improve community resilience to sea level change and coastal inundation. The Hawai'i site encompasses four sub-locations (right): French Frigate Shoals and Midway Atoll in the Papahanaumokuakea Marine National Monument, a portion of the Northwest coast of Hawai'i Island, and the ahupua'a of He'eia[1] on O'ahu. This summer I was a co-coordinator for the growing SSP in Hawai'i.



First, I met with community stakeholders who told me about their ongoing projects. Together with my supervisor, SSP Coordinator Doug Harper, I asked local resource managers what datasets would be of use to them as they make decisions to prepare for climate change. For instance, Kanekoa Kukea-Shultz, Nature Conservancy biologist and founder of non-profit wetland restoration and Hawaiian cultural project Kākoʻo ʻŌiwi, needs a model of predicted saltwater intrusion to show how sea level changes will affect his new taro loʻi (waterlogged growing patches, similar to rice paddies). We also held meetings with local researchers to learn about their data collection efforts and data needs.

Next, I generated an overview map of the work within the four sites with He'eia as a starting point and model. Based on a tool developed by the Pacific Climate Information System (PacCIS), I developed a matrix of all the sea level rise science and outreach taking place for the Hawaiian Islands, including the geographic extent, target audience, and further research questions of each project. A list of data gaps found many common data needs among the managers and ranked the issues in order of common concern.

This project produced new collaborations and strengthened existing connections between researchers and managers. These synthesis tools will help potential funders target grant monies more effectively to key management issues. Currently the Hawaii Sentinel Site sits far ahead of its peers on the forefront of collaboration between managers and scientists, and may serve as model for the other sites or similar programs such as the Habitat Blueprint. Future work should deepen these connections and address these issues in other locations.

(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)

Advisors: L. David Smith, Biological Sciences and Douglas Harper, National Oceanic and Atmospheric Administration (NOAA), Pacific Services Center, Honolulu, HI

[1]An ahupua'a is the way the Hawaiian people historically divided their land into adjacent watersheds.

2013 Population Estimation of the NNCES Stock of the Common Bottlenose Dolphin, *Tursiops truncates,* of Pamlico and Albemarle Sounds, NC

Emily Clark/2015

This summer I worked as a NOAA intern to estimate the abundance of bottlenose dolphins (*Tursiops truncatus)* present in the Northern North Carolina Estuarine System stock (NNCES). We conducted a survey[1] during the summer of 2013 to estimate the dolphin stock. A similar survey was conducted in 2009 that examined both the northern and southern estuarine stocks. During the initial "Mark" period of the census, as much as possible of the Pamlico and Albemarle Sounds (NC) were sampled using predetermined tracklines. This reduced bias caused by increased surveying of areas where dolphins were found in past surveys. Three boats crewed by 3-5 individuals were used in the Mark period. After a significant period of rest where dolphins had the ability to move freely within the survey area, we conducted two "Recapture" periods in which as many dolphins as possible found in the Mark period were relocated. During these Recapture periods, two boats were used to survey the areas where the most dolphins have been found in past surveys and during the Mark period. Whenever dolphins were sighted in the survey, photographs were taken of each dolphin's dorsal fin by two photographers for identification purposes. We also recorded the number of individuals seen in each group, number of sub-groups observed, and number of young-of-year or neonates seen.

I was assigned as the data recorder and a secondary photographer when only a primary photographer was present. After each survey, I took part in the categorization of the photographs taken during the survey so they could be used to identify dolphins seen during the project that have already been documented in the NNECS dolphin catalog. These data are used by statisticians following the survey to estimate the NNECS stock population size. A stock assessment is conducted every 8 years to accurately calculate the Potential Biological Removal (PBR), the maximum number of animals, not including natural mortalities that may be removed from a marine mammal stock while allowing that stock to reach or maintain its optimum sustainable population. Bottlenose dolphins in the NNCES stock are most affected by bycatch from fishing. Therefore an estimate of the current population is important to understand how dolphin mortality rates from fishing will affect the future population. The final population estimation will not be available for some time after my internship, but if the PBR is seen to be less than the number of reported human-induced mortalities, then the National Marine Fisheries Service establishes a Take Reduction Team (TRT) that develops strategies to reduce mortality to sustainable levels.

(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)

Advisors: Paulette Peckol, Biological Sciences and Aleta Hohn, Director of NMFS Program, Antoinette Gorgone and Barbie Byrd, National Oceanic and Atmospheric Administration (NOAA), Southeast Fisheries Science Center, Beaufort Laboratory, Beaufort, NC

The protocol for the abundance estimate project was completed by Antoinette Gorgone, NOAA Affiliate, but has not been published. Permission of use was granted.

Distribution, Abundance, & Mortality of Marine Species in the Northern Gulf of Mexico

Victoria Dunch/2014

The Gulf of Mexico is home to several thousands of marine species. The health of many key or commercially important species has become a matter of great concern due to pollution in the Gulf. In recent years, the number of sea turtle interactions with recreational fishermen has been increasing in coastal Mississippi.[1] In most cases the turtles go for fishermen's bait and become hooked in the mouth, which can be fatal without rehabilitation. The majority of these interactions involve the Kemp's Ridley sea turtle *(Lepidochelys kempii)*, the most endangered sea turtle species and the most common in the Mississippi Gulf coast area. Typically these sea turtle-angler interactions go unreported.

To better understand the frequency and outcome of these interactions, NOAA and the Institute for Marine Mammal Studies (IMMS) collaborated to survey fishermen's practices on piers in coastal Mississippi. I and other interns conducted the surveys at six major piers at various times. At the end of the interview we gave anglers a card with IMMS's turtle rescue number and explained how best to proceed should a turtle become hooked or entangled on their line. We then entered the survey information into various databases. In the middle of this project I participated in a nine day ground fish survey aboard NOAA's research vessel, the Oregon II. The objective was to collect plankton samples at different stations in the Gulf, as far south as the tip of Florida, to aid in assessing the health of the Gulf. Samples were collected with both a neuston and bongo nets. At each site a CTD was lowered to collect data for that location, and then the nets were dragged at different depths to collect samples. Each sample was stored either in ethanol for DNA preservation to be analyzed and sorted abroad in Poland, or salt water and formalin for archiving and future studies.

Results for the ground fish survey are not yet available. Preliminary results from the 2013 Summer Mississippi Fishing Pier Angler Survey show that 14% of anglers surveyed had incidentally caught a turtle in the past year, but the majority of those were not reported to any state agency, although 50% of the time the gear was removed prior to release.[2] Surveying will continue to collect more data for better assessments of sea turtle interactions with recreational fishermen in the Mississippi Gulf coast.

(Supported by Smith College PRAXIS Summer Internship Funding)

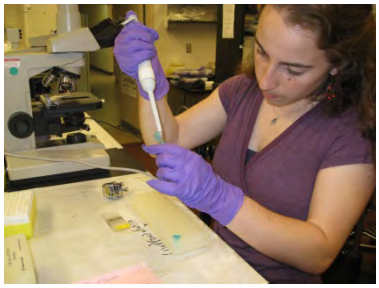
Advisors: Anne Wibiralske, Environmental Science & Policy Program and Andre Debose and Melissa Cook, National Oceanographic and Atmospheric Administration (NOAA), Southeast Fisheries Science Center, Pascagoula, Mississippi

[1] Lyn, H. et al. Displacement and Site Fidelity of Rehabilitated Immature Kemp's Ridley Sea Turtles (*Lepidochelys kempii*). Marine Turtle Newsletter 135:10-13, 2012.

[2] Mississippi Fishing Pier Angler Survey 2013.

Dolphin Health Assessment (Charleston, SC)

Eliana Perlmutter/2016



What can dolphins tell us about local ecosystems and even our own health? Dolphins are apex predators and therefore a good indicator species for the local marine ecosystem. As fish are the route of exposure to many chemical contaminants for both dolphins and humans, dolphin health is highly relevant to human health. The Dolphin Health and Environmental Risk Assessment (HERA) project is a collaborative multiyear study aimed at learning more about such topics from studying bottlenose dolphins (*Tursiops truncatus).*

Dolphin health assessments were conducted in the Charleston, SC estuarine waters from 2003 until 2005 and in August 2013. Similar assessments are conducted on the bottlenose dolphins of the Indian River Lagoon in Florida. The protocols at the two sites are standardized so data can be compared to learn the localized risks and health effects on the dolphins. Samples are collected from temporarily restrained wild dolphins by experienced marine mammal veterinarians. These include blood, saliva, blowhole, gastric, fecal, urine, blubber, and skin samples. Additionally, a tooth is extracted to determine the dolphins' age which provides important context to assess health. The animals are freeze-branded with an identification number and a roto-tag is attached to their dorsal fin. A wide variety of diagnostic tests and measurements are made to assess health status such as infectious disease, immune function, stress parameters, endocrine levels, and anti-bacterial resistance.

The aims of this project include investigating environmental and anthropogenic effects on dolphin health as well as connections to the health of the surrounding marine environment. This research contributes to understanding human impacts on the ocean and marine mammals. These studies also help us learn about the health effects on dolphins of chemicals like perfluorinated compounds (PFCs), mercury, and organochlorine contaminants as well as survey emerging diseases and their prevalence among dolphins.

Some emerging contaminants are known to have especially high concentrations in the Charleston dolphins. Given the 10 year time interval since the last dolphin health assessments in Charleston, it is especially important to find out how these contaminant levels are changing and how these changes impact dolphin health.

I played a key role assembling animal veterinary sampling kits and laboratory processing kits for the field operation. To be ready for the immunological component of the health assessment, I grew YAC-1 cells in the lab and prepared media. During the animal sampling I assisted the veterinarian technicians with use of the veterinary supplies and kits.

(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)

Advisors: Anne Wibiralske, Environmental Science & Policy Program and Patricia Fair, National Oceanic and Atmospheric Administration (NOAA), Center for Coastal Environmental Health & Biomolecular Research, Charleston, South Carolina

Coral Reef Ed-Ventures Program

Alyssa Stanek/AC, Kayla Clark/2014, Sarah Tucker/2013, Kiara Gomez/2014, Sarah Alper/2015, and Dena Greenstreet/2015

"I see brain coral!" "What kind of sea grass is that?" "Mostly manatee grass, some turtle grass." "Hey, a sea fan! A huge sea fan!" "Grouper fish!" As the glass-bottom boat floated through Hol Chan Marine Reserve, its young Belizean passengers shouted out the sights they saw as they peered through its transparent floor. The children were all students at the Coral Reef Ed-Ventures summer program, and had spent two weeks learning about the reef and nearby marine ecosystems.

Now in its fourteenth year, Coral Reef Ed-Ventures (Coral Ed) is an environmental education program run through Smith College's Environmental Science and Policy program in collaboration with Ambergris Caye's Hol Chan Marine Reserve. Coral Ed works to increase awareness and understanding of the marine ecosystems surrounding Ambergris Caye through free summer programs for local children. This year, six undergraduate students representing a variety of academic backgrounds, including geology, biology, sociology, education, and marine science, served as teachers for the program.

The students who attend Coral Ed all live on Ambergris Caye, Belize, an island near the Meso-American Barrier Reef. Spanning over 600 miles from north to south, this reef is the largest of its kind in the western hemisphere, and holds tremendous ecological and economic importance for the island and its residents. The well-being of other island ecosystems, including the mangroves, sea grass, and open waters over the fore reef also relates closely to the health of the reefs and of Ambergris Caye overall. The Coral Ed program works to educate local children about these relationships, as well as the importance of marine environmental conservation.

The educational program run by the Smith students includes a youth program intended for ages 7-12, and an advanced program for 12-16 year olds. The content and activities are designed so students gain significant knowledge of the local marine ecosystems and can identify reef organisms, understand adaptations and symbiotic relationships, discuss local environmental threats, and brainstorm possible solutions in the areas of sustainability and conservation.

This year, highlights of the youth program included making edible models of coral polyps, learning about fishing regulations from a local fly-fisherman, competing in a game show about mangroves, drawing elaborate "adaptation fish," and meeting a crocodile during a visit from the American Crocodile Education Sanctuary (ACES). To visually track their learning throughout the summer, students contributed each day to a mural that illustrated four marine ecosystems: mangrove, shallow sea grass, reef, and deep sea. The mural was displayed at the graduation ceremony, where students performed skits and songs, made and voted on conservation posters, and received "Coral Reef Expert" cards to show an audience of over 200 what they had learned.

During the weeklong advanced program, Coral Ed students learned about conservation through a crocodilian lens, thanks to a partnership with ACES. Advanced students had the chance to observe and participate in the work of ACES' research biologist and wildlife behaviorist through an evening field trip through the lagoon. The group ended the week with a wealth of knowledge about crocodiles, mangroves, and endangered species conservation, as well as with a collaborative map of Ambergris Caye, hand-drawn to depict the environment/community interdependence that they had discussed throughout the week.

In addition to planning and running the summer programs, the six Smith students worked with Smith faculty David Smith, Denise Lello, Al Curran, and Jon Caris to pilot several research projects. In collaboration with Hol Chan Marine Reserve, they used GPS/GIS technology and kite-assisted photography to create maps of sea turtle nesting sites and assess beach erosion along the island's north shore. The team also dove on snorkel and scuba, measured and diagramed coral mounds, and used underwater photography to document the abundance, diversity, and health of soft corals found in the Mexico Rocks patch reefs. These data were supplemented by kite-assisted aerial images of coral mounds at Mexico Rocks taken from a boat. Together, these methodologies will provide baseline information about hard coral distribution and soft coral diversity and condition so that changes can be monitored after Mexico Rocks becomes a protected marine reserve. Lastly, the group travelled to the mangroves to evaluate the possibility of establishing permanent monitoring sites to gauge sedimentation rates relative to sea level rise. Aerial images were also taken of the mangrove islands.

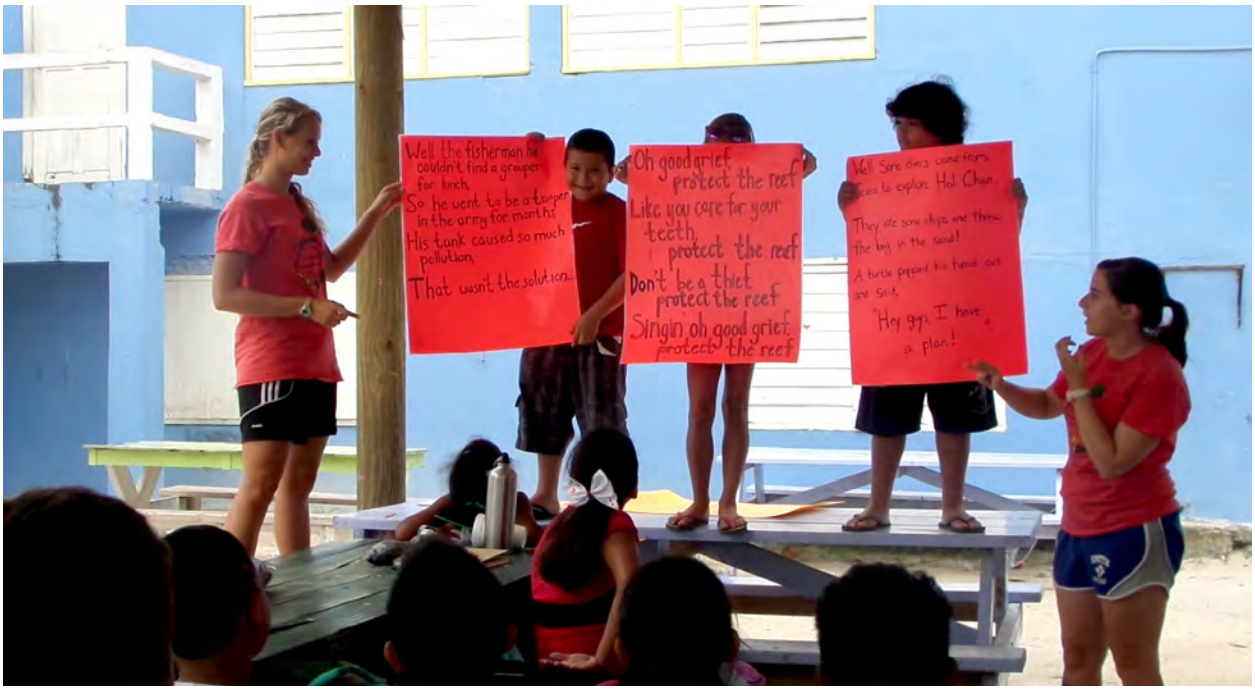
The collaboration between Smith students and professors, as well as the support of Hol Chan Marine Reserve and countless other members of the San Pedro community, has continued to add to the success of the Coral Ed program. This year brought new partnerships that strengthened the depth and breadth of Coral Reef Ed-Ventures, and we look forward to exploring how the program may grow and change in the coming years.

(Supported by the Agnes Shedd Andraea 1932 Research Internship Fund, B. Elizabeth Horner Fund, and Gift from Linda Salisbury '78)

Advisors: L. David Smith, Denise Lello, Biological Sciences, Al Curran, Geosciences and Miguel Alamilla, Jr., Manager, Hol Chan Marine Reserve, San Pedro, Belize

Students in the advanced program work with Dena on a map to show environment/community interactions on andaround Ambergris Caye

Sarah A and Sarah T record observations of soft corals



Kayla and Alyssa lead the camp song at the youth program's morning meeting

Coral Ed students enjoy a glass-bottom boat trip to Hol Chan Marine Reserve

Harmful Algal Blooms and Their Pacific Northwest Impact

Elizabeth D. Wright/Ada Comstock Scholar

The world's oceans are teeming with phytoplankton, diverse single-celled organisms. Several species of phytoplankton produce a suite of toxins that cause alarming symptoms in humans and marine mammals. *Dinophysis*, a common phytoplankton in the Pacific Northwest (PNW), produces okadiac acid, which causes diarrhetic shellfish poisoning (DSP). *Dinophysis* enter the food chain through filter feeders like shellfish that concentrate toxins. When in great abundance, *Dinophysis* create a harmful algal bloom (HAB).

Sequim Bay State Park in WA is a known "hotspot" for shellfish-related illness. Due to the unique, enclosed features of the bay, seawater is not as regularly flushed out, making it an ideal place to study phytoplankton blooms. On July 2 and 3, 2013, we used a Niskin bottle as well as an extraction of a whole water net tow to measure algae from the park dock at 0.5, 1.5 and 2.5 meter depths, taking samples every two hours for 48 hours. Measurements of phytoplankton from these samples will help us better understand their behavior and location based on time of day and tidal cycle, key information for creating models that forecast phytoplankton blooms.

We filtered water samples from the bay through Millipore filters to capture the toxins, then froze them at -20 °C until analysis with a protein phosphatase assay test (PP2A) to measure toxin levels. The results of the PP2A for all samples were below the limit of detection for the test, indicating low concentration of toxins. Additionally, I performed manual cell counts to determine relative abundance of *Dinophysis*. Both tests showed a lack of *Dinophysis* in the bay, which is unusual in July. These results suggest insufficient nutrients for the HABs and that perhaps our timing was poor. Further testing is needed to clarify this.

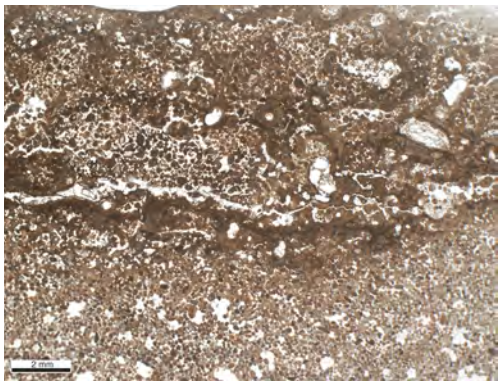
In addition to my work in Sequim Bay, I participated in a research cruise as a part of ocean acidification research on the West Coast. As oceans warm, they become increasingly stratified with warm surface water acting as a lid on deeper cold water. *Dinophysis* and other toxic phytoplankton seem to thrive in warmer water and bloom, impeding water circulation, thus becoming a potential threat to the oceans' overall health. I sampled seawater at 3 meters at more than 40 stations along the West Coast, between Seattle and Newport OR, to be analyzed for HAB and their toxins. Once analyzed by the scientists at NOAA, these results will help us understand what advantages a warmer, more acidic ocean provides for toxic phytoplankton.

(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)

Advisors: Jesse Bellemare, Biological Sciences, and Vera Trainer and Nicolaus Adams, National Oceanic and Atmospheric Administration (NOAA), Northwest Fisheries Science Center, Seattle, Washington

Analysis of Distribution and Origin of Caliche Crusts in Carbonate Eolian Deposits on San Salvador Island, Bahamas

Sarah Brisson/2014



In January 2012 I conducted geological fieldwork on San Salvador to examine unusual features of eolian dune deposits formed by sedimentation and lithification of wind-blown carbonate sand. Last summer through the SURF Program, I carried out petrographic and stable isotope (oxygen and carbon) analyses of collected samples. This summer I continued analyzing these samples and I also examined new samples collected in June 2013 by Profs. Bosiljka Glumac (Smith College) and Mario Caputo (San Diego State University). Our findings so far are summarized in an abstract I co-authored for a presentation at the Geological Society of America conference in Denver in October 2013. Research I conducted in the past two summers will be used as the platform for my Honors Thesis this coming year.

My research focuses on numerous thin caliche crusts present in eolianites of the upper North Point Member of the Rice Bay Formation exposed along the northeastern coast of San Salvador. These eolianites formed during Holocene sea-level rise about 6 to 5 ka and consist of complexly stratified deposits produced by wind-ripple migration, grainfall and sandflow processes[1]. Such caliche crusts are not commonly present in the lower part of these Holocene deposits or in the older Pleistocene eolianites. I examined the distribution and composition of caliche to better understand their formation.

Caliche is a hard microcrystalline crust formed by dissolution and subsequent reprecipitation of carbonate. Observed caliche crusts are laterally continuous and uniformly coat prominent bedding planes. Weathering and erosion have stripped resistant crusts and eolian laminae to produce a terraced exposure of up to 12 crusts in strata about 1.5 m thick. Individual crusts are 1 to 4 mm thick and separate bedsets that thin upward from about 30 to 5 cm. Caliche crusts have a sharp, smooth upper surface with varying degrees of weathering which impart a pitted, irregular appearance. Associated with these crusts are dense, laterally extensive fossilized plant roots (rhizoliths) and impressions of plant prostrate stems and runners. The image shown above is a thin section photomicrograph of caliche (dark areas) developed on eolian sand (bottom) and associated with common rhizoliths (circular to elliptical features in the upper part of the photograph).

The distribution of caliche crusts and associated plant-related fossils suggests their syn-depositional origin on or near dune surfaces. Upward-thinning Holocene eolian bedsets composed of wind-ripple strata and separated by syn-depositional bedding-parallel caliche crusts suggest a transition from times of increased sediment production and supply, active dunes, and drier weather to times of reduced sediment production and supply, inactive dunes, and wetter weather. The presence of fresh water and plant material on dune surfaces facilitated the formation of caliche by dissolution of sand grains and precipitation of microcrystalline calcite. Holocene and Pleistocene eolian strata on San Salvador also contain post-depositional or penetrative caliche crusts that generally cut across multiple eolian beds, are irregular in distribution, and variable in lateral thickness. My ongoing research will emphasize petrographic and geochemical distinction between penetrative and surficial caliche crusts.

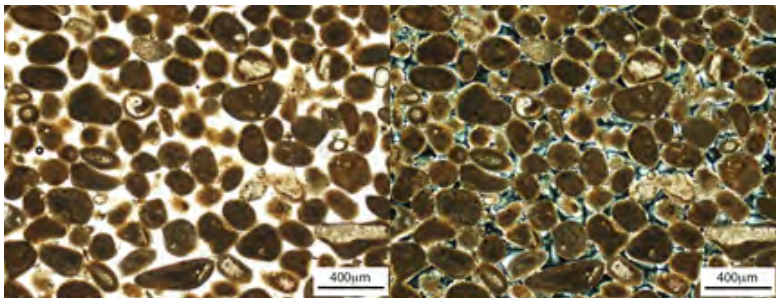
(Supported by the Schultz Foundation)

Advisor: Bosiljka Glumac, Geosciences

[1]White, B. and H.A. Curran. 1988. Mesoscale physical sedimentary structures and trace fossils in Holocene carbonate eolianites from San Salvador Island, Bahamas. Sedimentary Geology, 555: 168-184.

Testing the Hypothesis about the Influence of Grain Type and Texture on Formation of Polygonally Cracked Carbonate Grainstones in the Bahamas

Yumeng Melody Cao/2015



Polygonal cracking commonly forms by drying of exposed muddy sediment. A similar polygonal cracking can occur in Bahamian eolian (i.e., wind-blown) and backshore beach carbonate sandstone or grainstone without mud. The main objective of this study was to test the hypothesis from Glumac et al. (2011)[1] that the paucity of documented examples of polygonal fractures in mud-free grainstone requires desiccation of sediment that consists of well-sorted, well-rounded spherical sand grains, such as ooids, of fine to medium sand size, unlike the texturally and compositionally more heterogeneous skeletal and peloidal sediment that is common in the Bahamas.

To test this hypothesis, I examined petrographic thin sections of polygonally cracked carbonate deposits from three different locations in the Exumas, Bahamas to specifically address the role that size and shape of sand particles play in the formation of polygonal cracking. I documented in detail the texture and composition of these deposits (see photomicrographs above; plain-polarized (left) and cross-polarized (right) light views). I also wrote and submitted an abstract for a poster presentation at the 2013 Geological Society of America's Annual meeting in Denver.

The samples examined were comprised of fine to medium sand (mainly 150-350 µm in diameter), with rare coarse sand grains (up to 800 µm). Sand grains were mainly well-sorted and very well-rounded ooids with thin cortices around nuclei of peloids or skeletal fragments. The cortices consist of a series of concentric layers of calcium carbonate crystals that precipitate around nuclei suspended in shallow marine waters by high-energy wave or tide currents. The sand also contained some rounded skeletal fragments (mainly mollusks and foraminifera), which are uncoated or have a vey thin surficial cortex. Grains may be heavily micritized by microboring, but there is no mud present in the matrix. Instead, the sand is loosely lithified by mainly clear, equant meteoric calcite cement with isopachous rims, and meniscus and pendant morphologies.

These observations generally support the hypothesis that homogeneous composition and texture are key factors for the development of polygonal cracking in carbonate grainstones. However, there could also be other factors such as: 1) air and sand moisture regime (i.e., composition and amount of fluids); 2) temperature, duration and rate of wetting and drying; and 3) depositional processes that produce unique sedimentary structures and fabric, including grain packing and porosity in sediment. With future studies on comparing texture and composition of polygonally cracked versus uncracked grainstones as well as examining conditions under which polygonal cracking is currently occurring, it should be possible to evaluate these additional factors to better understand the formation of polygonal fractures in sand and to predict situation where it might be possible to find more modern and ancient examples in the Bahamas and elsewhere.

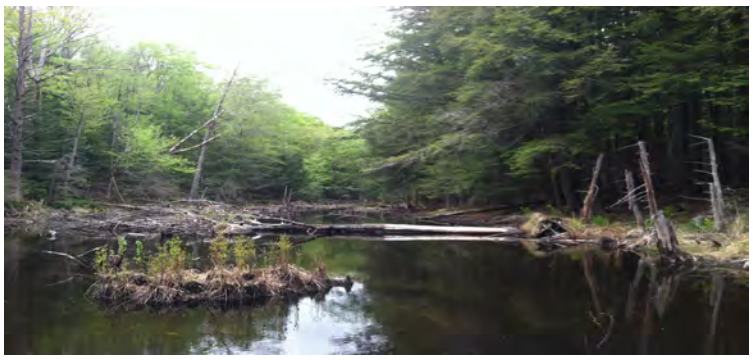
(Supported by the Schultz Foundation)

Advisor: Bosiljka Glumac, Geosciences

[1]Glumac, B., Curran, H.A., Motti, S.A., Weigner, M.M., and Pruss, S.B., 2011, Polygonal sandcracks: Unique sedimentary desiccation structures in Bahamian ooid grainstone: Geology, v. 39, no. 7, p. 615-618.

Role of Riparian Evapotranspiration in Mobilizing Methyl Mercury in Avery Brook, West Whatley, Massachusetts

Nicole Collier/2014



Atmospheric deposition of mercury (Hg) has greatly increased since the Industrial Revolution, primarily from the burning of coal. Once methylated, Hg can be concentrated as it moves through the food chain; often resulting in fish, concentrations above the EPA recommended limit. Methylation of Hg occurs as a biochemical reaction associated with the reduction of sulfate by sulfur reducing bacteria. This occurs in organic rich aqueous environments where dissolved oxygen has been depleted through organic decomposition.

This study was focused in the Avery Brook Watershed, a small headwater stream that provides water to Northampton's principle drinking water reservoir. In Avery Brook, methylation reactions are likely centered in beaver ponds and riparian wetlands. In my research, I examined the geochemistry of beaver pond waters and used these results to plan a sampling campaign to evaluate the hypothesis that daily evapotranspiration (ET) cycles force methylated Hg from riparian zones into the stream.

The basis of this hypothesis is that during the summer, riparian vegetation ET depletes water from areas immediately adjacent to the stream. At night, ET shuts down and groundwater from the adjacent hillside flushes through the riparian wetland moving the freshly methylated Hg into the stream and providing a fresh supply of sulfate for the sulfur reducing bacteria. The daily ET cycle thus acts like a pump that brings nutrients and Hg into the riparian wetland during the day where sulfur reducing bacteria methylates the Hg and at night groundwater flushes the now methylated Hg back into the stream.

Samples from Avery Brook were collected over a period of two weeks in May and June of 2013. Samples were analyzed for cations, anions, dissolved organic carbon (DOC), UV 254, and alkalinity. In addition, the delta(δ)18O and delta(δ)2H of water samples from both stream water and groundwater were determined.

Due to the timing of sample collection, it was difficult to observe the evapotranspiration cycles necessary for mercury methylation. Over the four week study period, heavy and frequent summer rains overshadowed the ability to see the beginning of the summer season evapotranspiration. However, an ISCO autosampler was used to successfully collect hourly samples over a 2 day period and the samples are currently being analyzed.

(Supported by the Schultz Foundation)

Advisor: Robert Newton, Geosciences

Investigation of Forest Soil Chemistry in Avery Brook, Massachusetts

Clarke Knight/2014

Accelerated soil decomposition due to climate change and invasive worm species such as *Lumbricus rubellus* (the red wiggler), are changing the environmental conditions of Northeast forest soils.[1] Chemical changes in the soil accompany accelerated decomposition with varying degrees of environmental impact. Rainwater leaching through the soils releases Dissolved Organic Carbon (DOC). DOC is a complex family of constituents (Figure 1) with several interesting and consequential properties. These were the focus of my SURF research.

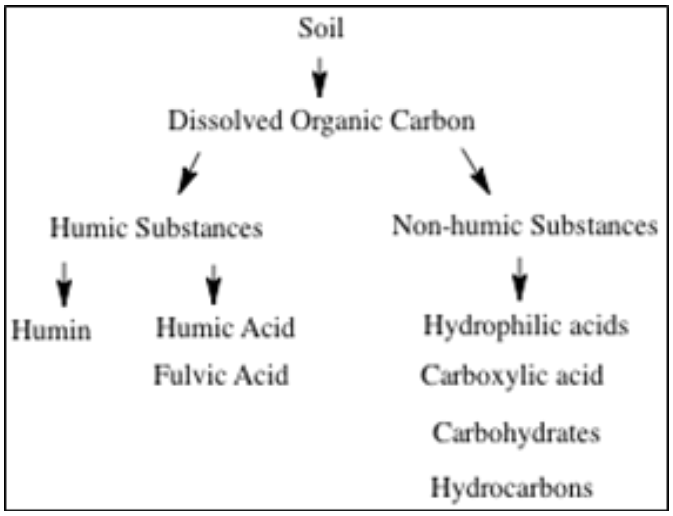


Figure 1. Outline of DOCs, reproduced from Michaels (2012)

Over the past three years, Smith College students and faculty have studied changing environmental conditions at the Avery Brook Watershed, located in West Whately, Massachusetts. In the fall of 2012, samples of organic soil horizons were collected from beneath hemlock and deciduous stands in the watershed for a project by Ravenhurst et al. that now serves as a baseline for further study. In these experiments, the soils collected under the two different canopy types were placed in a series of microcosms constructed from 2-liter soda bottles to investigate the impact of invasive worms on these two different soil types. Weekly simulated rainfall events were used to produce leachate for chemical analysis. Significant chemical differences were observed between the leachate from the two different forest stands. Hemlock stands yielded higher concentrations of mercury, DOC, iron, aluminum, phosphate, sulfate, calcium, and humic acids (separately quantified from DOC), while deciduous stands produced leachate with higher concentrations of silica. Mercury is of particular concern as it has accumulated relatively recently in forest soils because of atmospheric deposition from coal burning during the Industrial Revolution.[2] There is some concern that climate warming induced increased organic decomposition rates could release this mercury to surface waters. In addition, die back of hemlock stands due to the infestation of the woolly adelgid parasite could also increase forest floor temperatures resulting in higher decomposition rates.

This summer I conducted both laboratory and field research to further explore the chemical processes involved in organic soil decomposition and the release of mercury and phosphorous. This work will lay the foundation for my honors thesis. Building on past research, I collected soil, leaf matter, and cone/needle samples from Avery Brook under hemlock and deciduous stands. By using a Mercury Analyzer, I found elevated mercury concentrations in soil samples, varying by soil horizon. Unexpectedly, the needle, leaf, and cone samples contained less mercury than soil samples.

Besides exciting field research, one huge benefit I gained from my SURF research was learning how to independently operate the various analytical instruments available in the Center for Aqueous Biogeochemistry Research (CABR).

(Supported by the Schultz Foundation)

Advisor: Robert Newton, Geosciences

[1]Hale, C.M.; Frelich, L.E.; Reich, P.B.; and Pastor, J. 2005, Effects of European Earthworm Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, USA: Ecosystems, v. 8, p. pp. 911-927.

[2]Zheng, W.; Liang, L.; Baohua, G. 2011, Mercury Reduction and Oxidation by Reduced Natural Organic Matter in Anoxic Environments, Environmental Science & Technology, v. 46, 292 – 299.

Studying Soils and Grain Size at the MacLeish Field Station

Alana McGillis/2015

Across New England, Eastern hemlock (*Tsuga canadensis*) forests are disappearing due to the hemlock woolly adelgid (*Adelges tsugae*). Much is still unknown on how environments will be affected as hemlocks are lost and new successional deciduous forests grow in their place. This summer I was given the opportunity to join ongoing research at the MacLeish Field Station in Whately, MA, studying three plots, representing groups of hemlock, young black birch, and old deciduous trees; we measured factors such as soil pH, soil moisture, and nitrogen cycling, to better understand how the shift from hemlock to deciduous forests will change soils in the region.

In addition to this research at MacLeish, I conducted a grain size analysis to add to our understanding of the soils and geology of the area. Most soil can be categorized into three grain-sizes: sand, silt and clay. Finding out the percentage of each group at different levels in the earth can be a long and interesting process. It began on the 4th of July when I dug a soil pit close to our hemlock plot, collecting soil samples roughly ever 10 cm.

Back in the lab I dried those samples and performed a size distribution analysis. I determined the silt and clay fraction by adding calgonated water and dispersing the soil using a milk shake machine. I then wet seived the mixture through a 63 micrometer sieve. This separated the sand from the mud. From there, the sand portion was dried and filtered through another series of sieves, sized at intervals related to the Phi scale. The mud fraction was measured using stokes law of settling that says larger particles will settle out of suspension at a faster rate than finer particles. Using a hydrometer and recording the time and temperature of the samples, I was able to calculate the diameter and amount of the mud particles. Using this information I created cumulative curves for the soil at different depths (Figure 1).

I was also able to split my summer with the Smith Botanic Garden internship, where I helped teach some of the soil science techniques to fellow interns and expanded my knowledge on plant biology.

(Supported by the Shultz Foundation)

Advisor: Amy L. Rhodes, Geosciences

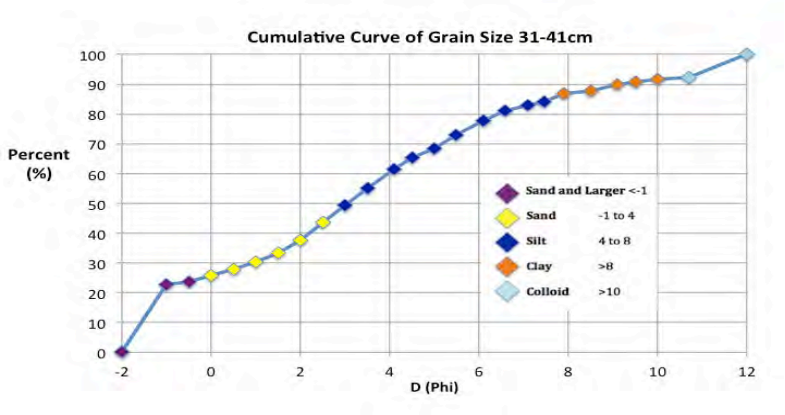


Figure 1: Cumulative grain size analysis of mineral soil collected at 31-41cm depth, hemlock plot (SEH), MacLeish Field Station

Neoproterozoic Drillcore Sample of Zambia

Kelsey Moore/2015

The fossil record of post-Snowball Earth times still holds mysteries today. My research focused on microfossils found in Africa from cap carbonates aged approximately 716 Ma overlying glacial deposits from the Sturtian Glaciation. I looked largely at drillcore samples from the Kakontwe Formation of Zambia, and incorporated samples from the Rasthof Formation at various localities in Namibia - including Okaaru and Ongongo, in order to compare equivalent cap carbonate formations of similar ages. These samples produced agglutinated testate microfossils resembling modern testate amoebae, and may contribute to our knowledge of the fossil record of the post-Sturtian interval.

My research was conducted through the extraction and examination of these fossils. I began by dissolving the carbonate rock samples in a solution of buffered acetic acid, which allowed me to remove the rock and isolate the fossils. I then filtered the residues left by the dissolving process and examined the residues under a stereomicroscope, where I could use a small paintbrush to pick out the 40 to < 100 μm tests. Once isolated, the tests were examined using the Scanning Electron Microscope. This instrument was used for two purposes. First, it allowed me to take images and look more closely at the tests to analyze the shapes and any distinguishing features of the fossils. Second, I was able to use the EDS program to analyze the chemical compositions of the tests and compare the mineralogy of tests of different localities.

These analyses demonstrate similarities in morphology between various localities, but show differences in chemical composition of the tests. The dominant morphotype of the tests found both in the Kakontwe Formation and in the Rasthof Formation is oval. The chemical composition of tests from the Kakontwe Formation, though, shows an abundance of biotite along with titanium bearing minerals. While samples from the Rasthof Formation at various localities contain biotite, they also contain a range of elements including calcium and sometimes phosphorous.

Results demonstrating the dominant morphotype of these samples are consistent with previous studies of the Rasthof Formation at four localities in Namibia—Okaaru, Ongongo,[1] Ombepera, and South Ombepera[2]—where both residues and thin sections showed oval or elliptical tests.[1] The differences in chemical composition may be the result of differences in mineral availability during agglutination, or the result of variation in taphonomic processes. These findings contribute to our knowledge of the fossil record of the post-Sturtian interval and allow us to make connections between formations of equivalent age at different localities.

(Supported by the Schultz Foundation)

Advisor: Sara B. Pruss, Geosciences

[1]Bosak, T, Lahr, D.J.G., Pruss, S.B., Macdonald, F.A., Dalton, L., Matys, E. 2011. Agglutinated tests in post-Sturtian cap carbonates of Namibia and Mongolia. Earth and Planetary Science Letters, 308: 29–40.

[2]Dalton, L.A., Bosak, T., Macdonald, F.A., Lahr, D.J.G., Pruss, S.B. 2013. Preservational and Morphological Variability of Assemblages of Agglutinated Eukaryotes in Cryogenian Cap Carbonates of Northern Namibia. Palios, 28: 67–79.

Comparison of Stable Isotope Composition of Carbonate Deposits from Multiple Lake Successions of the Jurassic Turners Falls Formation from the Deerfield Rift Basin in Massachusetts

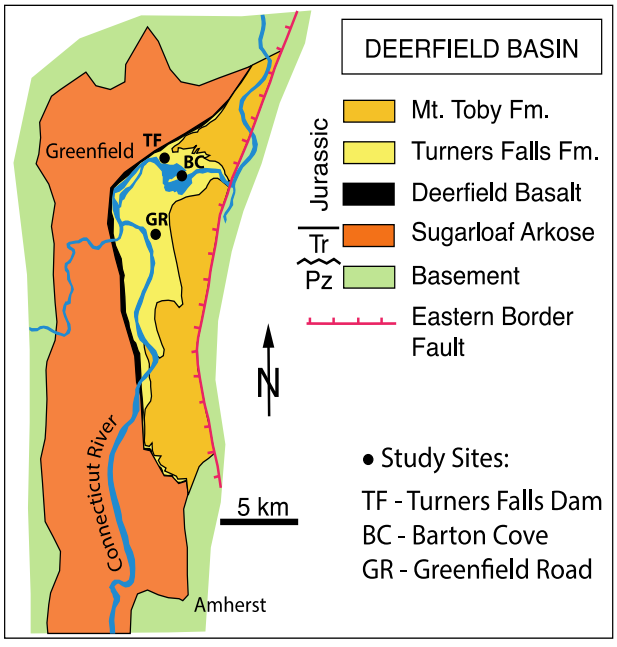
Sylvia Nashipae Mosiany/2016

During the early Mesozoic Era, the supercontinent Pangaea was splitting apart causing tectonic activity and formation of rift basins along the east coast of North America. Various Triassic and Jurassic-aged sedimentary rocks were deposited within these basins. Examination of these rocks provides unique information that can help reconstruct our local geological paleolandscape. In particular, this study compared stable isotope compositions ($\delta^{18}O$ and $\delta^{13}C$ values) of various carbonate components from multiple lake (i.e. lacustrine) successions of the Jurassic Turners Falls Formation from the Deerfield rift basin in Massachusetts. This was aimed at determining whether these lakes were hydrologically open or closed, and at correlating isolated outcrops of lacustrine deposits within this sedimentary basin.

This study is a continuation of my 2012-2013 Early Research project, which was presented at the Smith College Celebrating Collaborations and the Five College Geology Undergraduate Research Symposium. My SURF project focused on preparing the results of this research for presentation at the Geological Society of America (GSA) meeting and its publication in the Northeastern Geoscience Journal.

As part of this research project, new results from the Barton Cove and Greenfield Road sites[1] were collated with information on the Turners Falls Dam site.[2] Samples analyzed from the Barton Cove and Greenfield Road sites represent muddy to silty laminated dolostone, and breccias with dolostone and siltstone clasts. Clustering of isotopic data suggests the presence of three distinct lake successions. Although some of these lakes might be hydrologically closed, as evidenced by their positively covariant $\delta^{18}O$ and $\delta^{13}C$ values, they do not represent the same lake successions documented previously at the Turners Falls Dam site. Within a single set of samples the more silty and laminated carbonate deposits have more negative $\delta^{18}O$ values compared to homogeneous dolomicrite, reflecting the relative influence of precipitation versus evaporation. The strata exposed at Greenfield Road most likely belong to a single lake succession, which is similar to one of the Barton Cove lakes. The rest of the Greenfield Road data reflect the influence of later diagenesis (precipitation of coarse-crystalline calcite cement) and evaporation.

This work supports previous stratigraphic observations about the presence of multiple lacustrine successions in the Turners Falls Formation. Stable isotope analysis of various carbonate components can be used to distinguish among distinct lakes, most of which were hydrologically closed and characterized by formation of dolomite as a primary precipitate or early diagenetic replacement. All analyzed lake successions have similar later diagenetic signatures, and some samples from the new Barton Cove and Greenfield Road study sites reveal a more pronounced evaporation influence as reflected by their more positive $\delta^{18}O$ values and the presence of common evaporite molds.



(Supported by the Schultz Foundation)

Advisor: Bosiljka Glumac, Geosciences

References:

[1]Seidman, L.E., 2011, Origin of the enigmatic breccia and folds in the Turners Falls Formation, Deerfield Basin, Western Massachusetts: Senior Thesis, Smith College, Department of Geosciences, 49 p.

[2]Glumac, B., 2011, Lacustrine dolomite from hydrologically closed Early Jurassic lakes of the Deerfield rift basin in Massachusetts: Insights from isotopic analysis of various carbonate components of the Turners Falls Formation: Geological Society of America Abstracts with Programs, v. 43(5), p. 61.

Investigating the Longevity of Earthquake Segments along the Coast of Northern Chile

Seulgi Son/2016 and Sophie D'Arcy/2016

Interactions between the Nazca and South American Plates generate some of the world's most destructive earthquakes, producing incredible amounts of stress and shaping the geologic structures we see today. Lying directly above this tectonically active region, Chile exhibits an impressive array of surficial deformation lining its coast. Although we cannot observe subterranean stress, we can observe its effects on the earth, or the strain, in places like Chile to infer stress.

In continuation of our first-year STRIDE research project, we compared our mapped surficial faults along the coast of central-northern Chile to the stresses induced by the most influential earthquakes in our database. By creating this stress field, we can establish a relationship between current day structural geology of the Chilean coastline and the stress necessary to generate these features.

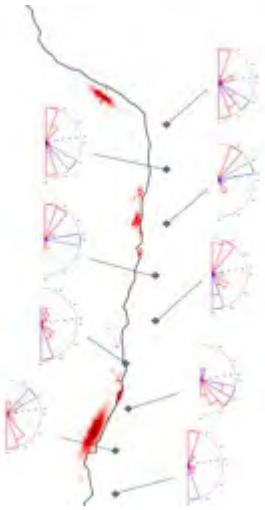
We selected the most significant earthquakes ($M_W > 7$) from the Global Centroid Moment Tensor's database of earthquakes since 1976. Stress tensors were derived from our previously calculated strike slip and dip slip values. Values representing maximum tensional stress (σ_3) were then singled out because movement on most faults likely results from applied tension.

As expected, our rose diagrams show that the stresses, marked blue, are generally 90° from the faults, marked red, at each point for all earthquakes, considering that north-striking faults are created when tensional forces tear apart blocks of crust in an east-west direction. Running along the length of the coastline is the expected "fanning" pattern, in which fault strikes trend north-south near the epicenter, while varying longitudinally the further away from the epicenter, and the major pulling direction is normal to the coastline. The consistency between fault strike and earthquake stress direction suggests that earthquakes have had similar locations and sizes for a long period of time, shaping the coastline faults in a consistent manner.

Our next steps involve a finer scale comparison between coastal faults and earthquake stresses. Additionally, our earthquake database provides us with geologic activity spanning a mere 37 years. By including historical earthquakes, we will be able to calculate slip and stress distributions in regions where there has been no seismicity since 1976. Finally, while we have utilized empirical relationships to calculate our slip distributions, we are aware that there are already calculated slip values for some recent major earthquakes. We plan on substituting these values for our own.

(Supported by the Schultz Foundation, Son and the Smith College Provost's Office, D'Arcy)

Advisor: Jack Loveless, Geosciences



Efficient Estimation of Partially Observed Clustered Data using Multiple Imputation

Kathryn Aloisio/2013

My summer research furthered my Honors Thesis work to help facilitate the appropriate analysis of incomplete clustered data using common statistical software packages.

Representing collected data properly for any analysis is crucial for obtaining appropriate inferences. Clustered data are particularly of interest because the classic assumption of independence between observations is undeniably violated and require sophisticated analytical methods. As an example, multiple-source reports are commonly collected in child and adolescent psychiatric epidemiologic studies where researchers use various informants (e.g. parent and child) to provide a holistic view of a subject's symptomatology. Fitzmaurice et al. (1995)[1] have described estimation of multiple source models using a generalized estimating equation (GEE) framework. However, these studies often have missing data due to multiple stages of consent and assent. The usual GEE is unbiased when missingness is Missing Completely at Random (MCAR) in the sense of Little and Rubin (2002).[2] This is a strong assumption that may not be tenable and may misrepresent the data. Multiple imputation is an attractive method to fit incomplete data models while only requiring the less restrictive Missing at Random (MAR) assumption.

Methods used to analyze partially observed clustered data are available in general purpose statistical software, though the difficulties involved in their use discourages wider uptake of appropriate missing data methods and remains a barrier for researchers less computationally limber.

This summer I submitted a subset of my thesis to a peer-review journal to facilitate the method using Stata.[3] In addition, I developed an R Markdown[4] script to simplify the process using R.

This research was presented at the Joint Statistical Meetings 2013 in Montreal, Canada on August 7, 2013 in a new and innovative SPEED session. The session consisted of 15 oral presentations of approximately five minutes each. These lightning oral presentations were followed by a poster session later on the same day.

(Supported by the Susan M. Rambo Fund)

Advisor: Nicholas Horton, Mathematics and Statistics

[1] Fitzmaurice, G M, N M Laird, G E P Zahner, and C Daskalakis (1995), "Bivariate logistic regression analysis of childhood psychopathology ratings using multiple informants." *American Journal of Epidemiology*, 142, 1194-1203.

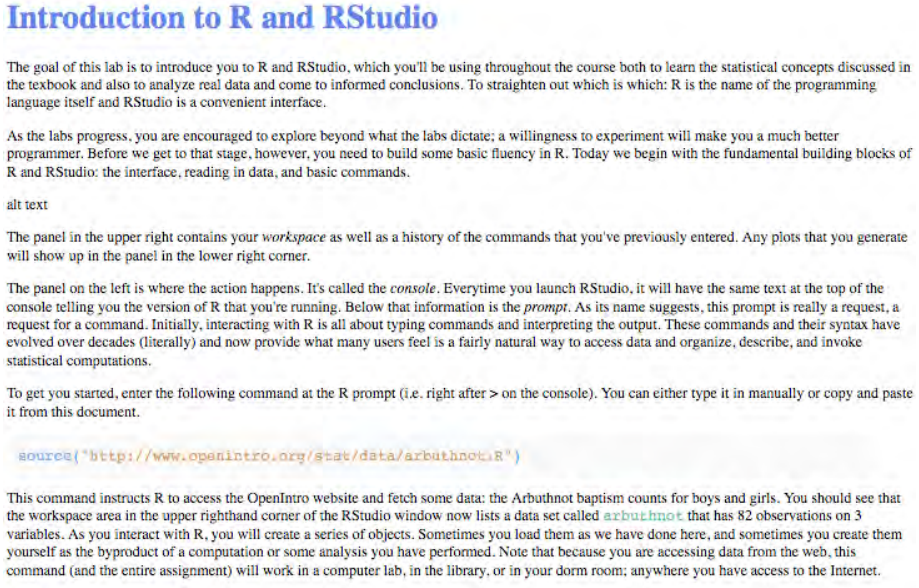
[2] Little, R J A and D B Rubin (2002), *Statistical analysis with missing data, 2nd edition*. John Wiley & Sons, New York.

[3] http://www.stata.com/

[4] http://www.rstudio.com/ide/docs/authoring/using_markdown

Developing Statistics Education

Yue Cao/2015



Introduction to R and RStudio

The goal of this lab is to introduce you to R and RStudio, which you'll be using throughout the course both to learn the statistical concepts discussed in the texbook and also to analyze real data and come to informed conclusions. To straighten out which is which: R is the name of the programming language itself and RStudio is a convenient interface.

As the labs progress, you are encouraged to explore beyond what the labs dictate; a willingness to experiment will make you a much better programmer. Before we get to that stage, however, you need to build some basic fluency in R. Today we begin with the fundamental building blocks of R and RStudio: the interface, reading in data, and basic commands.

alt text

The panel in the upper right contains your *workspace* as well as a history of the commands that you've previously entered. Any plots that you generate will show up in the panel in the lower right corner.

The panel on the left is where the action happens. It's called the *console*. Everytime you launch RStudio, it will have the same text at the top of the console telling you the version of R that you're running. Below that information is the *prompt*. As its name suggests, this prompt is really a request, a request for a command. Initially, interacting with R is all about typing commands and interpreting the output. These commands and their syntax have evolved over decades (literally) and now provide what many users feel is a fairly natural way to access data and organize, describe, and invoke statistical computations.

To get you started, enter the following command at the R prompt (i.e. right after > on the console). You can either type it in manually or copy and paste it from this document.

```
source("http://www.openintro.org/stat/data/arbuthnot.R")
```

This command instructs R to access the OpenIntro website and fetch some data: the Arbuthnot baptism counts for boys and girls. You should see that the workspace area in the upper righthand corner of the RStudio window now lists a data set called `arbuthnot` that has 82 observations on 3 variables. As you interact with R, you will create a series of objects. Sometimes you load them as we have done here, and sometimes you create them yourself as the byproduct of a computation or some analysis you have performed. Note that because you are accessing data from the web, this command (and the entire assignment) will work in a computer lab, in the library, or in your dorm room; anywhere you have access to the Internet.

The major goal of this summer research project is to develop new teaching strategies and lesson plans to transit from paper-based lessons to computer-based lessons, and to support OpenIntro, a scholarly community that devotes to contribute free and open-source educational materials, to make the source files (.rnw) for statistics labs as easy-to-use as possible for educators. The labs will be used in MTH245 in Fall 2013.

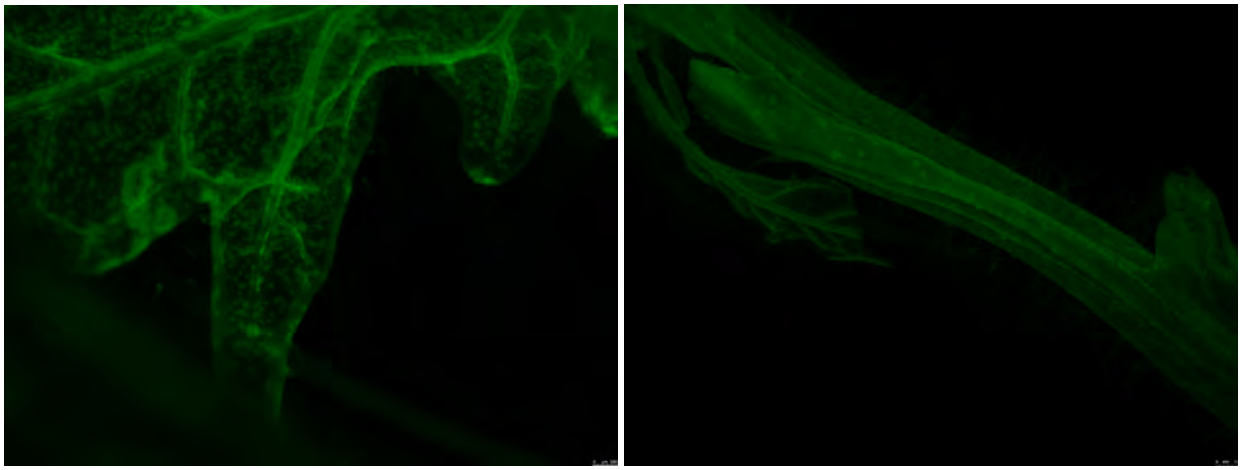
The project is divided into three parts. In the first part I planned syllabus and designed introductory statistics lessons for high school students with no past experience in statistics. The lesson plans were used in Census at School course during SSEP 2013. We prepared students for group presentations on statistical research projects with data imported from Census at School website and analyzed in Fathom and RStudio. In the second part I collected statistical studies and samples for Meeting within a Meeting (MWM). In the last part I reviewed all the labs that will be used in MTH245, converted the .rnw file from Sweave syntax to Markdown and utilized the MOSAIC package. An advantage of Markdown as opposed to Sweave is that the Markdown syntax does not depend on the package 'parser,' which has been orphaned and has been removed from the CRAN repository. Having the .rnw file in Markdown syntax allows educators to recompile those labs based on their needs at any time. While R and RStudio are powerful systems for statistical analysis, they may be frustrating to new users or first time learners. The MOSAIC package is designed to help shorten the learning curve for such beginners and make sophisticated statistical analyses easy to get started.

(Supported by Susan M. Rambo Fund)

Advisor: Katherine Halvorsen, Mathematics and Statistics

Phyllotaxis: mPS-PI Staining on Plant Vasculature

Karen Chau/2016



Phyllotaxis is the arrangement of organs around a plant's stem. If the left and right handed spirals formed by the organ arrangement are counted, the numbers will often be adjacent members of the Fibonacci sequence. When two leaves are aligned such that they form a vertical line, the pair of leaves belong to an orthostichy. It is presumed that the vascular traces to leaves of an orthostichy are derived from a common vascular bundle, discrete columns of vascular tissue arranged within the stem. However, the patterns of vascular tissue connections to leaves are not well understood. In addition, the phyllotactic spirals may change because of plant development, resulting in a transition to different adjacent numbers in the Fibonacci sequence. In my research, I am using fluorescence microscopy to examine how the pre-existing vascular bundles accommodate changes in phyllotaxis. A distinctive feature of plant vascular tissue is cells with walls reinforced by lignin (xylem cells) and sieve cells with callose deposits in sieve end plates (phloem cells). Staining techniques exist to visualize both of these features, but observing them in three dimensions has not yet been successfully accomplished.

The study subjects used were *Linum perenne*, *Linum usitatissimum*, and *Lycopersicon* (pictured). These were chosen because reports in the literature indicated that all three species show phyllotaxis transitions over relatively short temporal and spatial intervals and all are suitable to growth in a chamber. Plants were grown in 16:8 L:D at 23:16°C. We tried several staining and clearing procedures, but the most successful used a modified Schiff reagent with propidium iodide (mPS-PI) as described in Truenit et al.[1] with the additional step after fixation of 10-30 minutes in 80% ethanol at 65-70°C. After staining, the plant samples were cleared using the Clear(T^2) protocol following Kuwajima et al.[2]

Samples were viewed with a Leica205A fluorescent microscope using the mCherry filter that excites in the range of 587-610 nm because the peak excitation wavelengths of the propidium iodide stained callose in phloem cells are around 536 nm with peak emissions around 617 nm. By conducting a z-stack through the depth of the stems, partially focused images of the vascular bundles were obtained, with the most vibrant signal coming from leaf vascular bundles leaving the stem. Branching of vascular bundles within the stem remains unclear.

Therefore, the mPS-PI staining is a promising staining technique, but two problems remain. The first is increasing the depth of tissue that can be imaged in three dimensions. The second is to clarify the vascular tissue imaging by removing background noise. One option to further improve the quality of the imaging is to differentiate the signal of the fluorescence coming from the vascular bundles from that in background tissues. During the academic year, I plan to explore confocal microscopy and alternate sectioning and imaging techniques to refine the results.

(Supported by the Schultz Foundation)

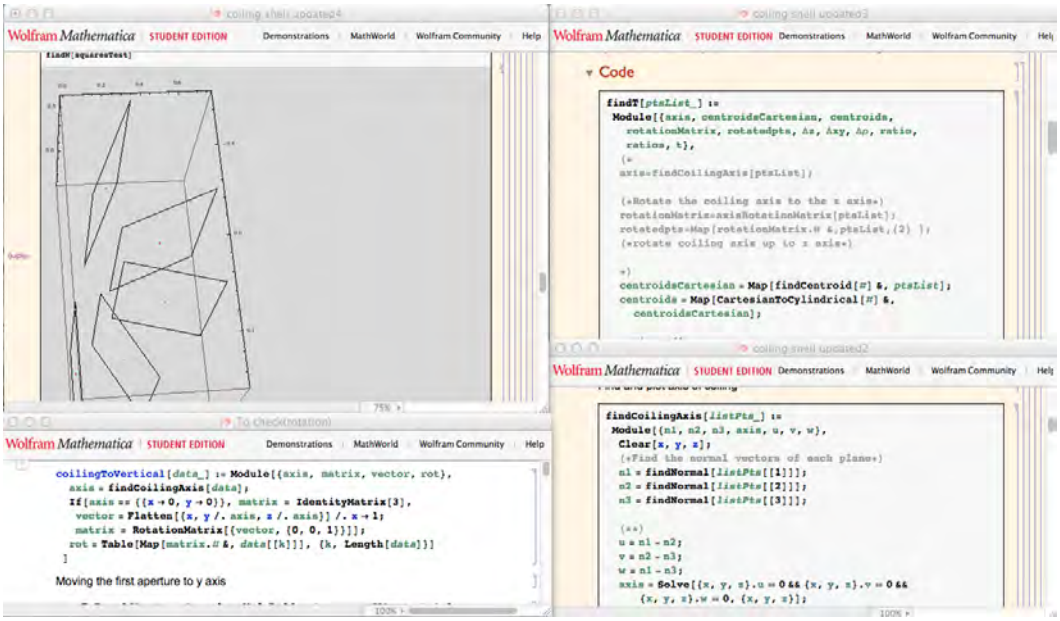
Advisors: Denise Lello and Christophe Golé, Mathematics and Statistics

[1] Truenit, Elisabeth et al. "High resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in Arabidopsis," The Plant Cell, Vol. 20 (2008) 1494-1503.

[2] Kuwajima, Takaaki, et al. "ClearT: a detergent-and solvent-free clearing method for neuronal and non-neuronal tissue." *Development* 140.6 (2013): 1364-1368.

Geometry of the Ecological Arms Race Between Crabs and Snails

Yidan Jin/2015



This project focuses on the geometry of a predator-prey arms race between the population of crabs and snails from New England area. In our experiments, we focused on the east coast of Maine. My research is a collaborative work, conducting experiments to show the structural changes of both crabs and snails in response to the environmental cues during development. For the modeling part, I corrected and improved models created by Linnea Yeazel using Mathematica and wrote new code modeling the geometry of snail shells. This project can help us understand the role of the species interaction has on the phenotypes of individual animals during their lifetime, and in turn the role these changes play in the ecological interaction between the two species.

My research is to make mathematical modeling of the shell of snail by using Mathematica and get parameters of the growth of snails from original data. We consider two parameters in modeling the growth of shells: the rate of whorl expansion (W), which measures how fast the aperture size increases, and the rate of translation (T), which measures how pointed the shell is. Using mathematical knowledge of three-dimensions, I improved the previous code by assuming the roiling axis of shells is vertical. Then I was able to use simplified code "findT" and "findW" to find the value of these parameters by using artificial data that I generated. I wrote code converting raw data from fluorescent microscope to working data, which has vertical roiling axis, so every step of finding parameters is clear and correct. When graphing the shell, I simplified code in one command "creatingspiral" which contains all the factors determining the shape of snail. In order to be closer to a real case, I made the program be compatible with inclined apertures of snails and irregular shape of apertures. This model basically presents the shape of snail but the irregular apertures part still need to be enhanced.

In the lab, we worked as a group doing four experiments separately in June and July: feeding experiment, choice experiment, snail crushing experiment and shape experiment. We mainly used size scaling to get raw data and compare crab foraging performance and defensive effectiveness of shells. We later used statistics software JPM to analyze and check the statistical difference between these experiments.

I will continue finishing the snail shape model in the following 2013-14 academic year and solving the problem of irregular shape of apertures.

(Supported by the Susan M. Rambo Fund and Ellen Borie Fund)

Advisor: Christophe Golé, Mathematics and Statistics

Mathematical Modeling of the Predator and Prey Induced Strengthening of Snail Shell and Crab Claw on the Northeast Coast

Yu Jin/2014

Professor David Smith and his research group in the biology department have previously discovered that snails can thicken their shell to protect themselves when facing their predator crabs, and crabs can also grow and strengthen their claw to increase their ability to crush their prey. Such co-evolutionary or phenotypic behavior and its outcome could be analyzed using game theory and dynamic systems and they might eventually help biologists to make quantitative predictions.

My summer research is comprised of two stages. In the first stage, Professor Golé and I studied game theory using textbooks and online literature. In the second stage, I researched on existing mathematical models based on game theory and tried to construct crab and snail models by adapting existing models. Besides, I also collaborated with the biological group working on the same topic. I helped them to record lab data and we went to a field trip in northeastern Maine.

We studied various characteristics of asymmetric matrix games and evolutionary games, understood the definition of best response, dominant strategy, Nash equilibrium, Evolutionary Stable Strategy, etc. After the study of game theory, I started to construct crab and snail models via imitation. I made a first attempt on Reinganum's heavily algebraic model of a dynamic game on competitive innovation and patent protection.[1] This model provided me great inspiration on how to abstract real life event into its mathematical form. However, since our model shared limited aspects with her model, we later switched to a simulation model using Mathematica.

So far, I observed that game theory is a powerful tool to describe interactive social and natural behaviors. However, computation difficulty might be a disadvantage when we try to analyze a large complicated model. This summer research is one part of my Honor thesis, and I will continue to work on this project during the next academic year.

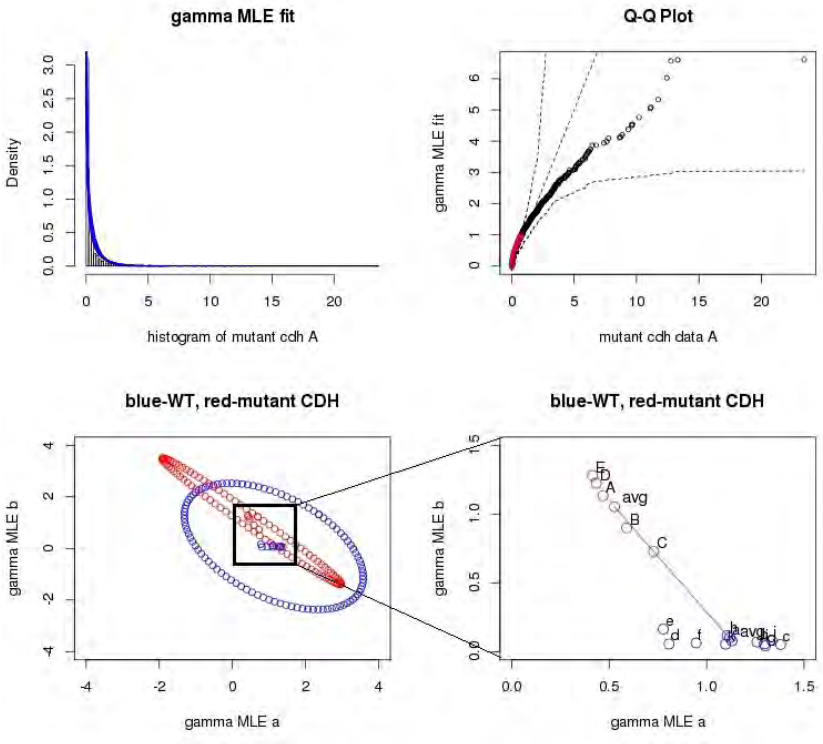
(Supported by the Ellen Borie Fund)

Advisor: Christophe Golé, Mathematics and Statistics

[1]Reinganum, J.F. 1982. A Dynamic Game of R and D: Patent Protection and Competitive Behavior. Econometrica, Vol. 50, No 3: 671-688.

Analyzing the Time Intervals of Action Potentials of Sensory Neurons in Zebrafish

Ji Ah Lee/2014



The research focused on analyzing the statistics of time intervals between action potentials of zebrafish. Action potentials, electrical impulses transformed from mechanical stimuli in hair cells, were generated spontaneously and their timings were recorded in Josef Trapani's lab (Amherst College). The first goal of the research was to find the best fitting statistical model for the distribution of time intervals between action potentials. The second goal was to analyze the difference in the frequency of electrical activities generated in wild-type zebrafish and mutant ones to gain further insights into the regulation of hair cells. The results may answer how sensory information is transformed into meaningful neuronal information and may also have broader impact on other systems such as hearing in our ears, also regulated by hair cells in the inner ear.

My advisors and I tried to fit a gamma distribution first. I used a statistical technique called MLE, maximum likelihood estimation, to estimate the parameters of the gamma distribution. Then, through RStudio, an open source environment for statistical computing, I fitted the original data on the estimated gamma distribution and checked how well that gamma distribution fits. Also, I compared the wild-type data and mutant data to see the change. To see how other distributions fit, I did the exact same process with a weibull distribution.

The estimated gamma distributions generally fit well. However, a few data sets did not quite follow a gamma distribution. Also, the estimated weibull distributions fit just as well as gamma distributions, which means a gamma distribution may not be the best model. Also, I saw that the time intervals of action potentials in mutants are generally longer than those in wild-type ones. However, I could not conclude how the distribution of time intervals changes, because there was too small number of mutant data sets to draw statistically significant results.

Due to some limitations the gamma distribution showed, my advisors and I have been trying to set a new statistical model that includes biological processes of action potentials. Looking into other papers, we have been working on a model combining two processes, excitation period and recovery period. This research will continue into the new academic year as special studies.

(Supported by the Ellen Borie Fund)

Advisors: Katherine Halvorson and Nessy Tania, Mathematics and Statistics

Orderly Tangles: Rebuilding and Cataloging Alan Holden's Polylinks

Emerson Lynch/2015

From 1935 until his death in 1985, Alan Holden was a crystallographer at Bell Labs. As a hobby during that time, he began building models of polyhedra and the linking shapes he built from them, which he termed "polylinks." Interesting due to their multiple axes of symmetry, these models have been studied by mathematicians and artists alike, building the models in new ways, whether it be with different materials that are aesthetically pleasing, or by representing them with three dimensional computer modeling and 3D printing. When Alan Holden died in 1985, he left his collection of models that he had built to Smith College in hopes that they would become a teaching tool for students to study symmetry. At the time, they were displayed briefly and then stored in the basement of the Science Center. Since then, they have gathered dust in storage and many have fallen apart.

In September, I began taking the models out of storage to restore them to exhibition quality. In addition to needing archival glue to put the connections back together, several of the models have collapsed completely, requiring hours of careful, detailed work to restore the structure and rigidity of the links. Once each model is restored, I examine it to determine the shape of the rings, number of rings, and the number of linkages so it can be classified as a tetralink, hexalink, octalink, pentalink, or icosalink. These classifications are made based on the regular polyhedra that provide the basis for the polylinks. As most of the models have been pictured in Holden's book,[1] I also note what figure for easy reference.

As a resource to the mathematicians and artists around the world that are interested in Holden's models and in anticipation of the UN designated 2014 International Year of Crystallography, I have made a website with information about the models and an index of models. Further work will involve continued restoration of the models and exploration into 3D printed models that can be used as durable classroom tools.

(Supported by the Ellen Borie Fund)

Advisors: Pau Atela and Marjorie Senechal, Mathematics and Statistics

[1]Holden, A. 1983. Orderly tangles: Cloverleafs, Gordian knots, and regular polylinks. New York: Columbia University Press.

A Biological and Mathematical Model of Phyllotaxis

Jordan Menter/2016

The regularity with which plant organs are naturally arranged around a stem is known as phyllotaxis. This phenomenon is characterized by a relatively constant angle of divergence present between consecutive plant organs (e.g. leaves). Mathematical explanations of phyllotaxis have often incorporated the frequently observed occurrence of the Fibonacci series in these patterns. Our research attempted to link these mathematical explanations with a key biological influence on phyllotaxis: specifically a plant's vascular tissue arrangement. In doing so we hoped to find connections between these two integrally related patterns that would account for such an elegant mathematical occurrence in nature.

Our methodology included three sub- projects: construction of an apparatus to acquire a set of images of plant stems that could be transformed from cylindrical to planar form, adapting software that permits analysis of our geometric model of phyllotaxis, and vasculature imaging through microscopy, First, we engineered a device that would rotate each sample 360 degrees in 10 degree increments. Using a digital camera, an image was taken of the stem every 10 degrees. To "stitch" these images into an image of an unrolled stem, we utilized the computational software program *Mathematica*; we wrote a program that compiled the 36 images of each subject. The stitched image was then imported into a different *Mathematica* program that allowed us to visually select the leaf nodes on each sample. These selections were entered as data in the form of coordinates; we were thus able to convert a flattened image of a plant into data applicable to other programs and essential to the identification of phyllotactic patterns.

The objective of our vasculature analysis was to explore the structure of the vasculature at phyllotactic transitions. To do this, we used a Leica M205 fluorescent microscope. We found a modified pseudo-schiff propidium iodide staining (Truemit et. al.) with a Clear-T 2 clearing method (Kuwajima et. al.) gave the best results. We also stained (aniline blue) and viewed cross-sections of sample stems at various locations along the stem.

While we made substantial programming progress and successfully "stitched" images, we have not yet been able to use this data in the modeling program. Likewise, our fluorescent images of vascular tissue have not yet reached the level of clarity we need. We are encouraged that we have identified a usable protocol that will yield usable data the near term. We plan to continue refining our program and our microscopy techniques this fall.

(Supported by a National Science Foundation UBM grant)

Advisors: Denise Lello and Christophe Golé, Mathematics and Statistics

Kuwajima, Takaai, Austen A. Sitko, Punita Bhansali, Chris Jurgens, William Guido, and Carol Mason. "ClearT: a detergent - and solvent-free clearing method for neuronal and non-neuronal tissue." *Development.* 140. (2013): 1364-1368. Web. 9 Sep. 2013.

Truernit, Elizabeth, Bauby Helene, Dubreucq Bertrand, Grandjean Olivier, Runions John, Barthelemy Julien, and Palauqui Jean-Christophe. "High-Resolution Whole-Mount Imaging of Three-Dimensional Tissue Organization and Gene Expression Enables the Study of Phloem Development and Structure in Arabidopsis." *Plant Cell.* 20. (2008): 1503. Print.

Phyllotaxis: Spirals in Nature

Mursal Naderi/2016

Phyllotaxis is the study of the arrangement of plant organs.

Our research team were particularly interested in plants that feature Fibonacci numbers of spirals in the arrangement of their organs and several hypothesis for why Fibonnaci phyllotaxis is dominant. We wanted to study the transitions between the different kinds of patterns observed in the models and in plants and their connection to the plant's vasculature. We began by first examining the transition between the different kinds of patterns observed in nature through perceptive work on growing and dissecting the plants, microscopic imaging, data gathering, modeling and mathematical analysis. Also, to better understand mathematical models and phyllotactic theories, we read published articles by other research groups on protocols and concepts to enhance our knowledge of the biological and mathematical aspects of fibonnaci phyllotaxis, and to analyze it in possible research methods.

The first aspect of our research project was to investigate the plant's development of vascular tissue using fluorescent microscopy to determine the visibility of leaves and stems for different kinds of specimen. We also hoped to discover how changes in phyllotaxis interface with preexisting vascular bundles. During this period we discussed Kuhlemeier's paper and thought that it would be a good idea to look at meristems under the microscope.[1] We stained most of our plants in a fluorescent solution and dissected out the meristem of several plants. The samples were stained with propidium iodide and metabisulfate, further cleared and mounted using Visikol. After the samples were stained, and cleared, a thorough analysis of the plant's stem using the z-stack feature of the fluorescent microscope showed the meristem and the interior vascular configuration. We adapted a few staining techniques and protocols, from which Clear^{T2} solution using PEG (polyethylene glycol) worked well.[2] The samples used for this study were Linum preenne, Linum usitatissimum, Lycopersicon, Calendula, and Zinnia.

The second aspect of this project was the advancement and execution of several mathematical models that display the phyllotactic growth and development of plants. One such model allowed us to responsively collect points in a node from a photograph of rolled plant material (i.e, clay, dental hardening, and pie crust). With these rolled plant material for mathematical model, we attempted to convert the cylindrical plant information into planar data that allow us to recreate meristem foundation on phyllotaxis. This later provided for us a visual representation of the parastichies and orthostichies present on the plant stem. This genuine data plotted equidistant nodes by computing average vectors between consecutive points in each node and took the average of these averaged vectors to plot it on top of the original data. The ultimate goal here was to plot a third group of nodes using the average of the averaged node vectors to eventually create a graph that would display all three groups of nodes that can be looked at for visual patterns in the nodes and to pinpoint potential orthostichies. Similarly, Mathematica stitching program created a stitched picture of multiple photos. This image-stitching program ran on 36 photos and was able to successfully align the pictures, and gave us a fully stitched image of a plant stem. In order to collect data for these models, we built a device that allow us to rotate the plant's stem every 10º while the microscope takes images.

Eventually a third aspect of this project is to develop a (3D) three-dimensional exterior rotation imagining of the plant stem. To do so, we will build on our rotating device and make it bigger than it's current size. Since the fluorescent microscope is not able to produce a good z-stack of the entire plant stem, this will give us a 3D image. We are considering all of our alternatives, but the next type of microscopy we may try this coming fall will be confocal.

(Supported by the Ellen Borie Fund)

Advisors: Denise Lello, Christophe Golé, Mathematics and Statistics

[1] Kuhlemeier's paper

[2] *Clear^{T}*: a detergent- and solvent-free clearing method for neuronal and non-neuronal tissue

http://dev.biologists.org/content/140/6/1364.long

Pattern Formation in Forager-Exploiter Model

Jize Zhang/2014

My SURF project is an exploration on particular animal foraging strategies, known as "producers and scroungers," using partial differential equation (PDE) models. "Producers/foragers" tend to look for food by themselves while scroungers/exploiters tend to exploit others. Ecologists have long observed that interactions between the two types of strategies are common in environments where preys are scattered in patches.

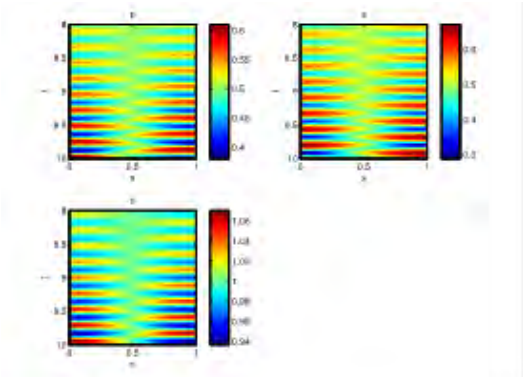
Previous studies by Prof. Tania and collaborators have shown that the "producer-scrounger" behavior does impact the formation of spatiotemporal population patterns. The model I developed for this project is an extension of their work.[1] In this model, foragers, exploiters and prey are all assumed to have random motion. In addition, the foragers are assumed to be attracted to high prey density, while exploiters are expected to move up along the gradient of foragers. The model also includes growth and death rate for each group. The growth of prey follows the logistic model, while that of foragers and exploiters is the same as in the classic predator-prey model. Also, prey growth should have a much shorter time scale than foragers/exploiters growth. In other words, the coefficients for foragers/exploiters growth and death rates are set to be of smaller magnitude. The full model after nondimensionalization is presented as the following:

$$\frac{\partial p}{\partial t} = \frac{\partial^2 p}{\partial x^2} - v_p \frac{\partial}{\partial x}(p\frac{\partial c}{\partial x}) + \delta pc - \delta p$$

$$\frac{\partial s}{\partial t} = \frac{\partial^2 s}{\partial x^2} - v_s \frac{\partial}{\partial x}(p\frac{\partial p}{\partial x}) + \delta sc - \delta s$$

$$\frac{\partial c}{\partial t} = d\frac{\partial^2 c}{\partial x^2} - \lambda(p+s)c + kc(1-\epsilon c)$$

where *p, s, c* stand for the population density for foragers, exploiters and prey respectively, and *x, t* by convention are variables for position and time. The patterns can be observed visually through numerical simulation.



There are several questions I intend to answer through this project. The major one is under what conditions the steady-state solution of the system will have instability and form spatiotemporal patterns. Before answering these questions, I first examined the simple forager model without exploiters as a comparison. Through linear stability analysis I found that the system always tends to a spatially uniform state in the long run. However after including the role of exploiters, oscillatory patterns can emerge. Using linear stability analysis, I was able to derive analytical condition for which pattern formation will occur. Briefly, I found that increasing the mobility of prey, foragers and exploiters promote pattern formation. Increasing growth/decay rate of the prey population (faster dynamics) also has a destabilizing effect. Meanwhile parameters controlling forager/exploiter growth have little impact.

In the future, I would like to explore more realistic modifications to the model by incorporating different effects such as direct competition between foragers and exploiters, prey evasion, or switching strategies.

(Supported by the Ellen Borie Fund)

Advisor: Nessy Tania, Mathematics and Statistics

Investigating the Neurotoxic Effects of Isoflurane on the Cytoskeleton of Developing Murine Neurons

Jamila Barger/2015

How exactly do anesthetics work? The medical procedure of general anesthesia, a reversible state of unconsciousness and unresponsiveness, has been practiced for over a thousand years, yet the mechanism and effects on the nervous system are not fully understood. Recent studies have shown that general anesthetics can lead to structural and functional changes in the brain. If during key periods of development, anesthetics could lead to widespread neurodegeneration, including long lasting cognitive and behavioral deficits after exposure (Culley et al., 2013). It has been suggested that some of the adverse effects might be due to anesthetic-induced alterations in cytoskeletal dynamics. Isoflurane is a volatile anesthetic used on neonates, children and adults. The aim of the study is to understand the mechanism responsible for the neurodegenerative effects of Isoflurane. We hypothesize that isoflurane activates the RhoA-Lim Kinase-cofilin pathway, increasing the phosphorylation of cofilin, a cytoskeletal modulator.

The study used Western blot techniques and densitometry to analyze the changes in expression of cofilin, phosphorylated cofilin and Lim Kinase-1. Cortical tissue samples were collected from C57 mice post natal day 12, exposed to clinical concentrations (1.5%) of isoflurane for 1 hour. The relative expression of phosphorylated cofilin to cofilin levels were calculated and averaged in treated and untreated samples.

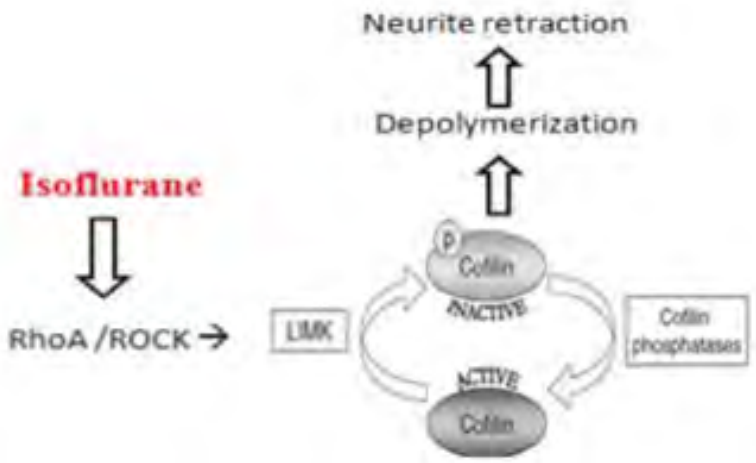
In our lab, previous in vivo experiments have shown an increase in relative phosphorylated cofilin to cofilin levels as high as 160%. My experiments continue to support this evidence. In animals treated with isoflurane for 1 hour, phosphorylated cofilin levels increased as high as 250% compared to untreated animals. The same group also displayed a 156% increase in Lim Kinase-1 levels.

These results suggest that our hypothesis is correct. However, more experiments must be done to show the cytoskeletal changes accompanied with increased phosphorylated cofilin. I will be continuing with this project during the school year, in which we will be planning experiments that allow us to show the cytoskeletal rearrangement and degradation of neurons after exposure. We will also begin looking into pharmaceutical agents that could be used to inhibit Isoflurane's neurotoxic effects.

(Supported by the Howard Hughes Medical Institute Fellowship)

Advisor: Adam Hall, Biological Sciences

Culley, D.J. et al. (2013). Isoflurane affects the cytoskeleton but not survival, proliferation, or synaptogenic properties of rat astrocytes in vitro. British Journal of Anesthesia, 110 (51): i19-i28.



Developing a Model of Fatigue in Middle-Aged Female Mice

Elissa Carney/2014

Fatigue, characterized as unusual tiredness, is a symptom of a variety of conditions including depression, multiple sclerosis and Parkinson's disease, among many others. The quality of life is negatively impacted for those who suffer from the diseases of which fatigue is a symptom and unfortunately there are few effective treatments. Development of an animal model therefore has huge implications, particularly for women, who are the majority affected.[1] Building upon the connection other researchers have made between an increase in specific cytokines in the brain and symptoms of fatigue, the Harrington lab has modeled acute fatigue by the single administration of the inflammatory cytokine, interleukin 1- β, which causes a dose-dependent reduction in wheel running activity in mice. My SURF project focused on improving this model through multiple interleukin 1- β injections.

C57BL/6 female mice (n=8) were administered a 400ng dose of interleukin 1-β or saline over three days, an hour before their dark cycle began. The experiment was a counterbalance design, for a total of four runs each with an 11-day cycle- five days of baseline, three days of injections, two days recovery followed by a cage change. After each run, wheel running and motion activity immediately following the injection period were compared to baseline activity to determine level of fatigue.

On average, mice that received interleukin treatment displayed a 20-40% decline from baseline in wheel-running activity compared to 90% and upwards in saline treated mice. This drop in activity was short-lived, as recovery in interleukin 1-β treated mice was seen by the first post-injection day. In the motion data, we saw a similar trend until the third round of injections where the motion activity for both treatment groups was relatively close to baseline.

Since the interleukin treated mice recovered wheel-running activity following the injection period, we again seemed to have modeled acute fatigue. In an effort to achieve a chronic model, another student in the lab decided to administer interleukin 1-β over five days instead of three. Additionally, other researchers have used Poly 1:C, a synthetic virus, to model fatigue in rats. Currently I am running a dose finding experiment in mice, in which I administer either a low or high dose of Poly I:C. In the coming semester, I will administer both interleukin 1-β and Poly I:C in hopes of modeling chronic fatigue.

(Supported by the Howard Hughes Medical Institute)

Advisor: Mary Harrington, Psychology

[1]Harrington ME. 2012. Neurobiological studies of fatigue. Prog Neurobiol, 99(2):93-105.

Oxytocin Receptors and Developmental Change in Affiliative Behavior in Hamsters

Emma Cooke/2014

In Syrian (*M. auratus)* and Siberian *(P. sungorus)* hamsters, pups display a shift in social behavior during development: while sociable from birth to around the time of weaning, pups subsequently become solitary and remain so for the duration of their adult lives. The polypeptide hormone oxytocin has been shown to play a role in diverse social behaviors including development of interpersonal trust[1] and formation of both same-sex[2] and opposite-sex[3] pair bonds. However, the role of oxytocin receptor (OTR) distribution in developmental behavioral changes has not been elucidated.

In this study, we utilized slides prepared from brains of *P. sungorus* (n=15) and *M. auratus* (n=16) sacrificed at 15 or 60 days, and used OTR autoradiography to compare receptor distribution in a variety of regions previously associated with social motivation or anxiety. Regions scored were the prefrontal cortex, lateral septum, endopiriform nucleus, bed nucleus of the stria terminalis, and central, basolateral, and posterior amygdala. All scoring was conducted using MCID Analysis.

A significant decrease in binding was observed in Siberian hamsters, in the endopiriform nucleus from d15 (0.193 ± 0.013) to d63 (0.145 ± 0.012, p=0.0104) as well as the basolateral amygdala (0.057 ± 0.003 to 0.038 ± 0.003, p=0.012). In Syrian hamsters, a decrease was observed in the lateral septum: binding was reduced from 0.145 ± 0.016 to 0.079 ± 0.010 (p=0.0035). Sex differences were generally not observed, although there was significantly more binding in Siberian hamster females (0.065 ± 0.003) than males (0.048 ± 0.001, p=0.012).

The fact that these species underwent changes in entirely different brain regions raises additional questions about the nature of neural development in these species, and how it relates to potential changes in their social behavior. Further research will include behavioral testing to establish the social correlates of these neural changes, and will hopefully gain insight into how these species' social behavior changes during maturation.

(Supported by the Howard Hughes Medical Institute)

Advisor: Annaliese Beery, Psychology

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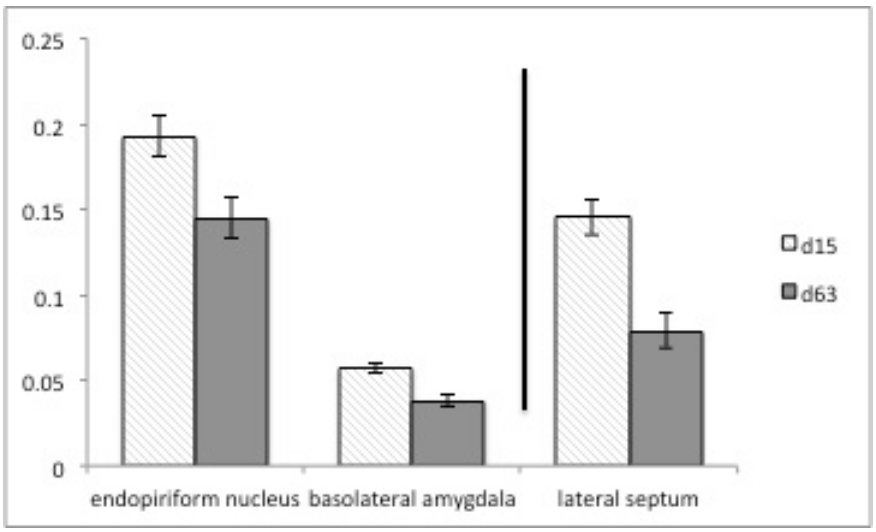

Oxytocin Regulation of Non-sexual Social Behavior in Female Meadow Voles

Nastacia Goodwin/2014

Though asocial during the longer day lengths typical of summer, female meadow voles form social nesting groups in the shorter day lengths typical of winter.[1] Seasonal changes in oxytocin (OT) receptor density have been previously found in several brain regions of meadow voles housed in long or short days.[1] The correlations found between oxytocin receptor (OTR) density and social behavior in brain regions not known to be associated with opposite-sex affiliation, including the lateral septum and central amygdala, cannot fully be interpreted without also exploring the changes in OT production occurring in the hypothalamus, and my research this summer focused on quantifying the total number of cells producing OT in the periventricular nucleus (PVN) of the hypothalamus to see if the increase of receptors coincided with an increase in actual OT production.

The brains for this project were stained using an established single labeling immunohistochemistry (IHC) protocol, and all photos were taken using an Olympus BX60 brightfield microscope with AxioVision software, according to a protocol that I established earlier in the summer. Because there were several brains that were processed in multiple IHC runs, a significant component of this project involved establishing which samples would be scored. This involved investigation of the quality of staining of the tissue, as well as balancing all of the runs so that there were equal amounts of long and short day voles represented per run and so that as many unique samples were included as possible. Once the runs were balanced and all of the slices with any PVN present were photographed, I began to score the slices using the ImageJ Cell Counter plugin. Computer assisted hand-scoring was used in place of computer scoring with either ImageJ or MCID due to the abundance of darkly stained OT fibers, which both automated programs consistently mistook for cells.

By the end of my fellowship, I completed the scoring for each brain once. In the Fall I plan to replicate the scoring for all of the brains and average the results in order to create accurate final cell counts. Understanding the circuitry of the OT regulation of sociality is particularly important as the incidence of social disorders such as autism continue to rise. Though there have been preliminary studies suggesting that the administration of long-term nasal oxytocin spray is promising and well-tolerated for treatment of social impairments of patients with ASDs,[2] research is in its infancy; the specificity and durability of effects remain unknown, and issues of safety and modes of delivery have yet to be addressed.[3] Understanding the relationship between OT and OTR changes occurring between the social short day voles and the asocial long day voles may provide significant insight into how OT production and reception relate to one another, and ultimately into the feasibility of OT as an augmentative treatment for social disorders.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Annaliese Beery, Psychology

[1] Beery , A. K. (2010). Oxytocin and same-sex social behavior in female meadow voles. Neuroscience, 169(2), 665-673.

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Elucidating the Molecular Mechanisms of Anesthetic Preconditioning

Bonnie Hawkins/2015

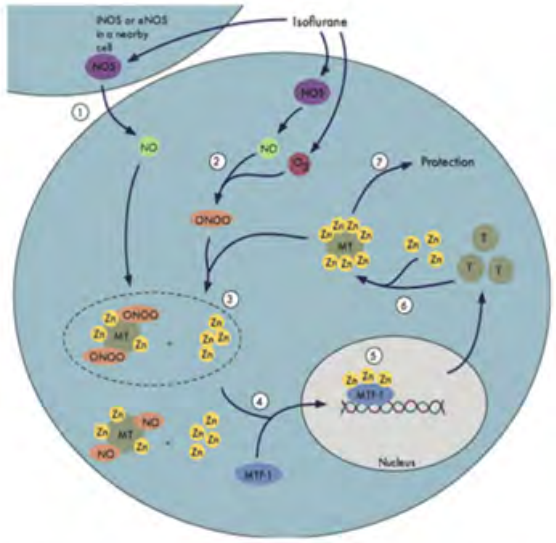

Figure 1: Proposed mechanism of anesthetic preconditioning (Edmands, 2009)

Anesthetic pre-conditioning is a phenomenon in which clinical doses of anesthetics result in protection against an ischemic event, such as in a stroke. It is estimated that 8.7% of strokes occur post-operatively,[1] and anesthetic pre-conditioning is a budding preventative treatment for patients undergoing surgery that are at risk for post-operative stroke damage.

The aim of this study is to elucidate and confirm a mechanism (figure 1) proposed by Scott Edmands,[2] as well as to identify upregulated factors. In the proposed mechanism, a critical step is the translocation of the transcription factor MTF1 into the nucleus. In order to confirm this step, neuron nuclear protein extractions[3] were performed on post-natal day 8 to 12 mice pups, both after anesthetic exposure to clinical doses of isoflurane (treated group) and after no exposure (control group). Western blots will be performed to confirm the presence of MTF1 in the treated mice nuclei and the absence of MTF1 in the untreated mice nuclei.

Additionally, post-natal day 8 to 12 mice cortices were snap frozen both 24-hours post isoflurane exposure (treated) and after no exposure (control) in both wild-type (WT) mice and in MTF knockout (KO) mice. Two-dimensional gel electropheresis will be performed to identify protective factors upregulated by the MTF pathway.

(Supported by the Smith College Provost's Office)

Advisor: Adam Hall, Biological Sciences

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Examination of Neural Oxytocin and Oxytocin Receptor Expression across Social and Solitary *Ctenomys* species

Yasmin Kamal/2014

Oxytocin plays a major role in same-sex partner preference and tactile stimulation. The peptide has also been implicated in social behaviors, such as parent-offspring bond formation, anxiety-reduction, and trust-formation. Differential expression of the oxytocin-receptor in mammalian brains has also been linked to differences in behaviors such as monogamy and promiscuity in some vole species. Furthermore, the neuropeptide is well suited to modulate behavior due to its localization in specific neural pathways, its slow enduring effects, and its durable response to physiological changes, which can be seasonally dependent.[1] Although most studies linking behavior with neural pathways focus on reproductive and parental care, a complete understanding of social behavior involves understanding the neural circuitry linked with non-reproductive social behaviors[1]. Thus the characterization of oxytocin and the distribution of the oxytocin receptor are important in determining the role of this peptide in the production of social vs. asocial behaviors. Therefore, in the current study, oxytocin receptor distribution was examined in species of the genus *Ctenomys*. These rodents display natural variations in social behavior and can provide a link between non-reproductive social behaviors and the neural circuitry underlining those social behaviors[2]. Using oxytocin receptor autoradiography assays, key brain regions expressing the oxytocin receptor were examined in three solitary and three social *Ctenomys* species. Using immunohistochemistry techniques, a protocol to label oxytocin in fresh-frozen tissue was developed so that oxytocin labeling may be possible in *Ctenomys* brain tissue samples collected and frozen in the field. Preliminary autoradiography analysis suggests there may be differences in the expression of the oxytocin receptor in the central and basolateral amygdala between social and solitary species. Additional analyses of these and other brain regions including the prefrontal cortex, lateral septum, and hippocampus are currently underway.

(Supported by the Howard Hughes Medical Institute)

Advisor: Annaliese Beery, Psychology

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Role of Orexin in Animal Fatigue Model with Interleukin 1β

Hyunji Kim/2014J

How do you describe fatigue and what are treatments for it? Deciding the definition and assessments of fatigue still has not been settled yet because of its subjective experience.[1] Ideal animal model of fatigue is required for the further investigation of fatigue, and ultimately chronic fatigue syndrome as well. Our lab has worked with interleukin 1β (IL-1β), a proinflammatory cytokine, to induce fatigue. We focused on the orexin-containing neurons, which interact with the circadian signals from the suprachiasmatic nucleus, to understand the role of circadian pacemaker on the neurological cascade of IL-1β-induced fatigue.[2] It has been found that suppressed orexin-containing neurons are mediating inflammation-induced lethargy.[3] More profound reduction in behavior symptoms were observed through inflammation in the ATx mice (orexin/ataxin-3-transgenic or orexin neuron-ablated) than wildtype (WT) mice.[4] Also orexin-A administration, either pre or post inflammation, decreased the volume of infarct in both WT and ATx mice. I hypothesized that orexin prevents inflammation-induced fatigue.

To clarify the role of orexin in a mechanism of fatigue and find the relationship between IL-1β and orexin, oexin knockout mice (n=4) were prepared with breeding and genotyping. Knockout (KO) and WT mice were administered with IL-1β or vehicle at age six months until. I collected voluntary wheel running activity, general motor activity, and video recordings under the dark condition to figure out whether fatigue will still be induced without orexin; I also gathered sleep cage data to make sure that there is no change in the total amount of sleep.

In general, orexin KO mice, an animal model of narcolepsy, shows reduced wheel running activity without a major change in general motor activity. With an administration of IL-1β, WT mice showed an acute effect, while KO mice were affected for a longer period till day P1 (Fig. 1). More data is still on the process of analysis. This will be presented at the end of next semester as a product of my honors thesis project.

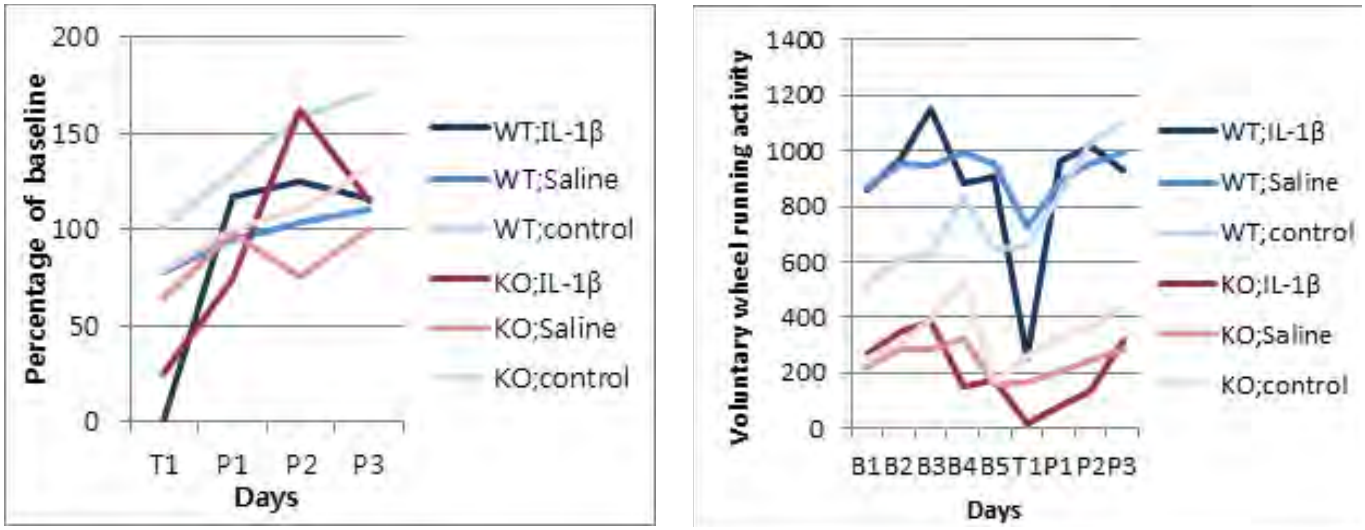

Figure 1. Voluntary wheel running activity with an administration of IL-1β or vehicle. Left) Five days of baseline were collected and averaged. Based on the number, each day's activity is shown in percentage. Right) Each day's activity is shown in graph. WT mice show an acute effect of IL-1β. KO mice have a reduced wheel running activity in general, and the effect of IL-1β lasts slightly longer, compared to WT mice. B1-5: baseline day 1-5; T1: treatment day 1; P1-3: post-treatment day 1-3.

(Supportecd by the Howard Hughes Medical Institute)

Advisor: Mary Harrington, Psychology

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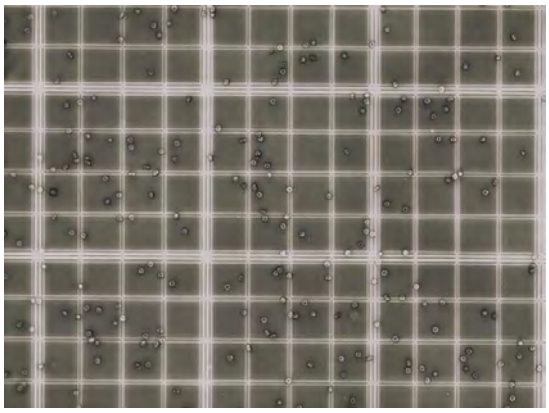
Anemia-associated Inflammation in Fatigue

Hailun Li/2014



In Dr. Mary Harrington's Lab, we have induced fatigue in mice through injections of interleukin-1β, which mediates a wide range of inflammatory responses in the brain. The goal is to expand research on the biological pathway of fatigue, and to improve the life quality of those who suffer from fatigue in the future. However, it has been implicated in the previous literature that fatigue might be merely a symptom of iron-deficiency anemia, which results in less oxygen carrying in the blood. To rule out this possibility, my work this summer focused on studying if anemia plays a role in our fatigue model.

Because anemia has been found to disproportionately affect seniors, I first studied the relationship between anemia and age with blood samples collected from nine mice of different ages. The level of anemia was quantified using two blood measurements: the plasma iron concentration and the number of red blood cells. Both would decrease as the level of anemia increases. The plasma iron concentration was measured using an iron assay kit, while the number of red blood cells was obtained through counting on a hemocytometer under a light microscope (as shown above). Relatively strong negative correlations were found between age and both measurements ($r=-0.692, -0.738$). However, because during their lifetime, the subjects were treated in different ways that might have affected the results, further experiments were needed in order to confirm the positive relationship between age and the level of anemia in the fatigue model.

In my current experiment, twenty mice have been divided into two age groups (young & old). Each mouse is randomly assigned to one of the two treatments: interleukin-1β (400 ng) and saline (400 ng). Terminal blood collection is performed four hours after the injection. In addition to brain samples, liver and spleen samples are also collected, from which tissue iron concentrations can be measured. It is hypothesized that with the same treatment, as age increases, both plasma iron concentration and the number of red blood cells will decrease. Within the same age group, if the two blood measurements are significantly lower in the interleukin-1β group compared to the saline group, anemia may have been induced in the fatigue model. If the tissue iron concentrations are higher in the interleukin-1β group at the same time, interleukin-1β may have induced the secretion of hepcidin, which leads to the sequestration of iron in the tissues, and thus decreases the plasma iron level. The experimental design above has been presented to the Harrington Lab; part of the experiment and data analysis will be conducted in the fall semester as part of my honors thesis project.

(Supported by tthe Howard Hughes Medical Institute)

Advisor: Mary Harrington, Psychology

Seasonal Variation in Corticosterone Levels of Female Meadow Voles

Kara Reitz/2014

In the summer (long day, LD), female meadow voles (*M. pennsylvanicus*) are solitary, territorial and aggressive toward conspecifics and usually will not form social bonds. However, in the winter months (short day, SD) they form communal nesting colonies, huddle together and are more likely to form bonds with other females.[1] There is great interest in determining what neurobiological mechanisms cause this shift, as there has not been adequate research on what facilitates same-sex partner preference in a non-monogamous species like meadow voles. Prior research has implicated the involvement of the hypothalamic-pituitary-adrenal (HPA) axis in the modulation of both anxiety and social behaviors. The activation of the HPA axis results in the production of the hormone corticosterone (CORT), which acts on different biological systems in response to stress. Levels of CORT have been demonstrated to vary seasonally in some avian and mammalian species[2] and therefore may be a link between stress and changes in social behavior.

In 2012 we performed both fecal CORT and plasma CORT assays that yielded opposite results from one another. The plasma CORT data suggested that there were higher levels of CORT in LD voles than in SD voles, and the reverse was true for the fecal CORT data. Due to this inconsistency we examined this phenomenon further by performing another run of the ELISA (enzyme-linked immunosorbent assay) using the same samples from the previous year.

For this study blood samples were obtained 5 hours after lights on from female LD (9-10am EDT; n=14), and SD voles (12-1pm; n=16) and processed using a corticosterone ELISA kit. The fecal CORT assay from the previous year had measured free CORT levels, while the plasma CORT assay utilized a reagent that displaces CORT from its binding protein and thus measured total CORT levels. Therefore, the re-run of the ELISA using the plasma samples was conducted using no steroid displacement to yield free CORT levels. The data obtained from this most recent assay indicates that the trend in plasma CORT levels of LD and SD voles are now in agreement with the fecal CORT data; LD samples were found to have higher overall CORT levels while SD samples exhibited lower levels. This is consistent with the current literature regarding seasonal changes in CORT of meadow voles[3] and suggests that the HPA axis may be involved in the shift in behavior seen in between the summer and winter months. Importantly, it suggests that seasonal regulation of corticosterone binding protein may play an important role in HPA axis functionality.

This study has set the groundwork for thesis research during the 2013-2014 school year where we will examine the effects of stress on the formation of a partner preference in female meadow voles.

(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)

Advisor: Annaliese Beery, Psychology

[1]Beery A.K., Routman D.M., Zucker I. 2009. Same-sex social behavior in meadow voles: Multiple and rapid formation of attachments. Physiol Behav., 97(1):52-7.

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Neurotoxicity Effect of Isoflurane on the Developing Brain

Maribel Santos/Ada Comstock Scholar

Anesthesia is a sedative agent broadly used to induce a state of unconsciousness. In the last decade new studies have investigated the neurotoxicity effect of anesthesia in the brain. This summer, I worked with Isoflurane, a volatile anesthetic, commonly used in adults, children, and neonatal. Previous work done in the lab has suggested that Isoflurane has an impact in cell morphology and dynamics of immature brain cells by affecting the cytoskeleton dynamic of neurons. My research is a continuation to the previous work that aims to elucidate the molecular mechanism by which Isoflurane induces neurite retraction.

To investigate the mechanism of neurite retraction, animal models were exposure to different concentration of Isoflurane. After Isoflurane exposure, the brain tissue was extracted (following Smith IACUC guidelines for use of animals) and used to measure the different levels of expressed cofilin and phosphorylated cofilin, LIM Kinase and phosphorylated LIM Kinase. In the two-week research I focused in investigating the levels of phosphorylated LIM Kinase using SDS-Gel Electrophoresis and immunoblotting with antibodies to detect the p=LIM Kinase. The results for these two-week investigations were inconclusive. However, further research will continue during the school semester as part of a special study. For future investigations, it is recommended that different concentrations of antibody be used. Once the proteins bands are determined, its levels can be analyzed using Image J program to determine the numerical concentration of p-LIM Kinase.

(Supported by the Blakeslee Fund in the Biological Sciences)

Advisor: Adam Hall, Biological Sciences

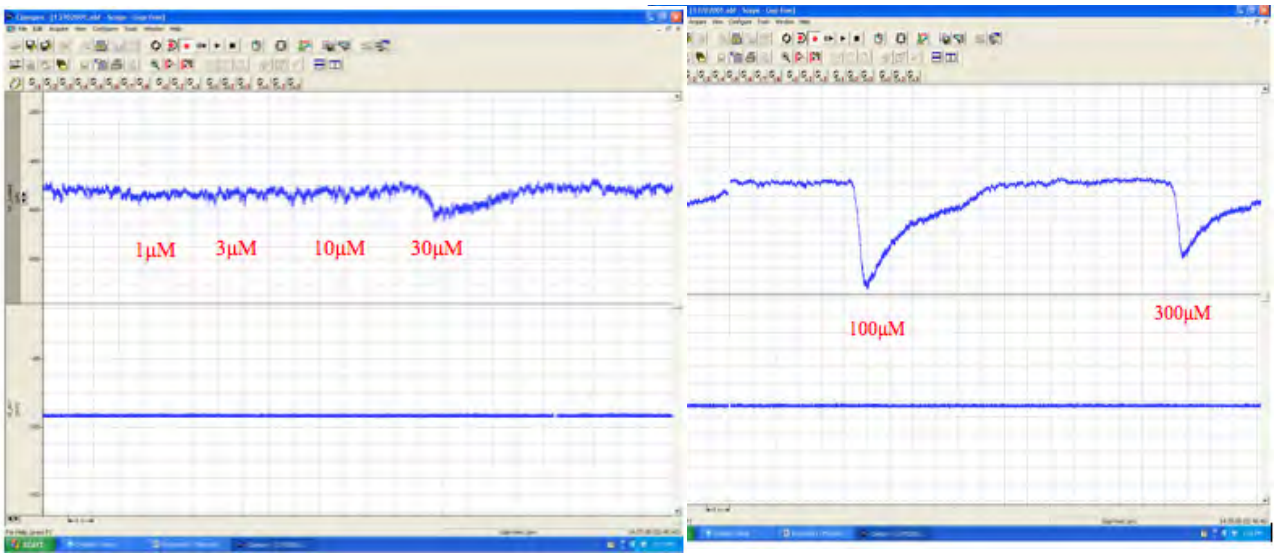
Patch-Clamp Investigation of Positive Modulators of $GABA_A$ Receptor Currents as Potential General Anesthetics

Naina Zaman/2016

How do general anesthetics affect the brain? Neuronal $GABA_A$ receptors are crucial due to their role as anesthetic targets in the mammalian brain. Anesthetics are positive modulators of $GABA_A$ receptor currents. Propofol, one of the strongest positive modulators of $GABA_A$ receptor responses strongly enhances inhibitory transmissions resulting in unconsciousness.

WSS-1 cells from Human Embryonic Kidney cell-line (HEK) were used due to large size and expression of human $GABA_A$ receptors with the endogenous β3 subunit to form receptors with the subunit composition of $\alpha_1 \beta_3 \gamma_{2s}$. Studies have shown that the β3 subunit to be imperative to evoke GABA currents using the patch-clamp technique (Davies, 2000). The patch-clamp is an electrical recording technique that allows for the measurement of the GABA receptor currents and their modulation by anesthetics.

Using this technique, I was able to record GABA currents using different concentrations of GABA in HEK cells.



It is seen from the figure above, that the higher the concentration of GABA, the larger the downward deflection. This means that the higher concentration of the GABA results in a larger current. I used the concentrations of 1, 3, 10, 30, 100 and 300 μM of GABA. It is seen that the 1, 3, and 10 μM do not visibly show any current the 30, 100 and 300 μM showed significant downward deflections. However, there seems to be a limit to this potency as the 100 μM has more of a downward deflection than the 300 μM. Experiments will now be conducted to observe the potency of potential anesthetics for positive modulation of the $GABA_A$ receptor currents which will be indicative of their potential for use as anesthetic agents.

(Supported by the Smith College Provost's Office)

Advisor: Adam Hall, Biological Sciences

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Mindfulness in Northampton Schools

Elizabeth Anderson-Krengel/2015

How do mindfulness exercises like deep breathing, yoga and meditation improve education in elementary schools? With help from Phil Peake and Nnamdi Pole, I attempted to answer this question alongside another Smith College undergraduate student, Jaime Rossow. As a team, we strived to determine how alternative exercises during school hours in addition to the strict English and Math curriculum could benefit overall wellness and speed of learning for children. Over the course of the year, a mindfulness curriculum at the Jackson Street School in Northampton was integrated for grades 1-5 (N=200). The project was a quasi-experimental study that looks at the effects of the mindful schools curriculum on the social and emotional learning skills, as well as the well-being of an intervention group in comparison to a cohort control group. This was a crucial research question to investigate because public schools in the United States are highly focused on testing and memorization. We were interested in integrating a wellness curriculum into the school week to see if their behavior and emotional wellness would change. Together, we looked at data that had already been collected at the Jackson Street School in Northampton, MA over the school year 2012-2013.

During the year, children received mindfulness classes from professional mindfulness instructors. These workshops included mindfulness breathing, mindful walking, mindful eating, meditation and journaling. The children completed self-report measures on three occasions and the teachers rated the children's behavior. Focus group interviews were used to learn more about how students at different grade levels responded to the intervention and to better understand how the teachers were able to integrate the program into their day-to-day teaching methods. The pre- and post-tests were already completed before this summer's research. I transcribed focus group audio tapes from each class interview and coded the results using the program Atlas. I inserted the pre- and post- quantitative data from both the teacher and student data files using SPSS.

Although I was unable to conclude concrete final results, I learned a substantial amount regarding the value of alternative learning in the elementary classroom. The most influential part of the research was the difference in behavior observed by parents at home and teachers in the classroom. More specifically, many children described the mindfulness classes being boring, making them feel tired, or preferring to focus more on English and Math lessons. However, when the children were asked if their parents had noticed a difference, they described a decrease in talking back, misbehaving with their siblings, and leaning on parents for emotional support. Children described arguing less with their parents and siblings. The children learned how to problem solve and separate emotions from reactions. In addition, teachers noticed that the children's overall good behavior in class increased as the mindfulness curriculum advanced.

We have just begun to understand how mindfulness exercises like meditation and deep breathing can positively contribute to the learning environment in school and in children's overall well-being. However, we still have yet to discover the long-term effects of mindfulness. Will children continue to benefit from mindfulness class throughout middle school, high school and college? Would it be appropriate to enforce mindfulness in public school curriculums throughout the United States? Are there better ways to integrate mindfulness strategies in very young children who become restless during sitting meditation or concentrated breathing? There are many questions we still have yet to answer about alternative learning curriculums.

(Supported by the Frances Baker Holmes Internship Fund)

Advisors: Nnamdi Pole and Philip Peake, Psychology

Neurobiological Studies of Fatigue in Aged Mice

Rebecca Broadhurst/2014

Fatigue is a common symptom that accompanies many debilitating neurological diseases such as Multiple Sclerosis and Parkinson's Disease. While no specific neural circuit or neurochemical change has been linked to what causes fatigue, finding an animal model that successfully represents fatigue could enable researchers to have a more comprehensive understanding of the molecular and cellular basis of fatigue and to explore new treatments that could reduce fatigue and improve a patient's quality of life. It has been found in previous literature, that aging is associated with chronic low-grade inflammation. Interleukin-1 beta (IL-1ß), an inflammatory cytokine, has been shown to be an indicator of fatigue symptoms; therefore IL-1ß might be an accurate representation of fatigue in our animal model (Bautmans et al, 2010). Over the course of the summer, I focused on how to create an animal model for fatigue with varying numbers of repeated injections of IL-1ß that could showcase a more chronic effect in older mice.

To measure fatigue in our research, 30 aged male and female mice were monitored by ClockLab. This data collection program constantly recorded how much voluntary wheel running and motion occurred during the light and dark periods. After a 5-day baseline data collection, mice were both repeatedly injected with saline or IL-1ß for 2 or 5 days, and then allowed to recover in a 2-day post treatment period. In addition, a bout analysis program was created in collaboration with Tanya Leise, which was used to more closely examine the running pattern and see if fatigue could be measured in the length and number of bouts the mice ran in the dark period.

Repeated 2-day injections showed different effects within genders. Aged female wheel running and motion were significantly knocked down after injection day 1, however were able to recover by post treatment day 1. Aged male wheel running and motion, also knocked down on injection day 1, showed the same reduction in injection day 2, not showing recovery until after the injections stopped. Using only aged males in the 5-day repeated injections, the results showed increased variability after injection day 3. The bout analysis revealed post treatment days had the same number of bouts but a reduction in length compared to baseline. However, these were also varied for both aged male and female mice.

From this study, I have learned that males seem more susceptible to repeated injections and that repeated injections should not go past 2 or 3 days due to an increase in variability. However it is possible data variability stemmed from individually age-related health problems. In addition, a saline effect was seen in the injections, demonstrating how sensitive the mice are to injections. This might also explain variability, as some mice might have had a higher coping ability. While still not a perfect animal model for fatigue, this research will help future investigations into what induces fatigue and how it should be measured. This project will be continued as an Honors Thesis this upcoming semester.

(Supported by the Howard Hughes Medical Institute)

Advisor: Mary Harrington, Psychology

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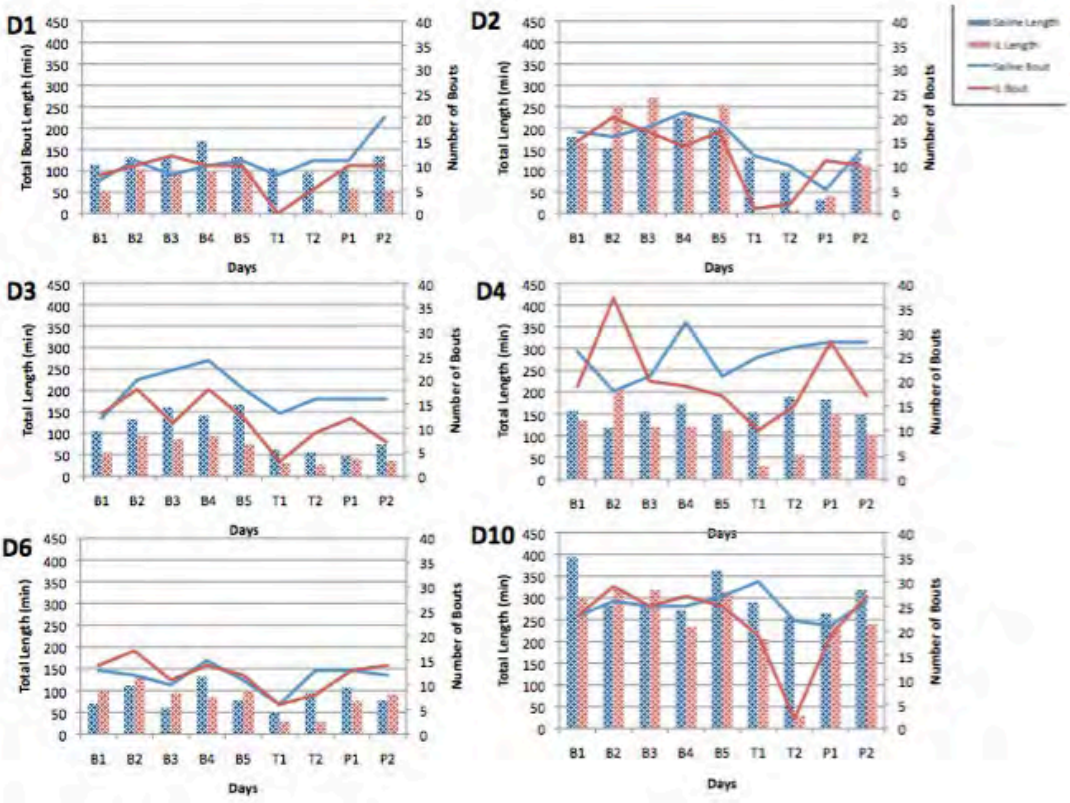
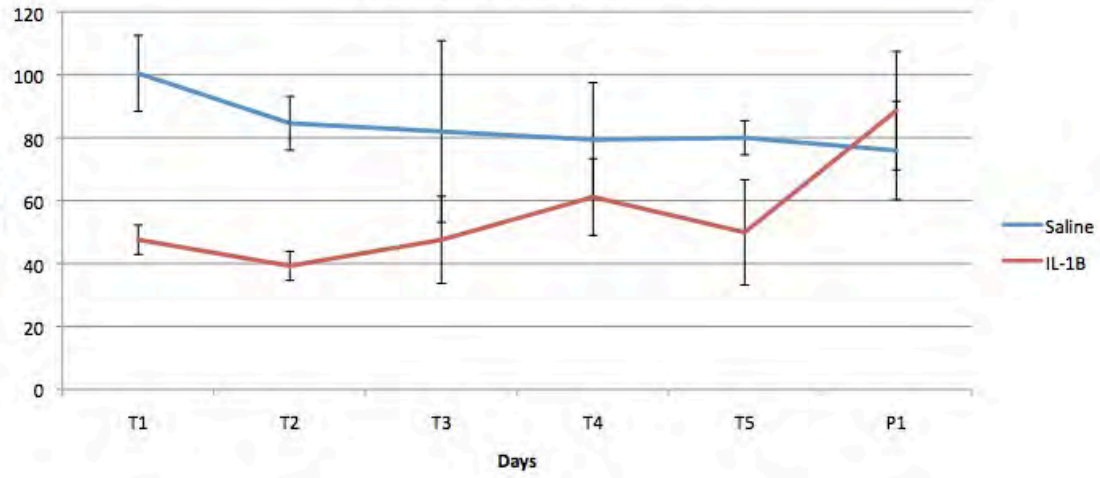
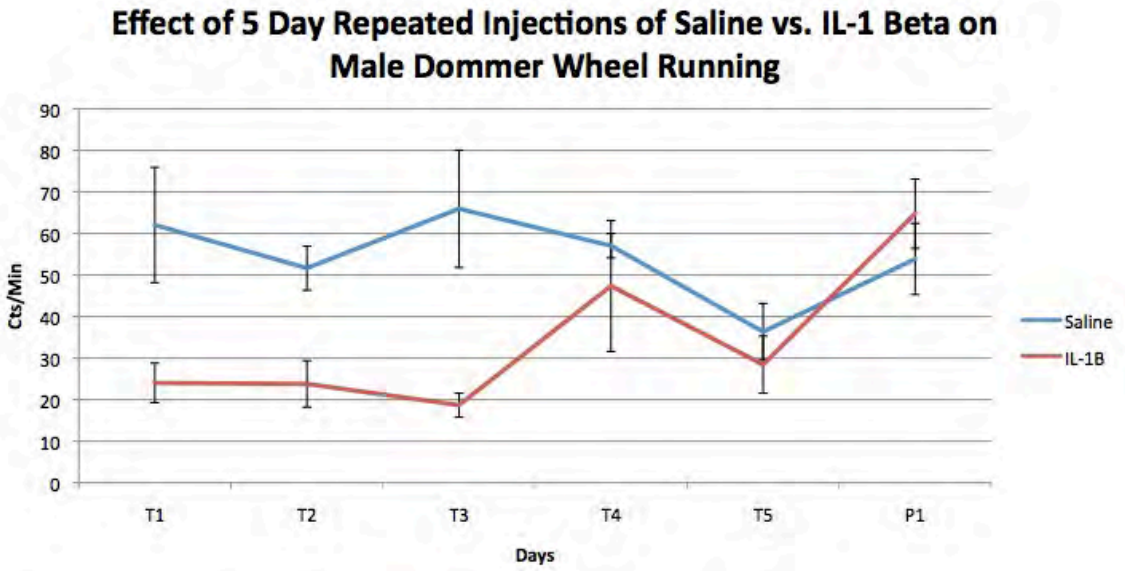
The Effect of Saline vs. IL-1B in Number and Length of Bouts for Dommer 1-6, 10



Effect of 5 Day Repeated Injections of Saline vs. IL-1 Beta on Male Dommer Motion




Effect of 5 Day Repeated Injections of Saline vs. IL-1 Beta on Male Dommer Wheel Running
Cts/Min
90
80
70
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T1
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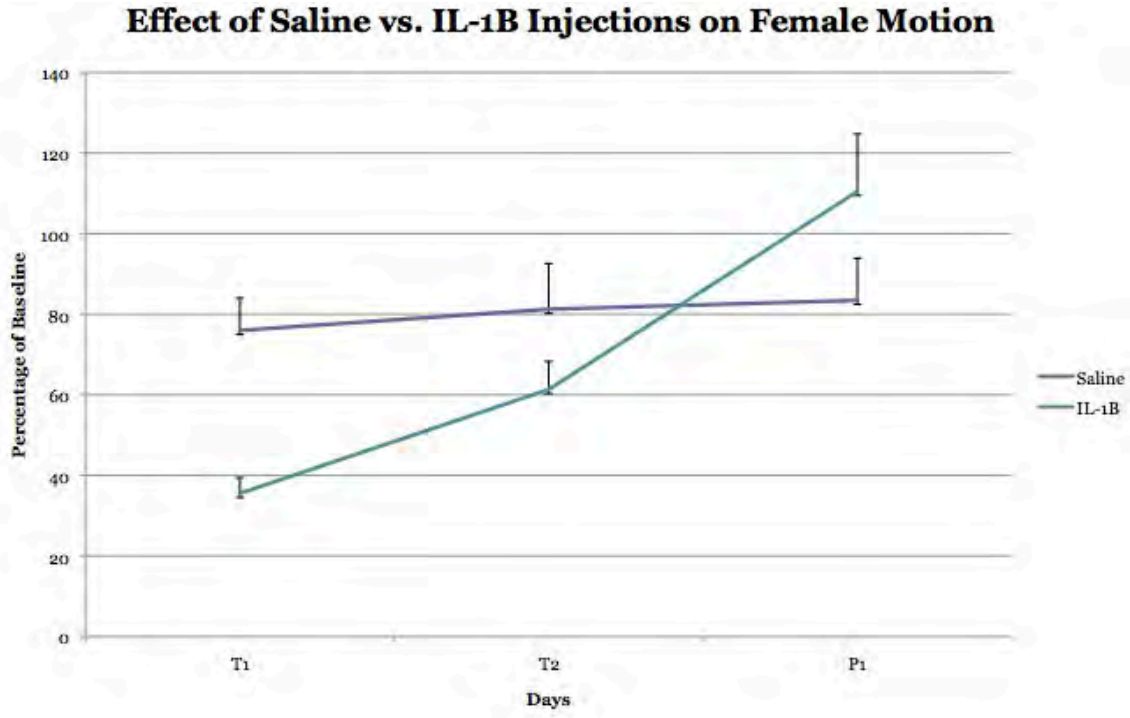
P1
Days
Saline
IL-1B


Effect of Saline vs. IL-1B Injections on Female Motion
Percentage of Baseline
140
120
100
80
60
40
20
0
T1
T2
P1
Days
Saline
IL-1B


Effect of Saline vs. IL-1B Injections on Female Wheel Running
Percentage Of Baseline
140
120
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40
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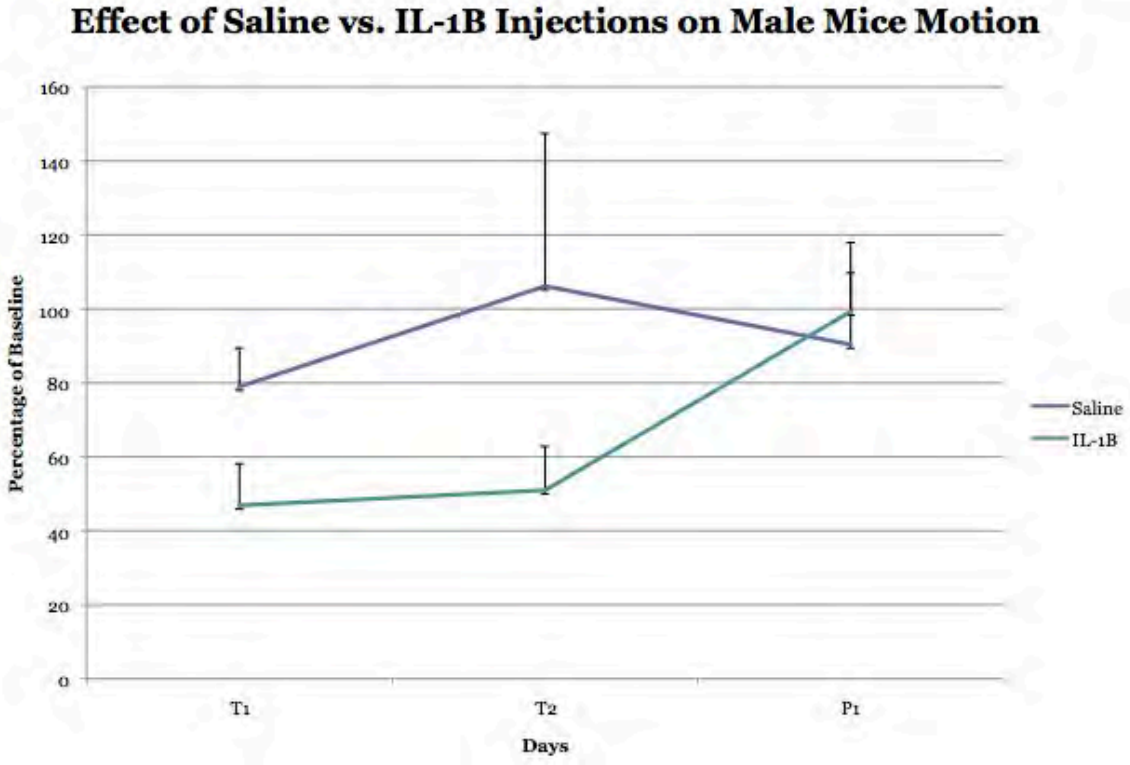
T1
T2
P1
Days
Saline
IL-1B


Effect of Saline vs. IL-1B Injections on Male Mice Motion
Percentage of Baseline
160
140
120
100
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T1
T2
P1
Days
Saline
IL-1B

Conversations Between Children with Autism and Their Parents

Meg Collins/2015J

Photo Credit : http://mentalhealthnews.org/relationship-with-autistic-children-improves-with-communication-therapy/841458/

Can children with autism carry on a successful conversation with a familiar adult? Do social skills improve spontaneously in autistic children over time, or are changes due to therapies? Autism spectrum disorders are marked by deficiencies in communication, as well as language and restricted, stereotyped interests. For my research I viewed videotapes of children with autism in 30-minute play sessions with a parent. They were instructed to play as they would at home, and were offered a variety of toys and activities. The study followed up on the participants with a second play session one year later.

The Children's Communication Checklist (CCC) (2008, Geurts, Embrechts), as well as the idea of contingent responses (1991, Tager-Flusberg, Anderson) were used to generate an original scoring system to measure conversational success. Transcripts of the play sessions were searched for desire verbs, cognition words and emotion words. The number of prompts by the parent was tallied, along with any instances of echolalia. The overall conversation was also rated for the frequency of inappropriate eye gaze, unusual intonation, failure to respond, and the child's level of focus on the parent. All of these variables were laid out in an Excel spreadsheet, which was added to as each video was viewed.

This is an ongoing project, and will be continued in the fall as a special studies with Professor Peter de Villiers. There are around twenty participants, and so far only five have been coded. I would like to compare the conversational scores of these children with typically-developing children matched for age and IQ to see the difference in the overall success of communication between children with and without autism. Preliminary findings indicate that there is a positive correlation between the number of prompts the parent provides and the child's display of echolalia and inappropriate eye gaze.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Peter de Villiers, Psychology

Geurts and Embrechts, 2008. Language Profiles in ASD, SLI, and ADHD. J Autism Dev Disord, 38:1931-1943.

Helen Tager-Flusberg and Marcia Anderson, 1991. The Development of Contingent Discourse Ability in Autistic Children. Association for Child Psychology and Psychiatry, 32:1123-1134.

Self-Objectification and Psychological Correlates: A Meta-Analysis of Survey and Experimental Studies

Cheri Eshete/2014 and Divya Chand/2014

Self-objectification is seeing oneself as an object whose value is based solely on appearance, and is ubiquitous in Western cultures. What are the psychological outcomes for people who engage in self-objectification? To answer this question, this summer we collected and coded data that will serve as the basis for a meta-analysis, in which results from previous studies done on self-objectification and psychological outcomes are pooled. For our research, we want to see what the results pooled from previous studies of self-objectification and psychological outcomes reveal; for example, how might the outcomes in women who self-objectify differ from the outcomes in men who self-objectify? In addition, we want to examine whether some psychological outcomes more than others show a higher correlation with self-objectification.

To perform this meta-analysis on the psychological outcomes of self-objectification, the first step was to gather past research done on this topic. We collected published experimental studies, survey studies, and unpublished dissertations through multiple academic databases such as ProQuest, PsychInfo, and Pubmed. Each article's title was then used to determine whether the subject matter was relevant to our study. Articles included based on title alone were reviewed and then excluded if they met any of the eight exclusion criteria decided on at the beginning of the project. Remaining articles could then be coded and entered to our growing database. In our first stages of analysis, we reviewed published studies and dissertations that reported survey research. For that task, we coded articles for demographics and correlates of self-objectification with psychological outcomes. To date, we have coded over a hundred articles out of 287 articles, coming from a pool of almost 2,000 potential studies. When the coding is complete, the laboratory team can begin the meta-analyses.

We hypothesize, as previous data have indicated that data pooled across many studies will show that women have higher rates of self-objectification compared to men. We also hypothesize that depression, eating disorders, cognitive deficits, and other negative psychological outcomes will be positively correlated with self-objectification, and that positive psychological outcomes will be inversely associated with self-objectification. This project will continue into the next academic year and, when finished, provide an important contribution to consolidating research on self-objectification and documenting the extent to which a seemingly harmless psychological state is associated with detriments in psychological functioning and well-being. Our findings will provide insight into the state of the literature and identify gaps for future research.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Benita Jackson, Psychology

Perspective-Taking in Preschool-Aged Children

Samantha Floyd/2014, Athulya Aravind, Lab Manager, Language Development Lab

Can children coordinate multiple perspectives? Do children generally have an egocentric bias when evaluating opinions? Can they utilize the syntactic structure to switch perspectives when needed? Can they coordinate relevant perspective to the main clause subject in embedding constructions? The language surrounding belief concepts is important as it can show us how humans compute others' mental states and use them to make predictions about behavior. Much of the research on developmental Theory of Mind looks at the ability to attribute false beliefs. When do children understand that mental states might diverge from reality, or "matters-of-fact"? Comparatively little has been done looking at how children fare with preference-based beliefs, which are idiosyncratic and vary by person and context. Children's ability to attribute preference-based beliefs is of interest to developmental psychologists and linguists. "Opinion" statements implicitly contain significant context and speaker information. They can inform us about children's capacity for perspective-shifting. We tested preschool-aged children (N=15) and adult controls (N=11) were given a set of scenarios followed by pertinent questions. We predicted that young children without experience reasoning about beliefs might not be able to distinguish between preferential and objective beliefs. Consequently, kids may find that only one opinion is correct. We predicted that they would find it difficult to switch perspectives when the relevant entity's opinions conflict with their own. However, children as young as 3 can accommodate multiple perspectives, even those that are different from their own. They are adult-like in their attribution of varying opinions to the right attitude-holders. The youngest children who might not have acquired complement-syntax show lower performance. Children tend to be more flexible than adults in accepting unusual preferences as valid on a general level. We expect this is because preschool-aged children may accept information readily from individuals perceived to have some expertise (Koening & Jaswal 2011). Children lack the world knowledge necessary to evaluate what tastes might be typical. As a result, they are much more willing to accept preferences as long as some entity finds that to be the case.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Jill G. de Villiers, Psychology

Koenig, M. A., & Jaswal, V. K., (2011). Characterizing children's expectations about expertise and incompetence: Halo or pitchfork effects? *Child Development, 82*, 1634-1647.

The Effects of Self-Objectification on Cognition

Helena Hassen/2014

The phenomenon of self-objectification—viewing one's body from an outsider's perspective— has been shown to lead to depression, in addition to many other psychological and physical health-related outcomes.[1] Research to date has focused mostly on the study of affective consequences of self-objectification, with less research on cognitive pathways related to performance, but we suspect that further study of cognitive outcomes resulting from self-objectification is warranted. Because high self-objectifiers have fewer cognitive resources,[2] we hypothesize that self-objectification will predict cognitive outcomes that indicate depleted cognitive resources, such as ruminative coping (perseverating on the causes, consequences, and symptoms of one's distress),[3] temporal discounting (showing preference for smaller but more immediate gains over larger but more temporally distant gains),[4] and generality of life-goal framing,[5] which have been linked to depression, respectively. The proposed project will test correlational and causal models of these three cognitive self-objectification outcomes. As young adult women in college are at particularly high risk for self-objectification, our samples will come from this demographic. Study 1 will be a correlational investigation in which participants complete measures of trait self-objectification, ruminative coping, temporal discounting, and life-goal specificity. Study 2 will be a between-subjects experiment in which participants will be assigned to either a self-objectification or control condition, then given measures of ruminative coping, temporal discounting, and life-goal specificity. In both studies, I will be continuing this project in the fall for my honors thesis, and I anticipate that self-objectification will predict cognitive outcomes of ruminative coping, temporal discounting, and life-goal specificity, in both correlational and causal models. The finding of a causal association between self-objectification and these cognitive outcomes would be an important contribution toward clarifying the nature and extent of the cognitive burden of self-objectification, especially for girls and women, and with further study, could inform design of cognitive treatments of disorders associated with self-objectification, such as depression and eating pathologies.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Benita Jackson, Psychology

[1] Quinn, D. M., Kallen, R. W., Twenge, J. M., & Fredrickson, B. L. (2006). The disruptive effect of self-objectification on performance. *Psychology of Women Quarterly, 30*, 59-64.

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[3] Grabe, S., Hyde, J. S., & Lindberg, S. M. (2007). Body objectification and depression in adolescents: The role of gender, shame, and rumination. *Psychology of Women Quarterly, 31*, 164-175.

[4] Lempert, K. M., & Pizzagalli, D. A. (2010). Delay discounting and future-directed thinking in anhedonic individuals. *Journal of Behavior Therapy and Experimental Psychiatry, 41*, 258-264.

[5] Dickson, J. M., & Moberly, N. J. (2013). Reduced specificity of personal goals and explanations for goal attainment in major depression. *PLoS One,* 2013 May 15;8(5):e64512. doi: 10.1371/journal.pone.0064512. Print 2013.

Young Adult Authoritarianism

Elysia Hung/2015

The present study examined the personalities of a Canadian sample with a focus on levels of authoritarianism. Authoritarianism is a cluster of personality characteristics that revolve around submission to authority, moral aggressiveness, respect for power and toughness, conventionality, lack of introspection, and intolerance of ambiguity. The subjects in the current study participated in extensive interviews when they were 26 and 31 years old. I quantified the age 26 interviews using the California Q-Sort to rate each participant's personality. I ranked each interviewee on a hundred different personality traits based on how pertinent the descriptions were. For example, item 1 of the Q-Sort reads: "Is critical, skeptical, not easily impressed." Item 2 reads: "is dependable and responsible." Each Q-Sort item is placed in one of nine categories, ranging from "9 = most characteristic" to "1 = most uncharacteristic" of the subject. After ranking each subject on the 100 Q-Sort items, I content coded parts of the age 31 interviews for authoritarian themes, including aggressiveness, submissiveness, self-discipline, conventionalism, intolerance of ambiguity, order and structure, and anti-intraception. When the interview content matched the authoritarianism descriptions established in the codebook, the theme was scored as present. After Q-Sorting each subject and content coding their interviews, I compared my coding with other scorers for reliability.

The result of this project used a psychometrically valid scale of authoritarianism collected at age 19 to predict age 26 Q-Sort data and age 31 content coding data. Although my internship ended before final analyses could be computed, the preliminary data suggested a relationship between the age 19 scale and later life outcomes. For example, individuals who scored high on authoritarianism at age 19 were described by the following Q-Sort items at age 26: "makes moral judgments" (r=.34), "judges other people" (r=.32), "behaves in gender-typed ways" (r=.34), and is NOT "introspective" (r = -.22). Furthermore, people who scored high on authoritarianism at age 19 told stories in their age 31 interviews that emphasized themes of conventionalism, self-discipline, and submission to moral authorities. These data showcase the continuity of authoritarianism over time across psychometric scales, observer-rated Q-Sort items, and life narratives.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Bill E. Peterson, Psychology

Traumatic and Psychotherapies Study

Yutong Jiang/2016

In SURF! 2013, I acted as research assistant for Professor Nnamdi Pole, doing research related to traumatic and psychotherapies studies. I have done three main tasks. The first one was to figure out and explore how to use Code-A-Text to analyze therapy transcripts, a computer program used to investigate the role of language, especially emotional language, in psychotherapies. The second one was participate in Stefanie Carreiro's (Smith School of Social Work) thesis study. The purpose of the study was to probe ethno-cultural differences among Blacks, Whites, and Latinos/as in trauma symptomatology. My last task was to check correctness of a longitude family study's database, examining the survey results and the records in computer.

Before my partner and I worked on the Code-A-Text, none of us knew how to use the program. We basically tried everything by ourselves to figure out how to utilize its functions. By the end, we solved the problems about how to format transcripts, count words, create and compare word groups, analyze results by using external dictionaries, and etc. We wrote a manual for other students who might use this program for further research. We encountered a lot of errors and obstacles, but finally solved them one by one. It taught me that research requires curiosity, patience and passion for your work, and that if you keep working on, you will make progress. Even though a small progress can be a meaningful one.

For Stefanie's project, I read through several articles related to the topic, and selected population size, measures, differences in PTSD, and other useful information in a table. This task gave me basic ideas about how to do a comparative piece of research. In the third project, checking data seemed monotonous, but made me understand that research requires precision and validity. A rigorous attitude is appreciated and more than necessary for researchers.

The reason why I selected the specific project was that based on my understanding and knowledge in psychology, I am particularly interested mental disorders and PTSD and I am impressed by the works and achievements of Professor Pole in this area. In this research experience, I gained a lot more than I expected. Every task I did deepened my understanding of the specific area, helped me build up skills and personality a researcher needs, made me clearer about my academic goals, and taught me that research is the foundation of almost everything.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Nnamdi Pole, Psychology

Creating a Mandarin Language Test in Order to Diagnose Specific Language Impairment in China

Tarra Murphy/2015

Currently in China, there is no field of speech language pathology. Speech language pathologists do many different things, and one of those things is to diagnose children with specific language impairment (SLI) so that they can receive the help they need. Working in conjunction with Dr. Liu, a Chinese speech language pathologist in Texas, and Dr. Ning, a professor from Tianjin University, Jill de Villiers has been working on creating a Mandarin language test.

Dr. Ning and his research team created a pilot test of their own and conducted research on about seven or eight hundred Mandarin-speaking children in China. The children varied in age, ranging from three years to nine years. The children were tested on many different things, including syntax, sentence production, pronunciation, tones, etc., for a test with almost 200 items.

The data from Dr. Ning's research was sent to our lab, and the data seemed to show that the children had scored along a favorable curve. I ran several univariate analyses of variance through SPSS, looking carefully at statistical differences for the scores on each portion of the test. From this we were able to compare how the children from each age group scored, and the significant differences between the age groups which would signal growth with age. It was concluded that some of the sections of the test were far too easy, and others far too difficult. Furthermore, there was very little growth with age by subtest. I used my basic knowledge of Mandarin as well as my linguistics training to help critique the items and suggest alternatives. After the statistics were compiled and analyzed, Jill de Villiers and Dr. Liu traveled to China to present the findings to Dr. Ning and give suggestions as how to improve the children's scores.

The research on this project is ongoing, and will continue through the school year. Also, per the request of Dr. Ning, the details of this project will not be disclosed until published. Now that the flaws of the pilot test have been identified, we hope to strengthen it by removing ambiguities, lowering the possibility of chance, and searching through published linguistic studies done on Mandarin to find inspiration for improving the test.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Jill de Villiers, Psychology

Using the California Q-Set to Quantify Narrative Data: The Futures Study

Janelle R. Olsen/2014

The California Adult Q-Set (CAQ) can be used to quantify aspects of narrative data.[1] We demonstrate in our study how CAQ items (based on narratives) can serve as criteria for understanding how authoritarianism (assessed by scale at age 18) manifests as personality characteristics at age 26.

The California Q-Set was developed by Block (1961/1991) to study personality functioning.[1] The CAQ consists of descriptors written on 100 cards (e.g., "Has high aspiration level for self"). In using the CAQ to describe individuals, a rater reads each item and places it on a continuum anchored by 1=extremely uncharacteristic for the individual to 9=extremely characteristic. The procedure limits the number of cards that can be placed in each of the categories so that the distribution resembles a normal one. The CAQ was developed to standardize different perceptions of the same person by providing a common descriptive language and formatting procedure.

Although the Q-sort method itself was originally created for assessing individuals after face-to-face interviews, researchers can also conduct "file-based" Q-sorts of narrative material. Based on interview transcripts, Prof. Bill E. Peterson, myself, and other raters conducted file-based Q-sorts of 103 emerging adults (age 26). Participants in the Futures Study were interviewed when they were 26 and each provided roughly 25 narrative pages. The narrative material was read by several raters who each Q-sorted the participant using the CAQ. A composite score was then created for each participant once the Cronbach's alpha for the combined raters' score reached .69 or above.

One way to use the CAQ data is to treat the items as criteria. For example, in the Futures Study a measure of authoritarianism was administered at age 18. What are the personality (CAQ) characteristics associated with authoritarianism? Upon running our analyses (using Pearson correlation), we discovered that, as expected, several predicted CAQ cards correlated with authoritarianism at age 18. For example, people who scored high on authoritarianism at age 18 were more likely to be rated high on the following CAQ card at age 26, "Makes moral judgments; judges self and others in terms of right and wrong" (r = .34, $p < .05$). Uncovering such a pattern of correlates suggests that personal narratives provide deep insights into the personality characteristics of emerging adults, and that these characteristics can be assessed reliably and validly by the CAQ. I will discuss the CAQ method and Futures Study findings in a Paper Symposia at the 2013 Conference on Emerging Adulthood Conference (Society for the Study of Emerging Adulthood) in Chicago, IL.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Bill E. Peterson, Psychology

[1]Block, Jack. *The Q-Sort in Character Appraisal: Encoding Subjective Impressions of Persons Quantitatively.* Washington, DC: American Psychological Association, 1960/2008.

Mindfulness in Elementary Curriculum

Jaime Rossow/2014

Under the supervision of Dr. Annemarie Gockel, I contributed to her research on the effects of mindfulness integrated into an elementary school curriculum, in both the general classroom setting and a specialized mindfulness class. The study seeks to determine what effects regularly-practiced mindfulness has on behavior, emotional state, concentration, and relationships in the classroom and at home.

Data collection was completed prior to the start of the summer. Interviews of children involved in classroom focus groups were collected and recorded by Dr. Gockel, as well as a variety of student pre- and post-tests addressing the target measures by self-report. The classroom teachers collected student mindfulness journals and filled out pre- and post-assessments of each child in their class, as well as keeping their own journals about implementation of the mindfulness curriculum in the classroom. The mindfulness instructors also submitted journals detailing the curriculum they taught and their observations of the students.

The majority of my involvement in this study was in processing the data. After transcribing the focus group interviews, my partner and I learned grounded theory coding methods coded the interviews in Atlas TI. We also coded the student journals in Atlas. Additionally, we entered post-test data from the student and teacher surveys into SPSS, and we entered the data from the teacher and instructor journals into Excel spreadsheets that we designed based on the data parameters. Throughout this whole process, we made notes on our observations of trends in the data that arose through this close contact with the data.

The work that we accomplished in the period of SURF prepared all the data for analysis, but we did not reach the point of data analysis by the end of the summer. Thus, there are not yet results for the study itself—these results will hopefully shed light on the benefits of mindfulness in primary education and lead to more in-depth research over a wider age range, which would give schools better tools to aid in the education and development of their students. This work filled in an important gap in my personal knowledge about the total trajectory of a study, in addition to the finer details of preserving confidentiality and organizing large quantities of data. Whereas before SURF I did not feel comfortable in my knowledge of the execution of an experiment, my hands-on experience has led me to feel confident in my understanding of the implementation of a scientific study.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Nnamdi Pole, Psychology

Are Adaptive and Maladaptive Perfectionism Related to PTSD?

Julia Sisson/2014

Perfectionism is understood as a multidimensional phenomenon in which individuals set excessively high standards and engage in overly critical self-evaluations.[1] These dimensions include *maladaptive evaluative concerns (MEC)*, which involve self-defeating doubts about actions and concern over mistakes, and *pure personal standards (PPS)* which involve high expectations for the self.[2] Perfectionism has been related to various anxiety disorders and depression, and its multiple dimensions have been specifically associated with certain cognitive experiences and symptoms.[3] In light of the multiple studies examining the relationship between perfectionism and anxiety, it is striking that relatively few have tested whether perfectionism is associated with posttraumatic stress disorder.[4] In my research, I focused on how the current literature relates experiences of post-traumatic stress and perfectionism, and whether data could support this rarely-explored relationship.

I first explored the most recent research available to establish a theoretical basis from which I could understand behaviors associated with perfection and PTSD, such as reflection and brooding, and how these could mediate – or indirectly affect -- the relationship between experiences of post-traumatic stress and multiple dimensions of perfectionism. I used the Preacher & Hayes[5] model of simple mediation to analyze previously collected data and understand if these associations existed. I then ran a bootstrapping analysis of the data to observe the power of the indirect effect, as recommended by Preacher & Hayes. Most behaviors were partial or weak mediators, but rumination fully mediated the relationship between multidimensional perfectionism and PTSD symptoms.

Post-traumatic stress disorder affects nearly 8 percent of Americans, though the complex array of causal factors associated with its development are far from understood. Behaviors and personality types that are associated with later development of PTSD can be targeted in trauma-laden fields, such as the military or police, and preventative measures can be taken for these populations. Furthermore, behaviors which indirectly connect personality factors with post-traumatic symptoms can be the focus of therapy in survivor populations, and lead to more effective treatment. This relationship will shed light on the factors leading to the development of PTSD, and offer key insights about how to properly address these factors.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Nnamdi Pole, Psychology

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[1] Frost, R. O., Marten, P. A., Lahart, C., & Rosenblate, R. (1990). The dimensions of perfectionism. *Cognitive Therapy and Research, 14*, 449-468.

[2] DiBartolo, P. M., Li, C.Y., & Frost, R.O. (2008). How do the dimensions of perfectionism relate to mental health? *Cognitive Therapy Research, 32,* 401- 417. doi: 10.1007/s10608-007-9157-7

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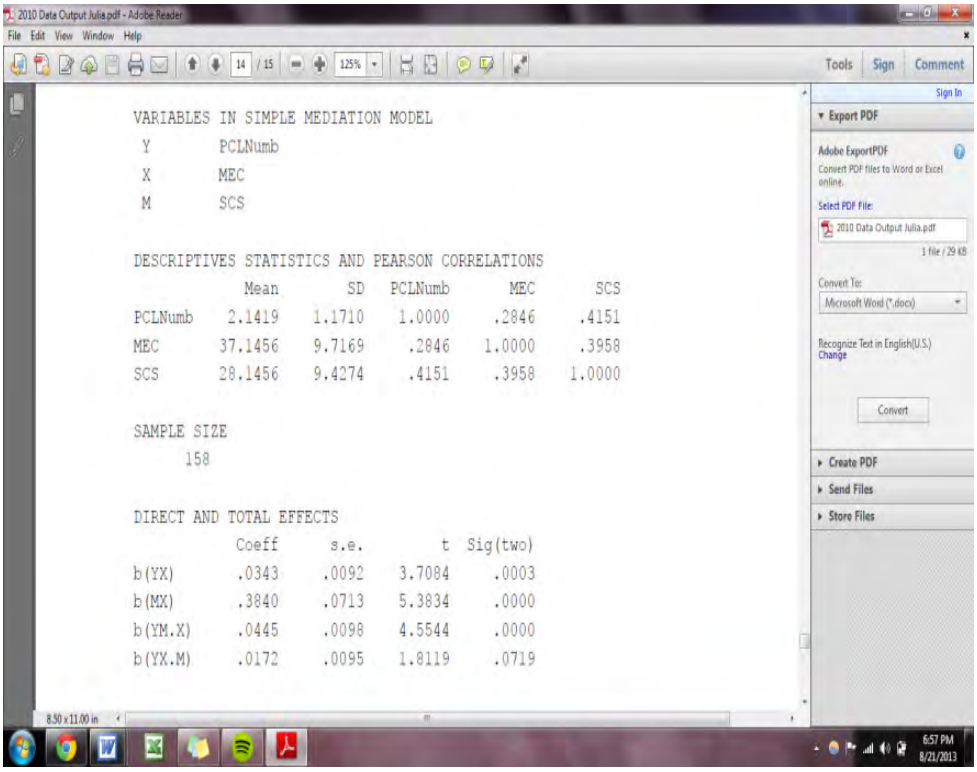
[4] Ibid.

[5] Preacher, K.J. & Hayes, A.F. (2004). SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behavior Research Methods, Instruments, & Computers, 36(4),* 717-731.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8/21/2013

It Only Takes One: Drinking Games and Prepartying among High School Students

Cara Tomaso/2016

Adolescence is often characterized as a time of exploration. One way in which adolescents might explore is by experimenting with alcohol. Indeed, research suggests that adolescents tend to experiment with high-risk drinking behaviors, including prepartying (i.e., drinking before going out) and drinking games (DGs), during high school.[1] These behaviors are troubling in their own right, as they have been linked to a range of negative drinking consequences, including hangovers, blackouts, and alcohol poisoning.[2] However, it is unclear whether involvement in more than one high-risk drinking behavior contributes to a multiple risk paradigm, wherein the risk associated with one's overall drinking is combined with the additional risk of rapidly ingesting alcohol as a result of either prepartying and/or participation in DGs. Thus, the aims of the present study were twofold: (1) examine whether current involvement in prepartying and DGs has synergistic negative effects vis-à-vis drinking consequences and (2) investigate group differences in drinking motives among those who engage in combinations of these high-risk drinking behaviors versus those who do not.

High school students (N=240; ages 14-18) were divided into three groups: (1) those who did *not* report playing DGs or prepartying (n=63); (2) those who reported playing DGs but did *not* report prepartying (n=79), and (3) those who reported playing DGs *and* prepartying (n=98). Participants completed measures of typical alcohol use and negative drinking consequences, drinking motives, and participation in DGs and prepartying over the past month.

We found significant group differences with respect to negative drinking consequences, as indicated by ANCOVAs controlling for age and typical alcohol consumption. Pairwise analyses indicated that involvement in two high-risk drinking behaviors as opposed to just one did *not* give rise to negative additive effects. However, (a) students who played DGs *and* prepartied and (b) students who only played DGs reported higher levels of negative drinking consequences than those who did not participate in either activity. Finally, adolescents who participated in both of these activities, as well as those who only played DGs, drank for social and enhancement (e.g., "to get high") reasons more frequently than adolescents who did not participate in either activity.

Altogether, these findings suggest that students who participate in just one high-risk drinking activity could be susceptible to similar levels of negative drinking consequences as those who participate in both behaviors. It is therefore important for health professionals and high school personnel who work with adolescents to (a) be educated about prepartying and DGs, (b) include items that assess these behaviors in their prevention and intervention efforts, and (c) be mindful of the health risks associated with adolescents' involvement in only one of these high-risk behaviors.

(Supported by the Frances Baker Holmes Internship Fund)

Advisor: Byron L. Zamboanga, Psychology

[1]Kenney, S. R., Hummer, J. F., & LaBrie, J. W. (2010). An examination of prepartying and drinking game playing during high school and their impact on alcohol-related risk upon entrance to college. *Journal of Youth and Adolescence, 39,* 999-1011. doi:10.1007/s10964-009-9473-1

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Smiling Across Cultures: Universal and Cultural Differences in Recognition of Facial Expressions of Emotion

Tingyu Zhang/2015

In order to understand human communication, one must understand conversational expressions and nonverbal expressions, like emotions. Language communications are culture specific. And so, the question of whether the recognition of facial expressions also varies across culture can be raised. In my research of the literature, I focused on universals and cultural differences in recognition of facial expressions of emotion.

Using photographs of posed facial stimuli[1][2][3] and spontaneous emotions,[4] researchers assigned participants from diverse cultural backgrounds to interpret facial expressions of emotions and rate their intensity. The main finding of this research is that the agreements in the judgment of facial expressions across culture are very high, especially among basic emotions.

However, differences still exist in levels of intensity.[5] Generally speaking, participants from western cultural backgrounds have higher intensity ratings than non-western participants. One possible explanation concerns the language barrier. In addition, context might influence the ratings. The results[6] showed that Asian observers were more influenced by context than Americans.

More research is needed to explore social emotions (i.e. embarrassment and guilt) in addition to the basic emotions. Because these emotions require the representation of the mental state of other people, a learning process is necessary for recognition.

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Advisor: Beth Powell, Psychology

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1 Ekman, P., Friesen, W. V., Chan, A., Diacoyanni-Tarlatzis, I., Heider, K., Krause, R., ... & Ricci-Bitti, P. E. (1987). Universals and cultural differences in the judgments of facial expressions of emotion. Journal of Personality and Social Psychology, 53(4), 712-717.

2 Ekman, P., & Friesen, W. V. (1971). Constants across cultures in the face and emotion. Journal of Personality and Social Psychology, 17(2), 124.

3 Biehl, M., Matsumoto, D., Ekman, P., Hearn, V., Heider, K., Kudoh, T., & Ton, V. (1997). Matsumoto and Ekman's Japanese and Caucasian Facial Expressions of Emotion (JACFEE): Reliability data and cross-national differences. Journal of Nonverbal Behavior, 21(1), 3-21.

4 Matsumoto, D., Olide, A., Schug, J., Willingham, B., & Callan, M. (2009). Cross-cultural judgments of spontaneous facial expressions of emotion. *Journal of Nonverbal Behavior*, *33*(4), 213-238.

5 Dailey, M. N., Joyce, C., Lyons, M. J., Kamachi, M., Ishi, H., Gyoba, J., & Cottrell, G. W. (2010). Evidence and a computational explanation of cultural differences in facial expression recognition. *Emotion*, *10*(6), 874.

6 Matsumoto, D., Hwang, H. S., & Yamada, H. (2012). Cultural differences in the relative contributions of face and context to judgments of emotions. *Journal of Cross-Cultural Psychology*, *43*(2), 198-218.

Examples of the photographs utilized in a study of how emotions are judges across literate cultures[7]

	Percentage Agreement in How Photograph Was Judged Across Cultures				
	UNITED STATES (J=60)	BRAZIL (J=40)	CHILE (J=119)	ARGENTINA (J=168)	JAPAN (J=29)
 Fear		67%	68%		66%
 Disgust		97%	92%	92%	90%
 Happiness	97%	95%	95%	98%	100%
 Anger	67%	90%		90%	90%

[7] Ekman, P, & Friesen, W. V (2003). *Unmasking the face: A guide to recognizing emotions from facial clues*. Ishk.

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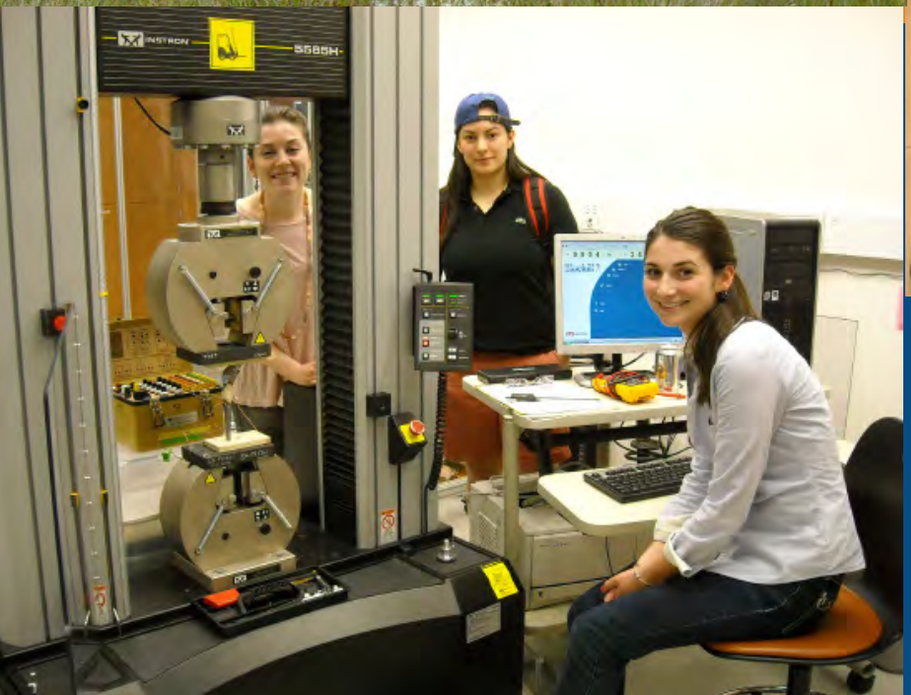
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