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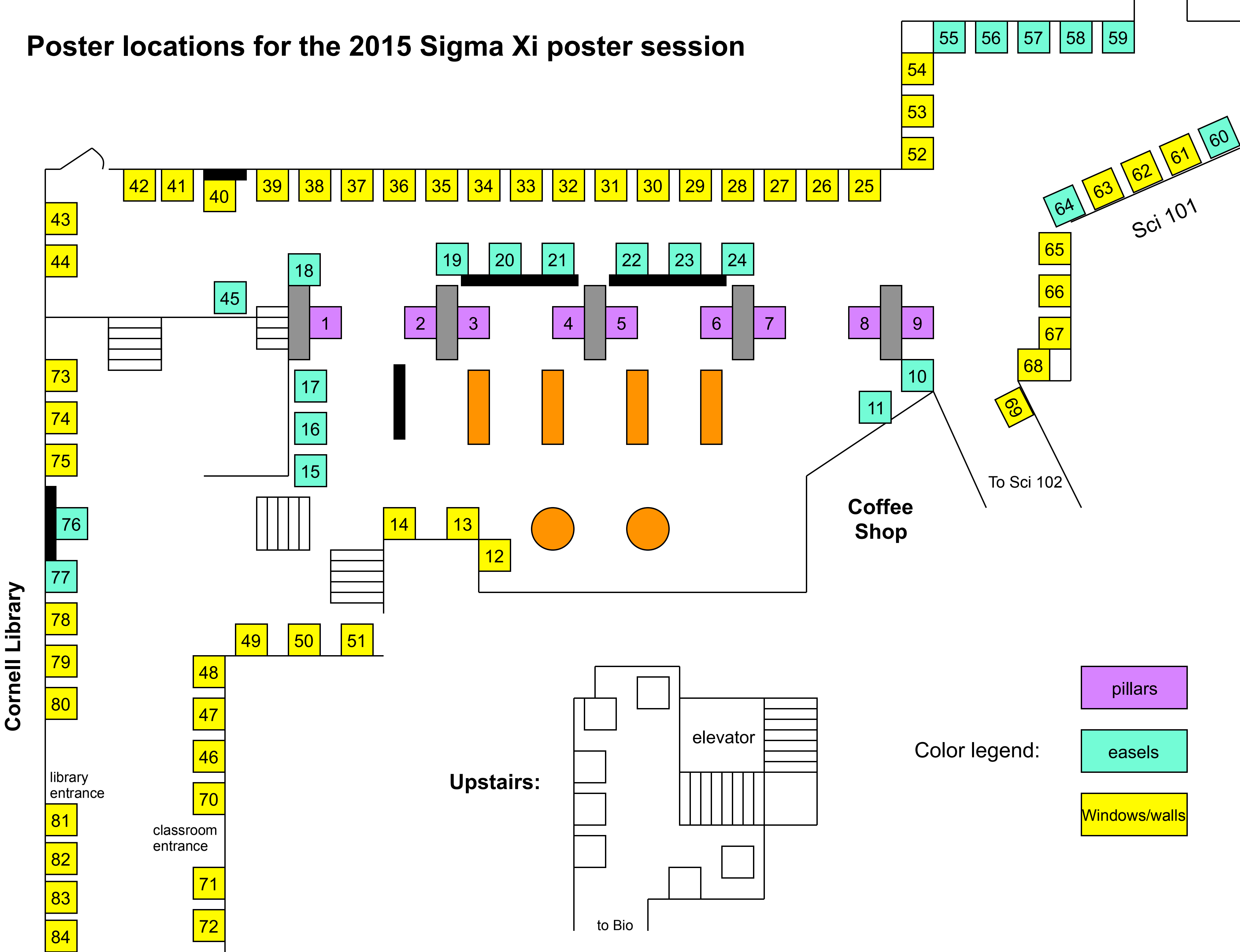

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Propranolol as a Potential Adjunct to Exposure Therapy in the Treatment of Pathological Fear Memory: An Animal Model

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Clinical studies have shown that a therapeutic procedure known as exposure therapy—the gradual exposure to a feared object in the absence of actual harm—is effective in reducing abnormal fear memory. The treatment, however, is limited: over half of the patients who undergo exposure therapy do not benefit and those who do often relapse.

Using an analogue of exposure therapy in laboratory animals—referred to as extinction—we investigated whether the effectiveness of extinction in reducing retention of fear (and by inference exposure therapy in humans) could be augmented by drugs. We chose the drug propranolol (a beta-adrenergic blocker) as a potential adjunct to exposure therapy for 2 reasons: 1) from a clinical perspective it is used to treat memory-related anxiety disorders such as post-traumatic stress disorder (PTSD) and 2) from an experimental perspective its neurochemical and neuroanatomical effects are well defined. What remained to be determined—and defined the purpose of the present study—is whether propranolol administered in combination with extinction is more effective in reducing retention of fear than either the extinction procedure or propranolol administered alone.

We used a modified extinction procedure in which laboratory rats, following fear conditioning, were returned to the apparatus for 60 sec in the absence of shock. In contrast to the typical procedure, the animals were injected 20 min prior to extinction—the time interval required for a drug administered systemically to reach the brain—with either propranolol (10 mg/kg, ip) or saline. Control animals received propranolol or saline in the absence of extinction. A retention test to assess fear memory was administered the next day. Fear was measured in terms of freezing behavior both during the extinction procedure (60 sec exposure) and the subsequent retention test.

The results were surprising. Propranolol administered prior to extinction not only failed to reduce retention of fear but rather created a state of fear during the subsequent retention test far more intense than that which existed with either treatment alone. In short, propranolol made the extinction procedure, which at best is moderately effective, worse.

In light of the preliminary nature of the experiment it would be premature to draw any conclusions regarding the effectiveness of propranolol (or more accurately, its ineffectiveness) as a potential adjunct to exposure therapy in the treatment of pathological fear memory until a more complete dose-response study is conducted. This defines the purpose of our current research.

[1] All co-authors contributed equally to the project. Supported by a grant from Swarthmore College.

Application of the Unscented Transform to Estimate Information Content of HIV-1 2-LTR Model Multivariate Distributions

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Abstract

In modeling HIV-1 dynamics, the formation of 2-LTR circles has been of interest to many scientists. In this study, the Unscented Transform (UT) is tested to estimate information content of multivariate distributions from a previously validated HIV-1 2-LTR model [1]. In practice, the model contains 5 random variables, but for the purpose of this study, a simpler 2-dimensional model is investigated.

Sensor limits of qPCR assay pose difficulties in using the UT to choose sigma points representative of the entire distribution, (for an N-variate distribution, the sigma points form a set of 2N+1 points such that the mean and covariance of this set is equal to that of the original multivariate distribution) so optimal point rotation methods are investigated.

The UT shows promise in estimating information content by preserving the optimal ordering of 2-LTR sample collection schedules, when compared to completely stochastic sampling from the underlying multivariate distributions. However, a drawback of this method is that, with point preservation, we lose information regarding the geometry of the qPCR sensored region, but given that the end goal of this project is to determine an optimal 2-LTR sampling schedule, we will mainly be concerned with patients whose 2-LTR levels lie above the qPCR sensor limit.

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Investigating the Function of the Bacterial Protein LsrE from *Salmonella Typhimurium*

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Abstract

Several species of bacteria produce and respond to small signal molecules termed autoinducers. With this process, called quorum sensing, bacteria can coordinate gene expression within a population. Behaviors regulated by autoinducers include biofilm formation, production of virulence factors, and more[1]. One kind of autoinducer, autoinducer-2 (AI-2, also referred to as DPD), is unique because multiple species of bacteria produce, recognize, and respond to it[2].

LsrE is a protein in the LuxS regulated AI-2 processing pathway of *Salmonella typhimurium,* encoded by the lsrACDBFGE operon[3]. Currently, it is the only protein in the operon whose function is unknown. Sequence analysis suggests that LsrE is homologous to genes that encode epimerases[3,4]. Epimerases are proteins that invert stereochemistry of molecules[5]. Specifically, many of the proteins that LsrE resembles catalyze the inversion of stereochemistry of phosphorylated sugars[4]. Based on the relation to other epimerases and the fact that LsrE is in the same operon as other proteins that act on phosphorylated AI-2 (P-DPD), we began by trying to see if LsrE acts on P-DPD. We conducted ^{1}H Nuclear Resonance Spectroscopy (NMR) experiments as a precise way to try to see subtle changes in molecular structure of P-DPD. This technique was utilized in previous publications [6,8].

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Investigating the effects of invasive species on leaf litter decomposition in conspecific and heterospecific canopies

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Abstract

Invasive plants can threaten ecosystems by altering decomposer communities in adjacent soil[1]. We studied this phenomenon in invasive Norway Maple (*Acer platanoides*) among native Sugar Maple (*Acer saccharum*) trees to examine the effect of invasive Norway Maple on leaf litter decomposition. We aimed to find out which hypothesis, the Home Field Advantage (HFA) hypothesis or the Substrate Quality - Matrix Quality Interaction (SMI) hypothesis, best describes how the canopy species affects leaf litter decomposition[2,3]. We performed a reciprocal transplant decomposition experiment in the Crum Woods, from which we quantified decomposition by the mass lost in the given time; we performed fiber analyses on the leaves from the transplant to characterize the amount of cell solubles, lignin, hemicellulose, and cellulose contents.

We found no significant difference in mass loss during decomposition for each of the five leaf litter combinations, regardless of the canopy under which they decomposed. The fiber analyses showed that the proportions of cell solubles were not significant between the five litter combinations. LC5 had the highest proportion of lignin, and this proportion was significantly different that LC1.

Whether our data supports the HFA or the SMI hypothesis is unclear at this point in time. Our data collection and interpretation will continue into the next several months, as this summer research endeavor was just the beginning of a two-year long experiment. It is difficult to predict in specific ways the effect of invasive trees on native leaf decomposition at such an early stage of decomposition. We hope that data in the next two years will provide more conclusive results on mass loss and the chemical content of this litter, both of which will provide a fuller picture of the decomposition process.

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Communication deficits in a 16p11.2del mouse model of autism spectrum disorder (ASD)

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Abstract

Autism spectrum disorder (ASD) is characterized by deficits in social interaction and communication, as well as repetitive or restricted behaviors and interests. ASD affects ~5 times as many males as females. The 16p11.2 deletion is the most common copy number variant (CNV) associated with ASDs, accounting for ~ 1% of affected individuals, and results in delays in speech and development as well as intellectual impairment (Yang et al., 2015). This genotype has been modeled in 16p11.2del mice containing a 0.39 Mb deletion of a conserved region on chromosome 7. Here, we investigate whether the mouse model exhibits deficits in communication similar to patients affected by 16p11.2-associated ASD.

Ultrasonic vocalizations (USVs) have been shown to be associated with pup-mother communication as well as male sexual behavior and have been used as a model of communication among mice, especially in the study of neurodevelopmental disorders (Scattoni, Crawley, and Ricceri, 2009). Yang et al. (2015) have shown that young adult male 16p11.2 deleted mice have decreased USVs in the presence of a novel estrous female, providing a model for ASD's core symptom of decreased social communication.

We sought to investigate whether communication deficits would be present at an earlier developmental age in 16p11.2del mice. We recorded USVs of 16p11.2del wild-type (Wt) and deleted (Del) pups at various infantile ages by separating them from their mothers for 5 minutes. Preliminary data indicated most robust differences at postnatal day 5 (PND5). At this specific timepoint, PND5 Del males had a significant decrease in vocalization rate compared to male Wt littermates, while there was no difference among females. Del males also had a significant increase in the percent of short vocalizations and a significant decrease in the percent of complex and one-jump vocalizations compared to Wt males. These Del-male specific communication deficits are consistent with both the male prevalence and communication deficits of patients affected by ASD. Future directions include determining the biological basis and the developmental trajectory of these communication deficits in PND5 Del male pups.

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Optimizing dynamic mate choice testing in grey tree frogs

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Abstract

Female *Hyla versicolor* localize sound to find and choose a male based on the characteristics of his breeding call. The goal of this study was to answer the question, ‘Does female reproductive condition influence mate choice?’ However, before this question could be fully explored, we had to develop a methodology that maximized responsiveness during phonotaxis testing. Amplectant and unamplectant female frogs were collected from Glassboro Fish and Wildlife Management Center, New Jersey. Females collected in amplexus were more responsive, completing significantly more trials than those that were collected out of amplexus. Prior to testing, females were either iced or left un-iced. The icing procedure is known to pause clutch development and experimentally lengthen the reproductively motivated period, thereby allowing testing at later hours. Responsiveness, latency to choice, and total path length to choice were not influenced by icing prior to testing, suggesting that icing females does not influence their behavior. In addition, night time testing was shown to increase responsiveness. Behavior was tested using a dynamic two-choice testing paradigm: different stimuli were played from opposite sides of a chamber, and were switched when the female crossed a decision boundary. A greater disparity between the attractiveness of the stimuli used increased reversal rate. In future dynamic two-choice phonotaxis tests, we should test amplectant females at night, ice females if needed, and use stimuli with at least a 10-pulse disparity. Further exploration and experimentation is required to develop a reliable methodology for encouraging pairs to return to amplexus and to monitor oviposition subsequent to testing.

How does reproductive state influence mate choice flexibility in grey tree frogs?

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Abstract

During a mating season, female *Hyla versicolor* localize males via phonotaxis and select mates based on the characteristics of their breeding call. We sought to answer whether females vary in the extent to which they alter their mating decisions in a dynamic calling environment and if so, what factors explain these individual differences. We formulated two hypotheses: 1) females that have invested more in developing a big egg clutch are less committed to their initial choice and more likely to reverse and seek another male; 2) alternatively, females closer to oviposition are more committed to their initial mate choice and less likely to reverse to find an alternative male, as doing so may jeopardize chances of her eggs getting fertilized. We collected amplectant *Hyla versicolor* pairs from Glassboro Fish and Wildlife Management Center, New Jersey. To determine individual females' degree of committedness in mate decision, we carried out dynamic two-choice phonotaxis testing with field-caught, gravid females. Following phonotaxis testing, we characterized individual females' reproductive condition by calculating the following biometrics: latency to oviposition, clutch mass, and residual body mass. We found that females that made greater reproductive investment (i.e. greater clutch mass) tended to be less committed, supporting our first hypothesis. Interestingly, we found that females closer to oviposition are not necessarily more committed, but are more motivated to mate as indicated by shorter latency to make a choice.

Assessment of Quasi-Palindrome Mutation Rates in *E. coli*

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Abstract

Quasi-palindromes (QPs) are almost perfect inverted repeats of DNA that form a hairpin and are capable of stalling DNA polymerase. The stalling of the DNA polymerase could cause template switch events and mutations, which are implicated in a wide range of human diseases including cancer and muscular dystrophy. Our goal is to quantify the mutation rate of QPs in *E. coli*. The Lovett Lab has created a QP reporter in *E. coli* for the leading and lagging strands by inserting four base pairs into the *lacZ* gene, which makes the strain *lacZ*$^-$. When a QP mutation occurs the strain becomes *lacZ*$^+$ and is identifiable by its blue phenotype on LacMinXI plates. Our strains were treated with 5-azacytidine, an FDA approved cancer drug that stimulates polymerase stalling, for a more robust mutational response. Wild type strains as well as strains lacking the SOS response protein RecA were used in the mutation rate assay. Our results indicate the leading strand has a higher mutation rate than the lagging strand and that 5-azacytidine stimulates further mutations in both the wild type and mutant strains. In the near future the Lovett Lab will begin to quantify the mutation rate in yeast to better understand quasi-palindrome mutation avoidance mechanisms in eukaryotes.

Non-adaptive and Memoryless Bounds for Dynamic Predecessor

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Abstract

Many important problems in theoretical computer science are known as data structure problems—the goal of these problems is to store data in a format which allows users to quickly respond to queries about it. In a dynamic data structure problem, the data structure must also be able quickly change in response to update requests. One well-studied dynamic data structure problem is Dynamic Predecessor, in which the data stored is a subset S of the integers [1, ... , m]. Updates insert and delete integers in the subset, and queries of the form query(x) must return the largest element in S that is less than or equal to x. In our research, we examined lower and upper bounds for Dynamic Predecessor in two restrictive models of computation: one with non-adaptive query and update algorithms, and one with memoryless query and update algorithms. We produced the following three theorems:

Theorem 0.1. If $w = \log(m)$, Dynamic Predecessor can be solved non-adaptively with query time $t_q = \log(m)$ and update time $t_u = 2\log(m)$.
Theorem 0.2. Any solution to Dynamic Predecessor with non-adaptive queries and non-adaptive, memoryless updates must have query time asymptotically greater than or equal to $t_q = m/w$, regardless of preprocessing time or space usage.
Theorem 0.3. Any non-adaptive solution to Dynamic Predecessor with update time t_u and query time t_q must satisfy $t_q > \log(m)/(3*\log(t_u * w))$.

Our upper bound for non-adaptive Dynamic Predecessor in theorem 0.1 is useful, as it shows how non-adaptivity can still allow a somewhat complex problem to be solved in logarithmic time. In contrast, our lower bounds in theorems 0.2 and 0.3 are significant because they are exponentially higher than Beam and Fiche's lower bound for the general case. This difference demonstrates how imposing a simple restriction on computation such as memoryless-ness or adaptivity can result in a very large loss of algorithm efficiency. Additionally, in proving our result in theorem 0.3, we discovered a method for finding lower bounds for algorithms that are non-adaptive but not memoryless. No such methods were previously known.

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Optimizing the Detergent Mediated Reconstitution of M2FL into Large Unilamellar Vesicles Using Dynamic Light Scattering and Fluorescence Correlation Spectroscopy

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Abstract

The Influenza A virus' M2 protein has been shown to induce curvature in Giant Unilamellar Vesicles (GUVs) and is thought to play an important role in viral budding. This function could serve as a useful assay for scientists studying M2 if a reliable way to incorporate it into GUVs can be found. Improving our understanding of M2 could enable production of antivirals that could prevent the spread of the Influenza A virus. The goal of my research this summer was to develop and optimize a protocol for incorporating M2FL into GUVs by first reconstituting it into LUVs and then electroforming the LUVs into GUVs.

A prior Swarthmore student, Tae Kim '14, started this project during his time at Swarthmore. While he was able to find a way to reliably transform proteo-LUVs into proteo-GUVs, he was unable complete the optimization of the reconstitution of the protein into LUVs, and had unanswered questions regarding the effect of the reconstitution on the size distribution of the LUVs. That is where my research began: optimizing the detergent mediated reconstitution of M2 into LUVs.

I used both dynamic light scattering (DLS) and fluorescence correlation spectroscopy (FCS) to optimize the reconstitution process. DLS allowed me to construct a phase diagram to find the point where the LUVs were saturated with detergent. This enabled me to optimize amount of detergent added during the reconstitution such that the LUVs were sufficiently destabilized but the formation of lipid-detergent micelles was minimized. DLS also allowed me to characterize the average diameter and size distribution of LUVs throughout the reconstitution process, which put to rest concerns Tae had held about the effect of detergent removal on the size distribution of LUVs.

While I had hoped to use FCS to track the removal of detergent by monitoring the presence of lipid-detergent micelles in solution, I was unable to reliably curve fit FCS data to yield a result consistent with chemical analysis. Despite several attempts to optimize curve fitting procedure, sample volume, dye concentration, and objective used, no clear trend in the fraction of micelles detected by FCS was established. This suggests that the mathematical model for FCS has too many free variables to effectively model the removal of detergent. Our results from DLS, however, were enough to optimize a low-detergent protocol for the reconstitution of M2 into LUVs. An EPR spectrum of our final product indicated successful reconstitution.

Characterization of Syntaxin Function in *Drosophila* Terminal Cell

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Abstract

Most biological tubes require cell-cell junctions. However, there are also seamless tubes which exist without the use of junctions. Terminal cells in *Drosophila* are highly branched cells of the tracheal system that form seamless tubes and deliver gas to internal tissues. It has been shown that vesicle trafficking is a major component in forming this internal lumenal space (1). Syntaxins are a class of target SNARE proteins that aid in the docking and fusion of vesicles to their target membranes. In this study, Syntaxins 1A, 5, and 18 were knocked down via RNA interference and the resultant phenotypes were characterized. Syntaxin 1A localizes to the plasma membrane, Syntaxin 5 to the cis-Golgi complex, and Syntaxin 18 to the endoplasmic reticulum. Knockdowns of these three Syntaxins results in reduced terminal cell branching and cell size. In addition, cytoplasmic vacuoles appear in the cell soma for Syntaxin 5 and 18 knockdowns. Although the cell morphology is altered in Syntaxin 18 knockdowns, the microtubule network appears wild-type. Syntaxin 1A oxer-expression also leads to a severe reduction in branching and cell size. We hypothesized that the secretory pathway in these cells would be disrupted when one of these Syntaxins is knocked down, and indeed, Golgi and ER markers in the terminal cell appear disrupted. Finally, overexpression of Sec31, a component of the Cop II coat complex leads to reduced branching and abnormal cellular morphology.

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Exploring alternative vertical growing media for application in edible plant production

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Abstract

Living walls, also known as green walls, are a bioengineering solution to solve or reduce some of the problems facing urban residents. Living walls are vertical structures (either independently supported or attached to existing conventional walls) with plants growing in a medium attached to the wall surface. The benefits of incorporating living plants into a built setting include pollution amelioration, aesthetic improvements, interior air quality improvements, therapeutic benefits, improved interior temperature regulation, reduction of the urban heat island effect through heat absorption, reduction of urban flooding through runoff absorption and reduced energy costs. There are three major green wall types – façade green walls, hydroponic walls and growing medium based walls. As green walls continue to increase in popularity, technology has improved. However, there are still very few successful indoor walls with edible plants.

My research was focused specifically on interior living walls. I conducted research by reading existing literature, by visiting existing walls and experts and by designing and running basic experiments. Initially, I began research with the intent to design a preliminary green wall for the new BEP (biology, engineering and psychology) building at Swarthmore. However, due to project delays, this became infeasible and I focused more on general applications and designs, particularly focusing my energy on growing edibles vertically.

I designed an experiment using a variety of sponges and quick-germinating edibles in which I concluded that natural cellulose sponges make the best short-term vertical growing medium of the materials I tested.

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Community Health Workers in Lesotho: Usage of Mobile Phones Provided for Health Work

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Abstract

The aim of this research was to explore the ways community health workers (CHWs) in Lesotho interact with mobile phones provided to them through the Bophelo Haeso project. The CHWs supplement the traditional health infrastructure of localized clinics serving surrounding areas by providing health education and basic care within each village. Often, the semi-literate CHWs forget their trainings, prompting the current system. Now, local nurses create multimedia content that is then loaded onto smartphones given to each CHW working at two rural clinics.

We first investigate CHWs' interactions with the provided health care videos through an examination of the pausing and playing activity. Secondly, we observe how CHWs used the mobile phones outside of the health care app, for non-prescribed usage. We find that while engagement and impact seem to be universal, interaction with the videos depends on three main factors: content, supervision style, and CHW personality. Notably, the CHWs demonstrated three personality types with regards to the frequency of playing and pausing. Furthermore, the behavior over time decreased in terms of video viewing but increased when considering non-prescribed uses. These findings portray smartphone usage for work and personal purposes by rural CHWs and the potential influencing factors for these trends.

Differential Expression Profiles for the Heat Shock Response in Arabidopsis

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Abstract

Temperature elevation above a normal physiological range results in protein denaturation in most eukaryotic organisms. To combat the deleterious effects of heat shock (HS), all organisms including plants have evolved a complex network of cellular-stress responses, including the expression of heat shock proteins (HSPs) and heat shock factors (HSFs). Collectively, this transcriptional response is known as the Heat Shock Response (HSR) (Qiao et al 2015). My research this summer focused on elucidating transcriptional phenotypes in the HSR of *Arabidopsis thaliana*.

Characterization of the HSR in Arabidopsis led to the isolation of *eg6,* a mutant in a small spliceosomal protein. *eg6* shows elevated expression and delayed attenuation of *Hsp17.6* expression in response to a HS. To identify whether the abnormal expression profile extends to other HSPs/HSFs in *eg6,* expression levels of genes upregulated in response to HS were compared in wild type (WT) and *eg6.* Expression data were generated by reverse-transcriptase polymerase chain reaction (RT-PCR). The genes tested showed higher expression levels in *eg6* than in WT. This suggests that *eg6*'s abnormal *Hsp17.6* expression profile is a reporter for other abnormal expression profiles in *eg6.*

HSFA2, a HSF required for basal and acquired thermotolerance in Arabidopsis, was selected as a candidate gene to study the effects of *eg6* on alternative splicing (AS) in Arabidopsis. There are three splice variants (protein isoforms) of *HSFA2* and they are expressed in a temperature-dependent manner (Liu et al 2013). We isolated a fourth, temperature-dependent, intron-retaining HSFA2 isoform using RT-PCR and characterized the abundance of each isoform in *eg6* and WT.

To further understand how temperature dependent AS is regulated by *EG6,* expression phenotypes for spliceosome subunits were also analyzed by RT-PCR (Palusa et al 2007). Preliminary studies show differential splicing of the genes selected across a variety of temperatures (22º, 37º, 40º, 43º, and 46º C). The expression phenotypes were different between *eg6* and WT across multiple temperatures using RT-PCR. *eg6* expression profiles resemble those of WT at higher temperatures. When taken together, *HSFA2* and spliceosome subunit splicing data suggest *eg6* plants think they are hotter than they actually are.

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Bumblebees vary landing strategy in the presence of wind

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Landing is a critical component of insect flight. In nectivorous insects such as bumblebees (*Bombus impatiens*), landing must be performed hundreds to thousands of times daily, and thus represents a key component of foraging behavior. Studies in *Drosophila* and Honeybees (*Apis mellifera*) have demonstrated that the visual system helps trigger preparatory landing behaviors, such as deceleration and leg extension (van Breugel and Dickinson 2012; Evangelista et. al. 2009; Chahl et. al. 2004), and controls flight speed (Fuller et. al. 2014). In particular, recent advances investigating the visual mechanisms of this behavior suggest that landing is regulated by a simple, yet robust, universal strategy whereby rate of image expansion is held constant to regulate flight speed during the approach (Baird et al. 2013). However, to our knowledge there has been no research investigating whether insects use this same strategy in the presence of wind, a ubiquitous challenge for insects flying in natural environments. In this study, we investigate the landing behavior of bumblebees in the presence and absence of wind, when landing on flowers that were aligned with or perpendicular to the flow. We filmed bees approaching and landing on real flowers in a wind tunnel using high-speed videography, and tracked body kinematics using a modified version of BEEtag, an open-source image-based tracking system (Crall et. al. 2015). Our results show that bees' approach paths to flowers vary highly in still air, but become more restricted in the presence of wind, irrespective of flower orientation. In still air, we also observe that flight speed declines gradually as the target is approached, as predicted by previous studies. However, in the presence of wind, flight speed remains constant throughout the approach and landing phases, peak acceleration is higher at touchdown, and body stability is reduced. These findings suggest that wind has a strong influence on landing strategies in insects, with important implications for both the design of flying micro aerial vehicles (MAVs) and the ecomechanics of insect flight in natural environments.

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Secondary Structure Investigation of (CAGAGG)n Repeats

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Abstract

Induced replication stress slows down DNA replication in various sites in mouse fibroblast cells. As ATR pathway (a protein kinase that stabilizes stalled forks) is inhibited simultaneously, the stalled forks collapse. Whole genome sequencing identified these fork-collapse break sites, and the repeat of (CAGAGG)n appeared in high frequency.

We hypothesize that (CAGAGG)n repeats are able to form secondary structures that obstruct replication fork progression. The hypothesis was tested using UV-vis, circular dichroism, HNMR spectroscopy, gel electrophoresis and crystallography studies.

Using HNMR information, we proposed a secondary structure that has a cubic core with alternating cytosines and guanines at each vertex connected with AGAG loops. We altered the number of repeats ranging from 4-15, and take off 1-6 nucleotides on both ends to create different variations of CAGAGG. Spectroscopic studies showed that oligos with over (>=) 5 repeats share similar CD signature of a positive peak around 265nm and a negative peak at 240nm as well as higher melting temperature generally above 48 C. For oligos with less than 5 repeats, no exact melting temperature was obtained, indicating their lack of structural stability. This data fits our "box" model as these shorter oligos lack the cytosine at 3' and/or guanine at 5' that are essential to core structure formation.

Gel electrophoresis was performed to help determine what oligos would be ideal for crystallization. Oligos that give a single band in electrophoresis were chosen for crystallization. After broad screenings, the condition of 0.05 M HEPES pH6.5, 0.005 M spermine, 0.91% (v/v) MPD yield the largest crystals that are suitable for diffraction. Hopefully, the diffraction pattern will give us more insights on the structure information of CAGAGG repeats.

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Will be included on the poster

Detecting rare and common germline variants associated with patient survival in bladder cancer using MSK-IMPACT panel

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MSK-IMPACT is a targeted sequencing panel designed to capture genetic aberrations in clinically actionable genes, and has been utilized in the search for somatic mutations in a wide range of solid tumors. Bladder cancer is the fifth most common cancer in the United States, and approximately 5% of cases present with high-grade tumors, which are associated with a worse prognosis and a higher rate of recurrence. A recent study by Kim, et al., applied the IMPACT panel to a cohort of 109 high-grade urothelial carcinomas of the bladder in order to identify recurrent somatic variants associated with patient survival. This study reported alterations in phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) and its pathways to be associated with more favorable outcomes. PIK3CA is a kinase crucial to a number of signaling pathways and implicated in a variety of cancers.

In light of the somatic IMPACT results, the Clinical Genetics Research Lab (CGRL) sought to determine whether germline variants affect oncologic outcomes. We will test this hypothesis first by investigating germline variants in PIK3CA and further expand to other genes represented on the IMPACT panel. Using the CGRL germline analysis pipeline, sequencing data from the IMPACT panel was jointly recalled to identify germline variants, specifically common variants and rare, recurrent truncating mutations. Within the germline consented group of 93 bladder cancer patients (high-grade bladder cancer n=83), rs2230461, a single nucleotide polymorphism (SNP) within PIK3CA, was observed with a minor allele frequency = 0.1. Association with oncologic and overall survival is being performed currently using log-rank tests and hazard ratios are being estimated by Cox regression analysis. The findings from the discovery phase will be confirmed in a separate validation cohort of 750 high-grade bladder patients. Knowledge of germline factors associated with survival may help assist clinicians in tailoring screening and treatment procedures for at-risk populations.

Citation: Kim, P.H., et al. Genomic Predictors of Survival in Patients with High-grade Urothelial Carcinoma of the Bladder. EUROPEAN UROLOGY 67 (2015) 198–201.

Inferring Primary Extinction Levels In Late Permian Food Webs Using Approximate Bayesian Computation

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Abstract

The end-Permian extinction was the most severe mass extinction of the Phanerozoic, yet its causes are not well understood. Here we use probabilistic food web models to explore how disruption of primary production could have caused the collapse of end-Permian terrestrial ecosystems. First, we simulate food webs (trophic networks) reconstructed for the Late Permian Dicynodon Assemblage Zone community of the Karoo Basin. Next, we perturb combinations of guilds in these food webs using simulated extinctions of taxa at varying levels of intensity. This probabilistic forward model allows us to estimate the effects of such perturbations on terrestrial communities.

We then use Approximate Bayesian Computation Sequential Monte Carlo (ABC SMC) techniques to solve the inverse problem: namely, inferring the level of perturbation responsible for the Permian extinction, as well as the pattern of extinction among guilds. ABC SMC works by randomly sampling perturbation values from a prior distribution and keeping only those that result in output similar to the observed data. This process is then iterated to sequentially narrow the range of plausible perturbations, thereby arriving at the posterior distribution of perturbation levels. Unlike other methods such as MCMC, ABC SMC does not require calculating the likelihood function, which makes it applicable in a wide range of problems.

Analyzing Reduction Benefits of Green Stormwater Infrastructure

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Abstract

Combined sewer overflows (CSOs) produce an overwhelming negative environmental impact in the City of Philadelphia to this day. Philadelphia's Green City Clean Waters is an unprecedented program to reduce the prevalence of these recurring CSOs, thus diminishing their environmental impact. Through large-scale, citywide implementation of green stormwater infrastructure (GSI), environmental and community benefits are to be accomplished over a 25-year period. EPA's Storm Water Management Model (SWMM) is used to analyze watershed runoff volume and sewer-shed outflow volume. Reductions in these values are examined when Low Impact Developments (LIDs, synonymous to GSI) are introduced into a fictitious SWMM watershed. These results will aid in making geographical and demographic decisions in the location of various types of GSI.

Green stormwater infrastructure is an alternative to "grey" solutions to CSOs, which capture sewer overflow at the output and treat it to the point where it can be released into the environment. GSI instead mimics the natural water cycle to reduce inflow to storm sewers at the source. Capturing runoff from pervious surfaces (roofs, streets, sidewalks, parking lots, etc.), GSI allows stormwater to gradually infiltrate into and saturate the ground, or store it and slowly release it to diminish the intensity of flow to storm sewers. If large-scale implementation of GSI can reduce the overall stormwater inflow into combined sewer systems sufficiently so that the outflow does not bypass the overflow weir, then all wastewater will be intercepted to a treatment plant. Thus, GSI can provide a solution to CSOs.

As parameters (width, length, depth, perviousness) and quantity of GSI increases, the total outflow runoff volume in sewers to the treated watershed will decrease. This leads to a positive trend in outflow benefits with augmentation of green infrastructure. However, with further increase in their parameters and quantities, a maximum benefit is reached. At this point, no matter how much additional resources are put into increasing aspects of GSI, the outflow benefits will not increase. By altering parameters using the SWMM graphical user interface and integrating the SWMM engine with python code, trends in runoff volume reduction can be observed and eventually fit to a function to find the maximum possible outflow benefits. The fictitious subwatershed created for the SWMM Applications Manual (Gironas, et al., 2009) is an effective model that can be altered to view these trends.

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Folk taxonomies, species naming and linguistic diversity: An ethnobiological approach to Micronesian fauna

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Understanding the connections that indigenous people draw between themselves and their environments is an important step in preserving ancient ways of life and understanding current human-environment interactions. It is made even more crucial by the endangerment of many indigenous languages and the loss of traditional environmental knowledge (TEK) that their extinction would represent. To that end, a survey of Micronesian fauna from the island of Pohnpei was collected via hand collection, photography and GoPro technology. Local consultants of Kapinga, an endangered language spoken on Pohnpei and Kapingamarangi atoll, worked with field linguists and biologists to identify specimens by both scientific binomials and traditional Kapinga names. Additional efforts were made to locate the organisms in the Kapinga animal folk taxonomy which resulted in a direct correlation between the perceived importance of each taxon to the Kapingese and the complexity of that taxon's folk taxonomy. Notably, the taxonomy for fish (which includes marine fishes, various marine invertebrates and whales) contained over twice the number of categories as the taxonomies for reptiles, birds and mammals combined. Interestingly, several Kapinga compound species' names serve to link taxa. The Kapinga name for 'rat', for instance, suggests that the animal is a meshing of two taxonomies – a combination of a reptile and a mammal. Similar names among all four studied taxa reveal that the Kapinga view their natural fauna not as separate taxonomies but as a single biological continuum. This research provides an example of an indigenous group using a technology to organize their world and resulting in a continuum rather similar to modern phyologenetics.

A Virtual Vocal Tract: A Novel Approach to Articulatory Speech Synthesis

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Speech synthesis is the artificial production of human speech. State-of-the art synthesizers are functional, but they fail to achieve the wide variety of sounds that humans can naturally produce. They rely on pre-recorded speech, making them accurate but inflexible. In contrast, articulatory synthesizers (i.e. virtual vocal tracts) produce speech like humans do, and are flexible, controllable, and refigurable. The purpose of this project was to build an articulatory speech synthesizer capable of producing all English phonemes. Additionally, our purpose was to validate a five-region model of vocal tract shape comprising only five concatenated tubes, proposed by Lammert (2014). We targeted articulatory and acoustic properties of each English phoneme: our program takes shape estimates based on real time MRI data and systematically adds variation to each of the five constriction values (Narayanan et al, 2014). It then calculates the resultant formant frequencies (peaks in the speech spectrum that are particular to the phoneme) and finds the best constriction values. With this method, we successfully produced a full set of English vowels, diphthongs, most approximants, and certain stop consonants. Furthermore, we learned that synthesis is more realistic with context-dependent, nonlinear transitions between phoneme shapes than with linear transitions. In future work, we hope to synthesize the remainder of the English phonemes and further validate the five-region model of articulatory synthesis.

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Sex but not social position predicts immune function in *Bolitotherus cornutus*

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Abstract

The immune function of an organism can be affected by factors ranging from genotype and sex to an individual's social environment. While position in vertebrate dominance hierarchies has been shown to affect immune function, more complex positions in social networks have been less studied (Bernard et al., 1998; Hawley et al., 2007). This study aimed to determine whether an individual's position in a social network, as measured by number of social partners, strength, and clustering coefficient, predicted phenoloxidase activity in *Bolitotherus cornutus,* a highly social mycophagous beetle. Immune function was quantified with a filter paper assay for phenoloxidase activity. While our results showed no relationship between PO activity and any of these social measures on immune function, sex strongly predicted PO activity, with males showing higher immune function than females. Our results suggest that immune function is a sexually dimorphic trait in which differing trade-offs or immunological pressures lead males to develop stronger immune responses than females.

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Characterizing the effects of following on pedestrian dynamics: Entropy

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Abstract

The study of pedestrian dynamics by modeling people as self-propelled particles is a subset of the field of jamming. Broadly, jamming concerns many-particle systems, particularly as particles come close together, and in some cases form rigid jams where particle motion is impossible. Pedestrian dynamics specifically are relevant to efficient building design, evacuation preparation, and crowd tracking and management.

Our simulation was based largely on the Social Force Model1 where social considerations, such as avoiding other pedestrians, are modeled as physical forces. Our experimental setup was a narrow hallway in which two groups of pedestrians, modeled as disks, tried to move in opposite directions. This condition of "bi-directional" flow is practically relevant and theoretically interesting.

The narrow goal of our research was to investigate how the tendency of people to follow one another affects their efficient travel to a destination. Thus, the most important variable in our research was the follow factor, which is a number between zero and one that controls how likely a pedestrian is to move towards the exit versus follow other nearby pedestrians. When the follow factor is zero, pedestrians only try to move toward the exit. When the follow factor is one, the desired direction of each pedestrian is simply the average of the directions of other pedestrians within a specific radius, which is a parameter of the simulation.

We found that when the follow factor is higher for pedestrians moving in one of the two directions, the flow of those pedestrians becomes less efficient. A signature of their less efficient motion is a higher average entropy in terms of the direction of their velocity, lower entropy in terms of their spee. Considering the magnitude and direction of the velocity, lower efficiency corresponds to higher entropy.

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Developing Electroporation as a Research Tool in the Sea Anemone *Aiptasia pallida*

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Abstract

Anthropogenic climate change threatens numerous ecosystems due to rapidly changing environmental conditions. As a result of global warming and increased ocean acidification, severe losses of coral reefs have been documented. Stressful temperatures cause a phenomenon called coral bleaching characterized by the breakdown in the symbiotic relationship between the coral and algae of the genus *Symbiodinium*. The endosymbiotic algae are expelled from the coral, severely weakening the host and potentially leading to death.

The sea anemone *Aiptasia pallida* serves as a model organism in the study of coral bleaching. Similar to coral, *A. pallida* can exist in a symbiotic relationship with *Symbiodinium*, but is also healthy without its endosymbionts, permitting the study of both states. Recently, both the genome and transcriptome of *A. pallida* have been sequenced, allowing researchers to begin exploring the molecular basis of the cnidarian-*Symbiodinium* relationship (Lehnert *et al.*, 2012).

In this study, we attempted to develop electroporation as a tool for studying gene function in *A. pallida*. Electroporation is a common biomolecular research technique in which an organism is exposed to an electrical current in a solution containing DNA. The shock opens temporary pores in the phospholipid bilayer, allowing DNA to rush in. The DNA construct includes a reporter gene, which translates into a protein that indicates if electroporation was successful. The ability to electroporate *A. pallida* would allow us to elucidate the specific function of genes and ultimately manipulate the symbiotic relationship.

We electroporated both adult and larval anemones and discovered that *A. pallida* has prodigious background staining, indicating endogenous enzymatic activity. It was impossible to discern any reporter expression from noise. Our results serve as documentation of this background staining and allow for more confident identification of positive results in the future. It is important to continue developing electroporation as a research technique in *A. pallida* because its successful implementation would greatly potentiate the study of the coral-alga symbiosis. Ultimately, this would enable us to better address the threat of coral bleaching in an increasingly warm world.

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Sleep plasticity due to sexual experience in *Drosophila melanogaster*

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Abstract

Sleep behavior is universal across taxa but the regulatory mechanisms underlying it remain mysterious. Drosophila sleep is plastic, and increasing literature suggests that social conditions are a source of such behavioral plasticity. We specifically examined the way sexual interactions affect Drosophila sleep behavior, at the level of individual mating events and general cohabitation, or socio-sexual interactions (SSI) between males and females.

It was recently demonstrated that female flies experience a post-copulatory reduction in daytime sleep mediated by Sex Peptide (SP) transferred to the female during mating via seminal fluid.[1] After successfully replicating these findings using electronic activity monitors to measure the sleep of individual flies, we discovered that the daytime sleep reduction lasted 6 days post-mating and had no apparent critical period with regard to female age or previous mating experience. We also established that Drosophila mating status has no bearing on circadian rhythmicity. This line of research provides evidence that sexual experience can temporarily modify an organism's sleep behavior and raises interesting questions regarding the mechanism by which SP reduces female daytime sleep.

Social enrichment via increased population density causes flies to consolidate their sleep into a small number of long-duration episodes - an architectural pattern thought to be beneficial for memory formation.[2] Analogously, we found that increased SSI caused a trend toward more consolidated sleep in both male and female flies - a result consistent with the presumably increased memory demands in a cohabitating environment. In another non-significant trend, parental SSI appears to promote less consolidated sleep in offspring. These preliminary findings offer the intriguing possibility that social effects on sleep plasticity may involve epigenetic mechanisms.

These results expand our understanding of the connection between an organism's sexual experience and its subsequent sleep behavior. Due to the neurochemical similarities between Drosophila and mammalian sleep, a biochemical understanding of the molecular basis of these forms of plasticity in Drosophila may provide mechanistic insight about social control of our own sleep patterns.

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Home Network Control Via TCP Modification

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Abstract

As the consumer demand for devices with Internet connection capabilities has grown, the world has seen a dramatic increase in the number of multi-device homes. In such settings, with devices ranging from smart televisions and game consoles to refrigerators and toasters, the need for network capacity begins to exceed the typical home ISP service package. Our work addresses the scarcity of network capacity by intelligently allocating bandwidth according to user preferences in order to achieve maximum quality of service and customer satisfaction within the constraints of the consumer's Internet service plan.

Our solution involved the development of an experimental toolset to allow the user to control their bandwidth allocation on a per-application basis across multiple devices. We provide this functionality by repurposing an existing field of the ubiquitous Transmission Control Protocol (TCP) called the receive window. The receive window's original purpose was to inform the sending device that the receiving device was getting close to exceeding its memory. Today, most devices have enough memory that this field rarely limits transmission rates in practice, and thus the field can be carefully repurposed to *lie* to the sender. In response, the sender will limit its sending to prevent overloading the receiver, which limits the bandwidth that the data transfer requires.

During the course of our research, we improved the project's ability to perform under more realistic testing conditions that more closely reflect a home network environment. The project will now allow for the dynamic identification of traffic from multiple devices and modify the receive window efficiently. Our experiments show that our methods are successful even in when latency and jitter vary significantly.

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Dynamin function is required for FGF receptor polarization prior to heart progenitor induction in *Ciona intestinalis*

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During asymmetric cell divisions, proteins and other molecules become polarized to achieve differential inheritance of these elements by daughter cells. These divisions are critical to development, in which a single cell must give rise to daughter cells with different fates. *Ciona intestinalis* is a simple chordate ideal for studying polarized division involved in heart development. The polarized fate induction in *C. intestinalis* heart progenitors is dependent on an asymmetric division. *C. intestinalis* pre-cardiac founder cells divide asymmetrically to produce a larger anterior tail muscle daughter cell and a smaller heart progenitor daughter cell. Previous research has informed a model in which polarized adhesions lead to a polarized distribution of the receptor tyrosine kinase, Fibroblast Growth Factor Receptor (FGFR), which is required for heart progenitor induction. The trafficking mechanism by which FGFR becomes polarized, however, was not uncovered. Here we show that Dynamin function is required for polarization of both adhesions and FGFR. These results indicate a role for endocytic trafficking in the model described above. They demonstrate that adhesive polarization occurs by directed endocytic trafficking. The adhesive distribution then serves as a cue for FGFR localization, possibly via an endocytic pathway. Our results are the beginning of a specific mechanism for directed trafficking of FGFR. They also indicate important roles for directed endocytic trafficking in mitotic cells. We anticipate this model to grow to include a mechanism for both adhesive and FGFR trafficking in mitotic pre-cardiac cells. These results may prove general to the complex trafficking mechanisms underlying the processes of asymmetric divisions in stem cells and other models.

Identifying mechanisms of left-right asymmetry in *Ciona intestinalis*

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Abstract

Correct arrangement and orientation of organs along the left-right axis is vital to organ function in many animals. In general, an initial breakage of symmetry activates asymmetric gene expression, which leads to morphological asymmetries. The signaling pathway *Nodal* is well established as the organizer of left-right asymmetry in all vertebrates and many invertebrates (Tingler et al, 2014). *Nodal* is expressed asymmetrically before morphological asymmetry arises and activates asymmetric expression of *Pitx*, a gene that encodes a transcription factor vital for correct development of morphological left-right asymmetry. However, drug inhibition of *Nodal* did not disrupt correct asymmetry of the heart in *Ciona intestinalis.* Here we show that drug inhibition of *Nodal* using the ALK receptor inhibitor SB431542 prevented expression of *Nodal* in stage 23 embryos. We attempt to identify alternate mechanisms controlling left-right asymmetry in *Ciona*. We found that our inhibition of *Nodal* was successful and screened several signaling pathways to identify additional pathways controlling left-right heart asymmetry. Inhibiting BMP and *Notch* pathways disrupted some morphological asymmetries but did not affect asymmetry in the heart, although further assays are needed to assess the effect of inhibiting the *Notch* pathway. These results suggest that *Nodal* is not the only or even the most important mechanism that creates left-right asymmetry in *Ciona intestinalis*. If this is the case, our findings cast doubt on scientific understanding of left-right asymmetry in chordates.

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A Systematic Review of the Human Milk Microbiota: The Prevalent Two

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Abstract

This systematic review characterizes the human milk microbiota by synthesizing results of existing studies. Relevant primary research studies were identified by conducting a comprehensive search of PubMed through July 2015. The search was restricted to studies conducted among human subjects (specifically, healthy mothers), written in English, describing bacterial diversity of human milk, using culture-independent methods, and reporting results at genus or species level. Of the 479 studies identified, 12 satisfied the inclusion criteria. Qualifying studies varied with respect to geographic location, study design, and milk collection, storage, and analytic methods. The genera *Streptococcus* and *Staphylococcus* were predominant in 6 studies (50%). Four of the 12 studies used next generation sequencing methods, of which all 4 (100%) identified *Streptococcus* and *Staphylococcus* as predominant genera. Next generation sequencing methods may be more sensitive to identify and quantify predominant genera. This suggests the predominance of *Streptococcus* and *Staphylococcus* may be underestimated in studies using older methods. These predominant genera: *Streptococcus* and *Staphylococcus,* the 'prevalent 2', may be universally predominant in the human milk microbiota, regardless of differences observed in study design, geographic location, or data collection methods. The authors conclude these genera, *Streptococcus* and *Staphylococcus*, are the 'prevalent 2' predominant genera in the human milk microbiota.

The role of the Plexin A3 receptor in the vertebrate Hedgehog signaling pathway

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Abstract

The Hedgehog (HH) signaling pathway plays a central role in vertebrate embryonic development and adult tissue maintenance (McMahon et al., 2003) HH signaling abnormalities can result in a range of defects in tissue patterning and formation. Recent findings also implicate abnormal HH signaling in the disease processes of several cancers, including medulloblastoma and basal cell carcinoma (Barakat et al., 2010). Relatively recently, a class of cell surface receptors called Neuropilins were identified as novel positive regulators of HH signaling (Hillman et al., 2011). This was a particularly intriguing finding because Neuropilins have a well-established role in the Semaphorin signaling pathway, which influences axon guidance and angiogenesis. When participating in Semaphorin signaling, Neuropilins interact with a class of co-receptors known as Plexins (Neufeld and Kessler, 2008). While the roles of the nine known Plexin proteins have been investigated in the context of Semaphorin signaling, their role in HH signaling has yet to be explored.

There are nine identified members of the Plexin family of cell surface proteins. These are Plexin A1-4, Plexin B1-3, Plexin C1, and Plexin D1, all of which are classified according to structural differences (Neufeld and Kessler, 2008). Preliminary findings from the Allen Lab at the University of Michigan suggests that Plexins A1 and A2 promote HH signaling in NIH/3T3 mouse embryonic fibroblasts, while Plexins B1 and B2 do not.

This project investigated the effect of Plexin A3 overexpression on Hedgehog signaling in cell culture and in the developing neural tube of chicken embryos. Preliminary data indicate that PlxnA3 promotes HH signaling in NIH/3T3 cells to a degree comparable to that seen in previous assays of Plexins A1 and A2. However, in the chick neural tube Plexin A3 overexpression does not appear to effect dorsal-ventral patterning.

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Purification of *Escherichia coli* C-LsrR and Characterization of DHAP Binding

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Abstract

Autoinducers, are signal molecules that are used for cell-to-cell signaling between bacteria to synchronize gene expression. Altering levels of autoinducer 2 (AI-2), an interspecies autoinducer has been shown to alter the composition of the gut microbiome in complex organisms[1]. Previous research has suggested that dihydroxyacetone-phosphate (DHAP) represses *lsr*, an operon expressed in the presence of AI-2 and required for the import and processing of AI-2[2]. This summer I improved the C-LsrR purification process established by Jason Hua by incorporating an ion exchange separation step to remove the remaining 6xHis-MBP tag. The tag was successfully separated from C-LsrR with the addition of a MonoQ column run at the end of the previous purification procedure.

Purified C-LsrR protein was used to conduct fluorimeter binding assays which were used to measure intrinsic Tyrosine fluorescence of C-LsrR exposed to varying DHAP concentrations. Fluorimeter traces and extracted data were compared to substrates with known binding properties. A sharp drop of fluorescent intensity in the range of ~70-200 μM DHAP in a solution containing about 3 μM C-LsrR suggests that a conformational change occurred in C-LsrR as a result of DHAP binding. Further analysis will look into calculating a binding constant for the protein-substrate interaction as well as crystalizing both C-LsrR and the C-LsrR/DHAP complex to elucidate the binding mechanism. A better understanding of this interaction will allow insight into how DHAP is involved in the negative feedback loop that controls AI-2 synthesis and absorption.

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Using candidate genes from GWAS review to search exome data for rare variants that cause UGI cancers

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Abstract

Gastric cancer (GC) and esophageal cancer (EC) cause more than 700,000 and 400,000 deaths, respectively, each year, and represent the second and sixth leading causes of cancer-related death worldwide. These upper gastrointestinal (UGI) cancers occur in the Taihang Mountains of North-Central China at some of the highest rates reported for any cancer. Risk factors for UGI cancer in this region include family history and dietary deficiencies and suggest a complex interplay of genetic and environmental factors. Several lines of evidence including familial aggregation and segregation analysis support the idea of high genetic risks in some multiple case families. However, since UGI cancer has low survival, it is very challenging to conduct informative linkage analyses. Exome sequencing offers a unique opportunity to search for high-risk UGI susceptibility genes in this population and to examine the evidence for genetic heterogeneity.

Literature review was conducted to find low risk SNPs associated with UGI cancers in genome wide and pathway based association studies. A candidate gene list (n=92) was then created from the genes most closely related to the discovered SNPs. The exomes of 183 UGI cancer patients from Shanxi Province were then searched for rare (in <1% of population) variants in candidate genes. These variants were then analyzed for further evidence of pathogenicity.

Subjects had 197 rare variants in 66 of 92 genes in the candidate gene list. 7 of these genes [with 24 rare variants] (*ADH4, ATP1B2, FUT7, NRG3, SHBG, TERT*, and *WDR65*) are not expressed as mRNA or protein in relevant tissues according to the Protein Atlas. Variants were deemed “of interest” (VOI) if 80% of *in silico* predictors predicted damaging or functional changes to the resultant protein or if the variant type was considered to be loss of function (LOF) as in the case of frameshift or stopgain alterations. Despite some promising suggestions of disease association, more work is needed to determine whether specific variants and/or genes may increase risk for disease. In particular, specific variants in *BRCA2*, *CHEK2*, *CYP3A5*, *CYP1B1*, *FUT5*, *MTRR*, and *RUNX1* are good candidates for genotyping in case-control studies. Most variants in *ALDH7A1, CHEK2, CYP1B1, FUT2, FUT5, GTF2H3, POLM, RUNX1*, and *SERPINB5* were VOI, suggesting that these genes could play a role in UGI carcinogenesis in this population, and should be examined using targeted sequencing methods.

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EPR Studies of a Budding Relevant Conformation of the Influenza A M2 Protein

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Abstract

M2 is a 97-residue protein encoded by the influenza A virus. M2 forms a homotetramer that embeds itself in the viral envelope and various membranes of the host cell to act as an ion channel in viral genomic unpacking and curvature generator in viral budding. The current work studies a conformation of M2 relevant to the protein's viral budding function. We hypothesize that this conformation is favored in high curvature lipid environments rich in cholesterol. CW EPR, Power Saturation EPR, and Dynamic Light Scattering were employed to glean insight into the conformational exchange occurring in lipid environments of varying curvature.

Survival post-infection in an immunodeficient zebrafish model

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Abstract

Here we present a model for a candidate gene causing immunodeficiency in a Palestinian family. In 2010, a 3 years and 10 months old boy was hospitalized due to recurrent pus-producing infections in the brain, lungs and abdominal cavity, suggesting a severe immunodeficiency. Given that he came from a consanguineous family, and other siblings had displayed similar symptoms before passing away, a recessive genetic disease is the most likely explanation for the phenotype. SNP arrays and whole exome sequencing revealed thirteen candidate genes. One gene- AIFM2- stood out in the context of this case, as it is highly expressed in B lymphoblasts and is involved in regulating levels of reactive oxygen species (Gong et al, 2007).

To further understand this gene and the mutation effect, we decided to develop a zebrafish (Danio rerio) model. We utilized the TAL endonuclease system to induce a frameshift mutation in the first exon of the zebrafish AIFM2 gene, effectively creating a mutant knockout line. We modeled septic injury by injecting embryos with fluorescently labeled E.Coli (Benard et al, 2012), and monitored injected embryos every 24 hours, for a total of four days. Movement of these embryos was recorded and quantitated in order to accurately measure survival post infection. Mutant embryos moved significantly less than wildtype embryos across all time points post infection, while uninjected mutant and wildtype embyros showed no significant difference in movement. This result indicates that mutant embryos had a lower survival rate after infection, implying a poorer immune response. Further experimentation, such as extending the monitoring period and varying bacterial load, is needed to conclusively assess the effects of AIFM2 mutations on immune response. This model remains a promising tool for evaluating the candidacy of AIFM2 as a causative gene for human immunodeficiency.

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Designing Software and Walking Algorithms for DARWIN-OP

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Abstract

The DARWIN-OP (Dynamic Anthropomorphic Robot with Intelligence - Open Platform) is a small but powerful humanoid robot is commonly used in research and Robosoccer. To overcome the convoluted and tedious manufacturer code that came with the robot, we wrote a new piece of software with the following goals in mind: reduced structural complexity, increased user readability and usability, separation of hi-level and low-level structures, and top-down design. After finishing the serial port communications, we then built more complicated bulk read, synchronous write, and other methods for controlling the motors and reading from the sensors. We also redesigned a trajectory playback program that was able to play simulation-generated trajectory files on the DARWIN-OP by updating each of the 20 motors for every time step.

When testing various walking algorithms on the DARWIN-OP, we first ran the walking algorithms in a simulation environment. Once the simulation passed, the walk would be safe to test on the robot by running it with the playback program. Past work implemented on the DARWIN-OP included stretched leg walking and Zero Moment Point Preview Control walking. We adapted a version Professor Matt Zucker's Static Walking code for HUBO to be compatible with the DARWIN-OP. With the new software and a lot of fine-tuning, we were able to get a 2cm step length, an improvement from the wobbly 1cm that we got before the new software. Since DARWIN-OP's motors were not strong enough for completely static bipedal motion, we determined that static walking was not suitable for it and opted to investigate quadrupedal walking gaits. The robot was put in a bridge position and two types of quadrupedal walking algorithms were implemented. The first method, in which each arm and leg was moved forwards one at a time, yielded a max step length of 5.5 centimeters. The second method, in which opposite arms and legs moved together yielded a max step length of 5 cm. The quadrupedal algorithms had better step lengths and were far steadier than the bipedal static walking one.

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Extending structural studies of the Influenza A M2 protein cytoplasmic tail

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Abstract

The influenza A virus impacts millions of lives yearly. The virus consists of four major proteins: the envelope proteins hemagglutanin (HA) and neuraminidase (NA) and the two matrix (M) proteins M1 and M2. The 97-residue homotetrameric M2 protein, with its highly conserved structure, is a target for a universal anti-influenza drug and has been partly characterized using site-directed spin label electron paramagnetic resonance (SDSL-EPR) spectroscopy.

EPR spectroscopy detects substances with unpaired electrons through the variation of a magnetic field. By introducing stable radicals known as spin labels, the position of a single protein residue may be detected through EPR. In order to attach the spin label, that residue must first be mutated to a cysteine, unique in its ability to link with the spin label. Using the SDSL-EPR technique, members of the Howard lab have demonstrated in the past that M2 residues 50-60 form a membrane-parallel amphipathic helix.

While the transmembrane and membrane-proximal amphipathic helix domains of M2 have been structurally characterized in detail, relatively little is known about the structure of the remaining residues of the cytoplasmic tail. This project extended structural studies further out into the cytoplasmic tail. A mutagenesis protocol was developed to create the proper cysteine mutants for SDSL-EPR. One mutant, P63C, was taken through the entire purification, spin labeling, and reconstitution protocol required to generate spectroscopic data. The resulting spectrum had a higher sensitivity and better signal-to-noise ratio than has been observed in the past. Power saturation measurements indicate that P63C has lower oxygen accessibility than any of the 50-60C constructs, suggesting that this residue is not as deep in the membrane. Without the oxygen accessibilities of preceding and following residues, it is impossible to say whether the amphipathic helix continues on a tilt after residue 60, or whether the trend loses its periodicity, indicating an end to the helical secondary structure. The protocol developed in this project will be used to collect spectroscopic data on residues 61-70 in the future.

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Reporter Transgenes Controlled by Medaka Twist1 and Osteocalcin Promoters Expressed in Zebrafish (*Danio rerio*)

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Abstract

Reporter genes encode for easily-identifiable proteins, including fluorescent proteins, that indicate where target genes of interest are normally expressed. Target and reporter genes are engineered to require the same transcription factors, regulatory proteins that control gene expression, and thus both genes are activated simultaneously. With modern sequencing technology, reporter genes can characterize the expression pattern of target genes implicated in molecular networks and congenital diseases. Various congenital musculoskeletal disorders involve gene networks with uncharacterized roles, and are the basis for our work on bone development shown here. We injected zebrafish embryos at the one- or two-cell stage with enhanced green fluorescent protein (EGFP) and Discosoma sp. red fluorescent protein (DsRed) plasmids attached to medaka (Oryzias latipes) regulatory sequences involved in osteogenesis. The EGFP plasmid was controlled under a medaka twist1 promoter activated by osteoblast precursors, while the DsRed plasmid functioned under a medaka osteocalcin promotor, which is expressed by medaka osteoblasts. In our injected zebrafish, the twist promoter was more active than the osteocalcin promoter in notochord cell expression, while the osteocalcin promoter was more active than the twist promoter in muscle fiber cells. The fluorescent-positive embryos were raised to adulthood, and will later be crossed to yield offspring with at least one of the two transgenes present in their genome. The offspring would ideally possess both fluorescent transgenes, and the developmental progression of twist to osteocalcin expression can be visualized within one embryo. The identification of bone-related regulatory roles may serve to progress the creation of muscle and bone tissue cultures from embryonic stem cells. Furthermore, findings on the network may specifically improve treatment of different musculoskeletal disorders.

Diffuse optical analysis of post-operative cerebral hemodynamics in neonates with severe congenital heart defects

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Abstract

This study used diffuse optical spectroscopy (DOS) and diffuse correlation spectroscopy (DCS) to measure cerebral tissue oxygenation and cerebral blood flow in neonates with severe congenital heart defects (CHD). Neonates with severe CHD need surgery during the first weeks of life. Perioperative hypoxy-ischemic (HI) brain injury (specifically periventricular leukomalacia, or PVL) commonly occurs among these newborns. In this study, we used DOS and DCS to measure post-operative cerebral tissue oxygenation and cerebral blood flow until the post-operative MRI (magnetic resonance imaging) was taken. By monitoring how cerebral hemodynamics recovered post-operatively, we sought to determine if absolute tissue saturation (StO2) predicted new or worsened PVL on the post-operative MRI. We also investigated whether there were any regional differences in baseline absolute tissue saturation by measuring both over the forehead and over the parietal region. Measurements were taken hourly during the first twelve hours following surgery, and then daily until the post-operative MRI was taken. Four measurements were made over each region. Before each measurement, a calibration measurement was made three times each on a calibration block and a check block, switching between the two. The probe used to make the measurements has eight source fibers and one detector fiber, meaning that there are four source-detector separations.

Before analyzing the DOS data, the calibration data was first used to calibrate the patient data. The natural logarithm of the amplitude (A) and phase (ϕ) of the (measured) modulated light intensity are linearly related to the source-detector separation (r). Separately graphing the natural log of the amplitude and the phase against the source-detector separation provided the slope values used to calculate the absorption and scattering coefficients, which were used to calculate the chromophore concentrations in the tissue. When the left forehead, right forehead, left parietal, and right parietal regions were analyzed individually, significant regional differences were not seen. When the forehead regions were combined and the parietal regions were combined, there was a significant difference seen between the forehead region and the parietal region ($p=0.47673$ for StO_2). Tissue saturation was also not seen to differ significantly between subjects with PVL on the post-operative MRI vs. subjects with no PVL on the post-operative MRI ($p=0.3037$). Further studies will be necessary to determine if these results were due to the small sample size analyzed.

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Tick species proteomic identification using MALDI TOF mass spectrometry

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Abstract

Ticks are important subjects of tropical disease research. Several tropical diseases transmitted through tick vectors are only found in certain species. We explored matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry as a method to identify tick specimens that were either fresh or had been preserved in ethanol for up to five years. Through generating spectra of several tick species, we attempted to create a database for future proteomic identification. Peak intensity and frequency of peaks were noted on each spectrum and a score representing the degree of similarity between an unknown spectra and an entry within the database was generated. High scores denoted a positive identification to the species level.

Preliminary results suggest that it is possible to identify preserved tick specimens accurately using only one pair of legs. MALDI-TOF mass spectrometry has the potential to identify tick specimens with greater accuracy than conventional taxonomic identification methods and is typically lower cost per sample than molecular methods. This application of species identification extends into several important areas of science today, not only in the medical field but also in entomological phylogenetic research, biomedical engineering, and tropical conservation

Regeneration of Functional Hematopoietic Stem Cells from Endothelial Cells

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Abstract

Hematopoietic stem cells are derived from the aorta-gonad-mesonephros (AGM) during development and colonize the fetal liver. During fetal development, a subset of endothelial cells in the AGM, termed hemogenic endothelial cells, change their cell fate to become hematopoietic stem cells (HSCs). HSCs are a self-renewing cell population that produces progenitors to all blood components. However, the molecular nature of their origin, hemogenic endothelial cells, is largely unknown. In order to address this, cell-intrinsic and cell-extrinsic factors that stimulate HSC production from endothelial cells have been identified. Harnessing these factors through the use of chemotherapeutic drugs may allow for the promotion of HSC emergence, ultimately allowing for HSC, or bone marrow, transplants from patient-derived cells. My project is to examine the effects of various chemotherapeutic drugs on the emergence of HSCs in the aorta-gonadal-mesonephros during development in *Dania rerio* using in situ hybridization.

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The effect of the presence of *Syringodium filiforme* on the physiology of *Acropora cervicornis* under ocean acidification conditions

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The increase of carbon emissions into the atmosphere is shifting the ocean chemistry, decreasing the pH and increasing the temperature, which can ultimately have detrimental effects on the physiology of calcifying organisms, such as corals. This study ran two short-term experiments, 5 and 2 weeks long, which evaluated the effects of *Syringodium filiforme* seagrass and of the projected oceanic pH in a century, pH 7.7, on the rates of respiration, photosynthesis, growth and calcification of *Acropora cervicornis* coral. Corals were placed in tanks with high pH seawater (pH 8.1) or low pH seawater (pH 7.6), where half of the tanks contained seagrass. In the five-week experiment, 13 corals under low pH condition suffered rapid tissue loss, some leading to mortality. The resulting corals showed no significant differences in coral physiology between treatment groups. The two-week experiment, however, showed increased growth rate in corals under low pH condition, and increased rate of photosynthesis in corals in tanks with seagrass present. Larger amount of CO_2 available in low pH condition in short-term, may have increased the carbon fixation by *A. cervicornis*, leading to a greater growth rate. The presence of the seagrass, on other hand, may have increased the concentration of ions used by the zooxanthellae within *A. cervicornis*, increasing the rate of photosynthesis. The study supports previous suggestions that the response of coral physiology to both the presence of the seagrass and the ocean acidification conditions is species specific.

A comprehensive analysis of classification algorithms for cancer prediction from gene expression

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Abstract

With the advent of inexpensive microarray technology, biologists have become increasingly reliant on gene expression analysis for detecting disease states, including diagnosis of cancerous tissue (Tan *et al.*, 2003). Microarray data sets are highly susceptible to the curse of dimensionality, as most have orders of magnitude more gene measurements (i.e., features) than samples (i.e., instances). Therefore, the classification algorithm must be robust with noisy and redundant data. While random forests (Breiman, 2001) and SVMs (Vapnik, 1998) have proven to be popular methods for expression analysis (Diaz *et al.*, 2006; Statnikov *et al.*, 2008) little work has been done to compare these methods with AdaBoost (Freund, 1997), a popular ensemble learning algorithm, across a wide array of cancer prediction tasks.

Our initial analysis compared several classifiers, including *k*-nearest neighbors, linear discriminant analysis, linear SVMs, among others on 24 microarray data sets (12 binary and 12 multi-class). However, the three algorithms mentioned showed the best results over others. Furthermore, our analysis shows that AdaBoost performs remarkably well on binary tasks, generally outperforming both SVMs and random forests. On multi-class problems, however, random forests and SVMs are indistinguishable from one another but generally outperform AdaBoost.

Our work differed from existing research in two important ways. First, our work provides a comprehensive analysis of the AdaBoost algorithm across a wide variety of cancer data sets. Second, our work shows that the choice of optimal algorithm depends heavily on whether the task falls under binary prediction or multi-class prediction.

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Distributed Genome Preprocessing using Apache Hadoop

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Abstract

Genome assembly is a long running, yet still relevant field of research. Assembling genomes allows us to get a glimpse into the genetic code that determines each living thing's traits. Genome analysis in the future could allow us to screen for genetic disease, identify cancerous cells, identify and differentiate new species, and many other interesting applications. Thus it is pertinent to ensure the accuracy and efficiency of genome assembly.

Genome preprocessing is the practice of analyzing and correcting genome read data, short snippets of genetic code, before attempting to assemble the reads into a genome. Preprocessing is a well-studied practice that has been proven to improve genome assembly accuracy and efficiency. Modern genome preprocessing relies on the fact that many of the reads for a particular genome sample usually overlap so that a statistical approach can be applied to determining trustworthy reads.

One popular method of genome assembly involves generating a structure called a De Bruijn graph, which rely on *k*-mers, substrings of a read of length *k*. Because of the popularity of the De Bruijn graph approach, many preprocessors analyze reads based on *k*-mers, including the one developed in this project.

Most preprocessing techniques have been designed for running sequentially, on a single machine. This project's goal was to design preprocessing tools that could run on multiple machines in a parallel, distributed fashion, in particular using Hadoop, a distributed computing framework developed by Apache. The Hadoop pipeline we designed to preprocess reads has proven to be both effective and scalable, showing that this approach is promising for creating more complex preprocessors in the future.

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Effects of Membrane Curvature on α-Synuclein

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α-Synuclein is a 140 amino acid whose biological function has been linked to Parkinson's Disease. Its association in Lewy Bodies, which are insoluble protein fibers, is a major component in Parkinson's making it a protein of interest. α-synuclein has been studied more thoroughly as a global structure, but nevertheless site-specific details are still lacking. α-Synuclein is divided into three domains: N-terminus region, Central region and a C-terminus region. α-Synuclein is originally disordered in solution but upon membrane binding it can conform into two conformations; a horseshoe conformation and an extended conformation. To further explore α-synuclein, Electron Spin Resonance (ESR) will be conducted to probe the conformation of the protein in vesicles of varying sizes and curvature. ESR is a biophysical technique in which electron spins are excited by a strong magnet allowing for a signal that is sensitive to the local environment of the protein. The collection of ESR data, was delayed by problems that were encounter with the protein expression protocol, however, this allowed for protocol optimization that allowed for better yield and purity of α-Synuclein. Protocols that were optimized were cell competency and protein purification. Results showed that imidazole concentration of 300-400mM yield the best purity and concentration of α-Synuclein. Also it was observed that a higher percentage of cells were competent when they were keep at colder temperatures during the protocol. These results implicate the necessity for protocol optimization for viable experimental materials.

Investigating the CtrA regulatory pathway of *Sinorhizobium meliloti*

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Abstract

Sinorhizobium meliloti is a nitrogen-fixing Gram-negative soil bacterium that engages in chronic host invasion of legume root nodules. The cell cycle of *S. meliloti* is regulated by the master transcription factor CtrA, whose activity is controlled by the histidine kinases DivJ and CbrA through regulated phosphorylation and proteolysis. We sought to better characterize this pathway by isolating spontaneous suppressor mutations that restore symbiosis to a Δ*cbrA* mutant and testing whether the second messenger c-di-GMP regulates this pathway. We conducted assays for exopolysaccharide production, sensitivity to high temperatures, and sensitivity to deoxycholic acid (DOC) on free-living bacteria. Several of the Δ*cbrA* suppressor strains produce wild type phenotypes of exopolysaccharide production and DOC resistance while still showing the Δ*cbrA* phenotype of increased sensitivity to high temperature. This temperature sensitivity is similar to a previously identified suppressor mutation in the kinase DivL. DNA sequencing will be necessary to determine the identity of the suppressor mutations studied here. Strains with constitutive overexpression of a c-di-GMP phosphodiesterase did not produce an observable effect in any of the strains tested, and thus we conclude that di-c-GMP does not regulate CtrA.

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Alpha-synuclein membrane binding with varying electrostatics and curvature

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Abstract

Alpha-synuclein (αS) is a small 140-residue protein that has risen to prominence in recent research as a result of its implications in Parkinson's disease. It is a major component of Lewy bodies, which are the aggregates of protein that reside inside nerve cells of Parkinson's patients. With a molecular weight of around 14.5 kDa, αS is small, soluble protein that is inherently disordered in solution. However, in the presence of lipid membranes, it experiences a conformational shift to a primarily α-helix structure. The protein-membrane interactions and associations of alpha-synuclein can be modulated by curvature and electrostatics of both lipid charge and charge on the membrane-associated protein (Middleton & Rhoades, 2010). In this work, we are looking at the binding properties of alpha-synuclein, by varying curvature and electrostatics.

To date, much of the studies done on alpha-synuclein involve fluorescence correlation spectroscopy (FCS). While a useful technique, FCS does not provide insight into the depth of the binding. However, it has established that alpha-synuclein preferentially binds to larger vesicles with less curvature and more charged vesicles (Chong et al, 2014). To further understand these interactions, this work will use electron spin resonance (ESR) spectroscopy to probe the binding of alpha-synuclein when varying both the charge of the membrane vesicles and the C-terminus of alpha-synuclein. The ESR experiments will probe membrane accessibility, which will allow us to more fully understand the orientation of the protein, and the depth of the binding. This work will provide more site-specific details into the effects of electrostatics on the membrane binding of alpha-synuclein. This feature of alpha-synuclein is thought to play a role in both the normal function of the protein and the diseased function with Parkinson's. In order to more fully understand αS's function in Parkinson's, it is imperative to understand its conformational shift as the lipid conditions change.

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Pursuing the Chemical Properties of Neonatal Odor

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Abstract

Chemical communication between conspecifics is essential to the relationships and survival of many mammals, including humans. One such relationship is that between mother and offspring. It has been shown, via fMRI, that baby odor activates reward centers in the brain and so is pleasing to women of maternal and pre-maternal status when reported in sensory studies (Lundström, et. al). The chemical compounds characteristic of adult body odors have been identified, such as 3-methyl-2-hexanoic acid (Zeng, et. al). Individuals are also able to discriminate body odor characteristic of elderly people and identify it as more pleasant than middle age and younger individuals (Mitro, et. al). The scent associated with neonates is not yet determined. This study aims to isolate and identify the chemical structure of that scent.

Sample clothing was gathered from the neonatal care unit at the Hospital at University of Pennsylvania and evaluated by scent judges for the presence of "baby odor". Potential odoriferous compounds were extracted and analyzed by gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O), and preparatory-gas chromatography (prepGC). Comparison of scent impressions revealed a distinct "baby odor" was noticeable in samples uncontaminated by commercial soaps. While scents from commercial products mask, enhance, or alter what we perceive as baby smell, this pilot study indicates that there is a distinct, recognizable scent emitted from infants as early as the first twenty-four hours following birth. Additional analysis will identify the chemical compounds that form this odor, with the subsequent goal of replicating this natural odorant.

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Process simulation of Tail-Gas Reactive Pyrolysis (TGRP) and Post-Processing for Guayule Feedstock using PRO/II Software

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Abstract

In light of increasing environmental issues surrounding fuel sources, various research is being conducted on alternative options for fuel sources. One of the growing areas of interest in alternative fuel sources is bio-fuels. Bio-fuels are fuels that are produced from living things or waste from living things.

The bio-fuel that was being studied was from the guayule plant. The guayule plant is one of the primary sources in the latex industry. After the latex has been extracted from the leaves of the plant, the guayule bagasse, the ground-up stems and branches, can be used as a viable feedstock in the pyrolysis process. The method that was being modeled was tail-gas reactive pyrolysis, where the non-condensable gases are recycled in the pyrolysis reactor aiding in the low oxygen content of the bio-oils produced. The more pure and desirable bio-fuel cuts were then refined by distillation followed by hydrodeoxygenation. The finalized products had various carbon levels similar to those found in gasoline, jet fuel, and diesel. Using SimSci PRO/II software, we were able to computationally model this pyrolysis and upgrading process. The model was based on past lab work done at the USDA using the guayule bagasse as the feedstock. It involves a component breakdown for each of the streams generated based on mass balance estimates of the pyrolysis and hydrodeoxygenation reactions. The process flow model developed is critical for a techno-economic analysis as well as an exergetic analysis. These are two important metrics to determining the validity of these processes.

Nswap2L: Heterogeneous Swapping for Cluster Computers

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Abstract

Most super computers these days are of a variety known as cluster computers. Cluster computers are built by combining many smaller computers, called nodes, and interconnecting them with a fast network so that they can communicate easily. Large jobs can be completed quickly by breaking them into small parts and assigning each node a part. Unfortunately, it is difficult to keep the workload even between all the nodes—it is often the case that some nodes will be using all their RAM while other nodes in the network have plenty of free RAM. Typically, when a computer fills up all its RAM, it will swap some memory from RAM onto a hard disk. This is convenient because hard disk space is much more cheap and abundant than RAM. The drawback is that hard drives are much slower than RAM.

Nswap provides an alternate method of swapping. Rather than send excess memory to the hard disk, Nswap finds a node in the network whose RAM has free space and sends the memory to be held there. Since network speed is much better than disk speed, this is a faster solution.

Nswap2L builds on Nswap by choosing whether to swap memory to network RAM, hard disk, flash drive, or some other storage device. This summer our main focus was the implementation of Nswap2L. One area we spent a lot of time on was the prefetch path, which moves pages internally between devices in order to balance loads and enable parallel reads from multiple devices.

When testing placement policies and prefetch policies, it is very useful for us to have an interface to Nswap2L which gives us feedback about how the system is performing, an allows us to change system parameters such as which policies to use or what size nswap cache to use without recompiling. To do this, we implemented an interface that lets us call special Nswap2L functions from user level by reading and writing to special files in the /sys directory.

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Tia Newhall and Douglas Woos,

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Our theories on the transmutation of species

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Abstract

We present a method for searching K2 lightcurves for transiting planets around eclipsing binary host stars using period detection tools. We search the K2 data from each campaign for eclipsing binaries and remove the signal of the binary to allow us to detect fainter, longer period signals such as those of planetary transits. Methods for the removal of the binary star signal include clipping out primary and secondary binary eclipses, as well as normalizing the data to the moving median of the light curve folded on the binary period. We then analyze the resultant light curve with planet detection software developed by D. Foreman-Mackey et al. The detection efficiency of our method is described with respect to various planetary system parameters.

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Statistical Course Recommendation

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This summer I performed exploratory statistical analysis on 10 years of course registration data. Though prior work had been done on course registration data it was not as extensive and did not involve a time series element. I started this project very simply, by cleaning the data. I began with a rather large dataset, encompassing 10 years of data with over 165,000 entries. After cleaning, the working data set contained over 95,000 entries. Cleaning steps included removing non-numerical identifiers from courses, normalizing semester numbers, and removing all students who did not take 7 or 8 semesters worth of classes. The first step of my exploration was creating a simple Python program that allowed a user to choose a course and then would list the other courses most strongly associated with it. using that as a starting place I gradually improved my selection algorithm, introducing information on a student's majors and minors as well as taking into account courses that would be offered in the coming semesters. The eventual model used a parent and child node relationship, modeled after a Dynamic Bayesian Network (DBN), however it was modified so as to not require the same assumptions as a true DBN (which were violated in this system). The enhanced code was then ported into PyQt4 in order to create a GUI for improved usability. Future work could include improving the system to recognize prerequisites by using natural language processing and screen scraping techniques to read from the online course catalog.

Analysis of Sex-Based Differences in a Mouse Model of Stress-Induced Analgesia

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Abstract

Although pain has evolved as a defensive response to noxious stimuli, the suppression of pain during stressful events is evolutionarily advantageous and known as stress-induced analgesia (SIA). Recent studies have drawn attention to unresolved sex differences in mammalian physiology, including differences in pain modulation. For instance, women tend to have a higher prevalence of chronic pain and increased pain sensitivity. Additionally, previous research has indicated that a specific stressor will induce different levels of SIA in male and female rats. Here, we investigated sex-based differences in a mouse model of SIA. We measured whether acute restraint stress would differentially regulate behavioral and molecular indices of pain following a standard noxious injury. Male and female mice were restrained to induce stress before receiving a formalin paw injection. Formalin injection is a common inflammatory pain model and induces both spontaneous pain-like behavior and mechanical hypersensitivity. The von Frey behavioral assay was used to test mechanical hypersensitivity after formalin injection with or without restraint stress. Behaviorally, restrained male mice demonstrated pain suppression compared to control males after an acute period of stress. Acute stress did not have the same effect on female pain-like behavior. On the molecular level, the signaling molecule extracellular signal-regulated kinase (ERK) was analyzed in the central nucleus of the amygdala (CeA). ERK is known to be phosphorylated (i.e. activated) in the CeA three hours post formalin injection and pERK expression was used as a marker of pain in the current experiment. Restraint in males decreased formalin-induced ERK phosphorylation in the CeA three hours post injection, supporting behavioral observations; female mice did not show a significant decrease in formalin-induced ERK phosphorylation with restraint. Additional support for the behavioral data comes from corticosterone blood concentrations. Restrained male mice demonstrated increased corticosterone blood concentrations compared to control males three hours after formalin paw injections. Restraint, however, had no effect on corticosterone levels in restrained females compared to controls. Formalin injections alone failed to significantly increase corticosterone blood concentrations compared to basal levels in both males and females. The results suggest that SIA might impact outward behavioral signs of pain through changes in ERK phosphorylation in the CeA. ERK phosphorylation in the CeA is a potential supraspinal mechanism for the observed sex-based differences in behavior in amygdala-dependent SIA. Understanding how males and females process and modulate both stress and pain can potentially lead to the development of better human pain treatments.

Development and implementation of a genome-wide screen for regulators of a *Vibrio parahaemolyticus* type III secretion system

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Vibrio parahaemolyticus is a gram-negative foodborne bacterial pathogen that is the leading cause of seafood-associated gastroenteritis. Previous work showed that the major virulence factor (type III secretion system 2 or T3SS2) of *V. parahaemolyticus* is induced in response to bile, and this induction requires two transcription factors, VtrA and VtrB (Gotoh 2010, Kodama 2010). We performed a genetic screen to identify potential factors necessary for expression of T3SS2. First, we created a reporter strain of *V. parahaemolyticus* in which *vtrB* was replaced with a tetracycline resistance cassette. Finally, we generated a transposon-insertion library that contained over 140,000 unique transposon-insertion mutants and screened for mutants that failed to induce the tetracycline resistance gene in response to bile. We culture the library under a control condition (0.04% bile) and an experimental condition (0.04% bile and tetracycline) while attempting to ensure an equivalent population expansion between the two samples using OD as a read-out. We then sequenced the genomic DNA flanking sites of transposon-insertion in the control and experimental samples. Using computational analyses, we identified genes necessary for induction of the tetracycline resistance cassette based on changes in the relative abundance of individual transposon-insertion mutants across control and experimental conditions. There were a total of 86 significantly enriched or depleted genes that could represent potential regulators of VtrA and/or VtrB, factors necessary for bile sensing/transport, or novel regulators of T3SS2. A western blot with a primary antibody against VopD2, a component of T3SS2, showed that one of our hits Δ*toxR* did eliminate the expression of T3SS2 while another hit Δ*hrpA* did not. Using cytotoxicity as a read out for T3SS2 activity, we found corresponding results for the effects of both knockouts. We would like to validate more of screening hits using knockout mutants to perform validation experiments of western blots and cytotoxicity.

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Spatio-temporal effects of urban gardens on the stability and abundance of agricultural pests and their natural enemies

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Abstract

Given the resurgence in interest in urban gardens in the US and recent studies showing that these gardens provide substantial resources to support a diversity of organisms, there is real potential for urban gardens as spaces for the conservation of biodiversity and increased food production. From a meta-population perspective, each garden represents a habitat patch interspersed within vast regions of inhospitable urban space (Hanski and Gilpin 1991, Parris 2006, Johnson et al. 2012). However, the long-term viability of urban biodiversity is still in question, and while many organisms inhabit urban gardens, agricultural pests and the natural enemies that control them are particularly important for the gardeners who maintain these garden habitats. In this study, we tested whether and at what scale the distribution of urban gardens influences the population dynamics and stability of pests and their natural enemies. To do this, we surveyed populations of aphids, Coccinellids, and parasitoid wasps over a three-month growing season (June-August) 2013 using sticky traps placed at two spatial scales across the entire city of Ann Arbor, MI. Statistical analysis of our population survey data combined with existing census data of urban garden type and coordinates (Hunter and Brown, 2012) found spatiotemporal effects for all three organisms. Garden density positively influenced the abundances of all three taxa studied, albeit at different spatial and temporal scales, and aphid populations were found to be most stable when garden density was highest at a landscape scale. These results demonstrate the importance of including spatio-temporal dimensions when assessing the effects of urban gardens on pest and natural enemy populations

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Parallel simulated annealing with MRAnneal

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Abstract

Simulated annealing algorithms, which repeatedly make small changes to candidate solutions to find approximately optimal ones, are a common method for approximating solutions to computationally expensive optimization problems. While using multiple machines to perform such computations in parallel is attractive as a means to reduce the running time, execution in a cluster environment requires substantial software infrastructure to cope with the challenges of a distributed system.

We introduce MRAnneal, a framework that simplifies the implementation of parallel simulated annealing algorithms. MRAnneal allows users to explicitly trade-off running time and the quality of approximate solutions by supplying only a small number of automatically tuned parameters. Our experimental results demonstrate that implementing applications using MRAnneal is straightforward and that such implementations yield approximate solutions quickly, even for applications without intuitive serial approximation heuristics.

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2DC Optical Array Probe:

Characterizing Time Response and its Effects

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Abstract

An Optical Array Probe detects cloud particles and measures their shapes and sizes as they pass through a laser beam and cast a shadow onto a receiving diode array. These measurements are used to estimate the physical characteristics of cloud drops and ice crystals and to generate particle size distributions. At high airspeeds, particles move faster through the probe and the duration of their shadow decreases. The quick response time of the diode array is critical to the recognition of small particles. As response time increases, there is a reduction in the probe's true sample volume for small particles, which must be compensated for to avoid low skewed concentration estimates. The time response was measured and characterized for each diode and the effects of these results on sample volume were modeled.

Pretty Good State Transfer in Coronas of Graphs

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Abstract

We investigate the continuous-time quantum walk on the corona of two graphs. This yields several new infinite families of graphs with pretty good state transfer. Our work extends the results of Fan and Godsil on double stars.

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The Sounds of Water

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Water is an integral component of a healthy society and ecosystem. It has a great deal of uses to humanity such as nourishment, agriculture, energy production, sanitation, and manufacturing. We cannot live without it. On top of that, only 0.5% of the world's water supply is available to meet the needs of our population and ecosystems. With this finite recourse, we should take regulate it religiously, and go to painstaking lengths to protect and preserve it.

Unfortunately, we aren't doing a good enough job. Every year, we waste about 1.3 billion tons of food, which translates to 45 trillion gallons of water-45 trillion gallons that could have quenched the thirst of the 2.5 billion people who go without fresh water every day. 1.7 billion gallons are wasted annually due to faulty water infrastructure. These huge numbers can be terrifying and overwhelming, but the good news is that they can be greatly diminished quite easily. We only have to buy the food we will eat. Saving leftovers instead of tossing them out will save billions of gallons of water worldwide if we all make the effort. Our problem is manageable, but we must take action immediately.

My research this summer consisted on inventing a device that would allow us to cut infrastructure water waste by monitoring leaks in pipes. (In apartments, offices, etc.) A former Swarthmore student, Tom Sahagian of Power Concepts Limited (PCL), approached Professor Carr Everbach and myself with an idea to help save some of that meager 0.5% of water we survive on. He wanted a device that could measure in real time, the water flow through a pipe, relay that information to a transmitter, which would then upload that information to a website where the water flow data could be analyzed and quantified. From that data, one could detect a leak in the monitored pipe.

Exercises with the Fetch Mobile Manipulator

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Abstract

Our research this summer focused on becoming more familiar with the new Fetch robot, which has an attached, seven degree of freedom mobile manipulator. The Fetch robot we received is the fifth that Fetch Robotics has ever produced; therefore, our goals were to program Fetch to pick various objects up, to then place those objects, to wave and also to identify and press an elevator button. Furthermore, we set out to create software that would allow us to audit the trajectory of the robot when it was performing these tasks. We used ROS, an open source robotics software to program Fetch, and we wrote our code using Python. Furthermore, we worked with OpenCV, an open source computer vision software, to write the program that would recognize circles in a frame, thereby allowing Fetch to press the elevator button. By the end of the summer Fetch was successfully able to grab objects of different shapes and sizes from a table or the ground, place those objects in a trash can, wave its arm back and forth, and push the elevator button on the third floor of Hicks. Additionally, before commanding Fetch to perform these tasks, we were able to see what path Fetch's arm would take to accomplish these tasks.

Identification of mechanisms impacting left-right heart asymmetry in *Ciona intestinalis*

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Abstract

Humans' bilaterally symmetric appearance disguises the asymmetric body plan that underlies their growth and health. Diseases resulting from defects in asymmetric development can result in congenital heart defects and lead to death. However, very little is known about the origins of these diseases and how to prevent them. Here, we use the simple chordate *Ciona intestinalis* to develop an understanding of early mechanisms determining heart asymmetry. In *Ciona*, it is known that nodal signaling, H^+/K^+-ATPase activity and neurula rotation all contribute to molecular asymmetries. However, how these mechanisms contribute to left-right heart asymmetry remains unknown. Recent assays have been developed to test these hypotheses by viewing both juvenile heart asymmetry and the earlier asymmetric shift of larval heart cells. Here, we show, that in *Ciona,* nodal signaling is dispensable for left-right heart asymmetry. We additionally show that H^+/K^+-ATPase activity is involved in left-right heart asymmetry and that in later embryonic stages, there is also role for MAPK signaling in *Ciona* heart asymmetry. These reports indicate a left-right heart asymmetry pathway, upstream of neurula rotation, that is ion flux-dependent and nodal-independent. Further research into other signaling pathways, such as sonic hedgehog (*shh)* and Wnt pathways, as well as the mapping of early genetic asymmetries using RNA sequencing, will aid our understanding of what processes are contributing to left-right heart asymmetry in *Ciona*.

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Metacognitive Regulation in Undergraduates in an Upper-Division Cell Biology Class

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Abstract:

Metacognition, which is the awareness and control of thinking for the purpose of learning (Cross & Paris, 1988), is an important component in learning. Metacognition has been correlated with learning outcomes (Wang, Haertel, & Walberg, 1990), problem solving ability (Rickey & Stacy, 2000; Sandi-Urena, Cooper, & Stevens, 2012), and performance (Vukman & Licardo 2009; Young & Fry, 2008). There are two components of metacognition: metacognitive knowledge and metacognitive regulation (Brown, 1978; Jacobs & Paris, 1987). Metacognitive knowledge is the awareness of one's own thinking. On the other hand, metacognitive regulation describes the action one takes in order to learn (Sandi-Urena et al., 2011). Student with good metacognitive regulation skills are able to select the most appropriate learning strategy for a task or assignment and adjust their strategy based on feedback.

Students' use of metacognitive skills is also affected by their attitudes and beliefs about learning, such as self-efficacy and agency (Ambrose, 2010; Chemers, Hu, & Garcia, 2001). Self-efficacy is one's beliefs in their capabilities to learn (Bandura, 1997; Estrada-Hollenbeck, Woodcock, Hernandez, & Schultz, 2011). Self-efficacy is also a strong predictor of performance and metacognition (Coutinho & Neuman, 2008). Self-efficacy is important because it may be a factor in the metacognitive regulation of undergraduate students.

In this study, we investigated upper-level biology students metacognitive regulation (n=73). Students were prompted to reflect on their metacognitive process with the use of evaluation assignments, administered after their first and second quiz throughout the course. We used content analysis to identify evidence of metacognitive regulations skills in the students' responses. We found that more students are able to monitor, evaluate, and plan, but there is still a range of metacognitive regulation in upper-level cell biology students. This suggests that there are differing levels of metacognitive development late in the undergraduate career. Furthermore, more interviews will be conducted to better understand self-efficacy's role in the development of metacognitive regulation.

A Computational Exploration of the Stability of Methylated Benzene Carbocations

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Abstract

This study used Density Functional Theory at the B3LYP/631G(d) level to analyze the relative stability of cyclic carbocations derived via the protonation of methylated benzene compounds. Optimization and frequency calculations were run on each structure and its carbocations and the SCF energies were recorded. The negative protonation energy (NPE) of each carbocation was determined and, in conjunction with data describing the substitution of each position on the cyclic structures, a multi-variable regression was created to fit the linear nature of the data. This regression was used to obtain calculated NPE values for comparison to the experimental values. The formula recreated with good accuracy the experimental NPE values. The data support the accepted hierarchy that tertiary (methylated) positions are superior to secondary (unmethylated) ring positions in stabilizing the positive charge of a carbocation.

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Analysis of Kepler Observations of ASAS Variable Stars

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Abstract

This study compares the performance of period-finding algorithms when using data gathered by ground-based telescopes to their performance when using data gathered by space-based telescopes. In order to make this comparison, the periods reported by the All Sky Automated Survey (ASAS) Catalog for Variable Stars in the Kepler Field of View (Pigulski et al, 2009), a study that identified targets for the Kepler Mission before its launch, were compared to periods determined by this study. Only targets that were identified in the ASAS Catalog and later observed by the Kepler Mission were selected for analysis, leading to a total of 599 targets. The observations gathered by the Kepler Mission were analyzed using three period-finding algorithms: the Lafler-Kinman algorithm (Lafler and Kinman, 1964), the Analysis of Variance algorithm (Schwartzenberg-Czerny, 1989), and the Conditional Entropy algorithm (Graham et al, 2013). These three algorithms analyzed the light curves of each target, and one of the periods produced was selected to be compared to the period found by the ASAS Catalog. The analysis of the two data sets highlights issues with the performance of period finding algorithms with ground-based data, leading to crude period estimates for all targets with periods longer than 10 days. Since the Large Synoptic Scanning Telescope (LSST), due for first light in 2020, will have a similar observation schedule to that of the ASAS survey, similar issues can be expected with the analysis of LSST data for long period variables, and follow-up observations may be necessary to validate periods found to be longer than 10 days.

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A Computational Study of Rotational Barriers in Atropisomeric Diphenyl Ethers

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Abstract

Diphenyl ethers form a basic structural unit seen in many important natural compounds such as the antibiotics vancomycin and bouvardin and the hormone T3. Additionally, that unit is also the basis of some polymers with useful thermal and tensile properties such as PEK, PEEK, PES, and PEES, as Strassner notes[1]. An interesting feature of diphenyl ethers is that unique isomers can be separated due to hindered rotation about a single bond; such isomers are termed atropisomers. For diphenyl ethers, this rotation is slowed by bulky *ortho* substituents on the two phenyls. However, there is little material in the literature that describes the minimum requirement of various substituents for an atropisomeric compound to retain one conformation for a specific period of time. Very few experimental papers exist discussing the topic, and computational ones are frequently concerned only with the unsubstituted diphenyl ether's interconversion barrier and potential energy surface, often calculated using semiempirical theories and small basis sets. This study aimed to fill the gap by identifying some specific requirements for ensuring a chiral product over a specific, long time frame using the relatively robust B3LYP/6-31G(d,p) level of theory and basis set.

Rotational barriers about the phenyl-oxygen bond of a series of diphenyl ethers *ortho* substituted with halogens and alkyl groups, and *para* substituted with various functional groups were studied. Barriers ranging from 1.1 to 44.7 kcal/mol were reported, with predictable correlation between increased *ortho* steric substitution and greater activation energy. *Para* substitution played a minor role in raising interconversion barriers, though it was not clear if the effect was electronic, steric, or some combination. Racemization half-lives were calculated at 273, 298, and 373 K. Also calculated were the storage temperatures required for each compound to undergo 1% racemization over the course of a year.

Though other combinations of substituents and temperatures are also sufficient to ensure long term chirality, the minimum conditions required of the diphenyl ethers studied are a tetra *ortho* substituted diphenyl ether, with two methyl groups and two bromine or chlorine atoms, stored at 273 K. We predict such conditions produce less than 1% racemization over a full year.

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Site-fidelity, body size, and mating success in *Bolitotherus cornutus*

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This experiment investigated the relationship between site-fidelity and body size and mating success of *Bolitotherus cornutus* (forked fungus beetle) found on a population of *Ganoderma applanatum* fungus. Beetles were observed daily and their locations, elytra size, and mating behaviors (if any) were recorded. Body size and mating success (measured by courtships and guards) were put into multiple regressions along with number of brackets each beetle was found on. Elytra length had a significant effect on the number of brackets visited, and this effect differed between males and females. Smaller males were more likely to visit more brackets than larger males. The number of brackets visited had no effect on the number of courtships or guards a male obtained. This shows that site-fidelity of male beetles is not predictive of mating success, but that body size clearly affects site-fidelity. It is possible that this pattern may be due to aggressive interactions between males of different sizes, and provides the framework for future work involving territoriality in forked fungus beetles.

Where Do Wildfires Stop: A Case Study in the Blue Mountains of Oregon

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Abstract

The purpose of this research was to study the relative importance of human and biophysical factors in influencing where wildfire cessation occurs. The majority of forested lands in the United States have moderately or significantly altered fire regimes. Logging, livestock grazing, and invasive plants, insects, and diseases have all contributed to these changed fire regimes, although the biggest contributing factors are climate change and historical policies of fire exclusion. Fires in the past 25 years have been growing in both size and intensity, and landscapes with altered fire regimes are more likely to have adverse ecological, economic, and social impacts. The direct and indirect effects of wildfires cost government agencies billions of dollars every year. As climate change continues and more humans move into the Wildland Urban Interface, the economic, social, and ecological effects of wildfires will continue to increase.

This research asks what biophysical variables are significant in stopping wildfires, and what role human creations like roads and trails play. The effect of human infrastructure in particular is understudied, despite the prevalence of roads, trails, and housing in and beside forests. Thus, the guiding working hypotheses were that wildfires would stop near human variables like roads, trails, and the Wildland Urban Interface, and near biophysical variables like streams, in ridges and valleys, and in cold, wet areas.

Six fires were chosen for study in the Blue Mountain ecoregion in Northwestern Oregon. The fires varied in size and location, but they were all at least partially in Wallowa Whitman or Umatilla National Forests. Geospatial data were obtained and assembled from federal agencies. Case points were created every 200 meters along each fire boundary. Then each case point was matched with 5 control points, each 100 meters apart on the interior of the boundary. After intersecting these points with the geo-processed independent variables and checking for multicollinearity, a matched case-control logistic regression analysis was performed, and models were selected and reported.

The statistical significance of the coefficients showed spatial associations between wildfire cessation and both human and biophysical variables. However, there was varied direction of influence between fires for many of the variables, which would take further, more detailed study to explain. Furthermore, the weather variables were not included in this model due to time constraints. While further study is needed to account for missing variables before drawing conclusions, it is evident that both terrain and human infrastructure significantly affect wildfire cessation in this preliminary research.

Diastereoselective Synthesis of Enantiomerically Pure Planar Chiral *N*-Oxazolidinoyl Diene Tricarbonyl Iron(0) Complexes

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Abstract

The use of chiral oxazolidinone auxiliaries allows for the synthesis of highly functionalized pure planar chiral molecules via a wide variety of reactions, such as regioselective stannylcuprations, modified Stille couplings, and complexations of iron metal fragments. Conjugate additions alpha to the diene backbone are possible, and diversify this class of compounds. Determining the functionality on side chains, and the effect these chains have on the yields and diastereoselectivities upon complexation of the iron metal fragment, is necessary to determine the outreach these planar complexes have.

Investigating the role of DNA methylation during changes in social status in *Astatotilapia burtoni*

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Social behavior is universal across the animal kingdom and is comprised of the complex set of interactions between organisms within a community. In the African cichlid fish, *A. burtoni*, males exhibit differences in social behavior through discrete dominant and subordinate phenotypes. Since males can switch reversibly between phenotypes, their underlying molecular mechanisms may include epigenetic changes to DNA. One such molecular mechanism is DNA methylation, a reversible covalent modification made to cytosine known to regulate gene transcription.

Two approaches were made: first, to examine the role of DNA methylation in gene expression and second, to find the proteins that methylate DNA. In the first approach, the mRNA transcriptome of possible methylated genes were examined. Differentially expressed mRNA transcripts were found between dominant and subordinate males, which could mean that these genes may be methylated in either dominant or subordinate phenotypes. In the second approach, cross-reactive antibodies for proteins that are key in DNA methylation were optimized. Future directions include immunohistochemistry for neuro-anatomical correlates of protein, ChIP sequencing of the genome, and mapping methylation of validated genes that are differentially expressed.

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Intensity in ENSO Forecasts

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Abstract

The North American Multimodel Ensemble went live in 2011, providing forecasts for ENSO events for many different models. These forecasts typically involve a three-category setup, where they display the probability of an El Nino, a La Nina, or Neutral conditions. However, this setup does not allow us to forecast ENSO events of different intensities. In this work, we took nine NMME models and devised five- and seven-category forecasts and tested the skill of these forecasts in comparison to that of the three-category forecasts. We also created a multi-model ensemble and tested its skill in comparison with the skills of individual models.

We found, not surprisingly, that the multi-model ensemble is more skilled than any individual model. Additionally, from cursory analyses of intensity forecasts for NMME models, we found that skill seems to drop for increased number of categories, though five- and seven-category multi-model ensemble forecasts remain somewhat reliable. For future research, we will conduct more comparative analysis about hindcast and real-time samples and look into the addition of duration in forecasts' effect on skill.

Loss of Huntingting (Htt) disrupts sleep in D. melanogaster

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Hungtington's Disease is a neurodegenerative, autosomal dominant disorder caused by the misfolding of the Huntingtin protein (more specifically due to an abnormally large number of CAG repeats in the molecule's Poly-Q region). Though the disease is rare, its progressively destructive effects on patient personality and motor control (culminating ultimately in death) have been well documented. Huntington's also has some effect on the circadian rhythm patterns of its sufferers, though this symptom is far less consistent between individual patients. Unfortunately, disrupted sleep patterns often further exacerbate the disease's other neurological symptoms as the brain loses valuable cleansing and recovery time. We attempted to further elucidate the abnormal circadian rhythm patterns associated with the disease by comparing the sleep schedules of *Drosophila melanogaster* fruit flies with mutated Huntington genes ($dHtt^{int}$) to those without (dHtt). We then attempted to rescue any potential mutation-induced circadian changes in $dHtt^{int}$ flies via the implementation of a GAL4-UAS system using an Armadillo (ubiquitous) GAL4 driver and a UAS-functional Huntingtin expression line. Results showed disrupted sleep behaviour in $dHtt^{int}$ relative to wCS flies, yet no circadian rhythm differences between the two groups. Attempted rescue of the wCS sleep phenotype was unsuccessful using drosophila Htt, yet was successful using human Htt. Though results were inconclusive, these data lead us to consider the importance of Htt in maintaining normal sleep behaviour.

Interactions Between G-Quadruplex DNA and Porphyrin Ligands

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G-quadruplexes (GQs) are non-canonical nucleic acid secondary structures comprised of π-π stacked units of four Hoogsteen hydrogen-bonded guanines. GQ-forming DNA sequences have been found in oncogenes and human telomeric DNA; thus, ligands which bind to and stabilize GQs are of interest as potential cancer treatments. This project investigated promising porphyrin ligands: N-methyl mesoporphyrin IX (NMM) and five derivatives of the well-studied GQ ligand tetra (N-methyl-4-pyridyl) porphine (TMPyP4). The interactions of TMPyP4 derivatives and GQ of human telomeric DNA were characterized with FRET assays, UV-vis absorbance titrations, Job plots and CD annealing/melting experiments. The derivatives were found to be less stabilizing of, but more selective for, GQs compared to TMPyP4. Results suggest a relationship between overall charge and GQ stabilization. Second, the kinetics of NMM-induced GQ structural rearrangement was studied by monitoring the fluorescence of NMM over time. It was found that rearrangement speed was dependent on temperature and indirectly dependent on the concentration of potassium, an ion important for GQ formation, in buffer. Third, the thermodynamics of GQ-NMM binding was investigated with absorbance and fluorescence titrations. NMM was shown to bind in a 1:1 or 1:2 stoichiometry, but not tightly. Data suggest that GQ-NMM binding mechanism may be similar for different GQs.

Characterizing the effects of following on pedestrian flow

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Abstract

The study of pedestrian dynamics by modeling people as self-propelled particles is a subset of the field of jamming. Broadly, jamming concerns many-particle systems, particularly as particles come close together, and in some cases form rigid jams where particle motion is impossible. Pedestrian dynamics specifically are relevant to efficient building design, evacuation preparation, and crowd tracking and management.

Our simulation was based largely on the Social Force Model[1] where social considerations, such as avoiding other pedestrians, are modeled as physical forces. Our experimental setup was a narrow hallway in which two groups of pedestrians, modeled as disks, tried to move in opposite directions. This condition of "bi-directional" flow is practically relevant and theoretically interesting.

The narrow goal of our research was to investigate how the tendency of people to follow one another affects their efficient travel to a destination. Thus, the most important variable in our research was the follow factor, which is a number between zero and one that controls how likely a pedestrian is to move towards the exit versus follow other nearby pedestrians. When the follow factor is zero, pedestrians only try to move toward the exit. When the follow factor is one, the desired direction of each pedestrian is simply the average of the directions of other pedestrians within a specific radius, which is a parameter of the simulation.

To characterize the efficiency of pedestrian movement, we looked at "burst" and "lags" as pedestrian groups tried to traverse the system. The distribution of lag durations suggests that the data follows a power law, as expected. Additionally, we calculated the average "current" of pedestrians through specific regions in the simulation. We found that when the follow factor is higher for pedestrians moving in one of the two directions, the flow of those pedestrians becomes less efficient.

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Determination of the downstream Ras effector pathway in *C. elegans* neuroblast delamination

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Abstract

The small GTPase Ras causes myriad changes in cell identity and function. Ras signaling is known to promote epithelial mesenchymal transition (EMT), a process by which tightly linked epithelial cells change state into more migratory types. Mechanistically, however, Ras's role in this process is obscured, as it acts through multiple effectors and pathways. The *C. elegans* excretory system provides a model for studying cytoskeletal remodeling and junction loss, key steps in EMT, as one of the constituent cells, the G1 pore, delaminates from the neighboring duct cell and migrates and divides to form two daughter neurons. It has been shown that Ras is necessary for proper junction loss, and that Ras signaling in pore delamination acts cell non-autonomously through the duct cell. A loss of function mutation in the guanine-nucleotide exchange factor *sos-1* leads to a decrease in activated Ras in the duct cell, which in turn causes incomplete junction loss in G1 pore cells. Here, we attempted to identify the downstream effector of Ras involved in G1 pore junction loss by trying to rescue proper G1 delamination in *sos-1* mutants using duct lineage promoter driven transgenes to express constitutively active versions of known Ras effectors. Among these, Raf was selected as the first candidate, as it is the best-characterized and most prominent Ras effector.

Raf/lin-45 gain of function and dominant negative constructs were made successfully and verified by direct sequencing. Constructs under the duct specific lin-48 promoter were not injected in time to create and test a stable transgenic line, however, a stable transgenic *sos-1* line with a heat shock promoter driven *lin-45(gf)* was generated and used for preliminary testing. Upshift experiments to temperatures non-permissive for the *sos-1(ts)* allele were unable to reliably produce the delamination defect phenotype, and, as such, could provide no information regarding Raf's role in the process. Because of inconsistent staging of embryos following pulse lays, upshifts were not timed properly to arrest pore delamination, and instead resulted in a phenotype with two pores and no duct in the excretory system, as was expected from previous studies on Ras signaling in excretory cell fate induction.

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Entomopathogenic fungi display sensitivity to fungicide practices in golf turf

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Abstract

Entomopathogenic fungi (EPF) such as *Beauveria bassiana* and *Metarhizium anisopliae* show promise as biocontrol agents against insect pests in turfgrass systems. In order to improve EPF effectiveness, however, it is necessary to understand how soil and turf management practices affect them *in situ*. We surveyed EPF abundance across eight golf courses with varying soil characteristics and fungicide application histories. Using soil collected in 2015, we employed the *Galleria* bait method to estimate EPF abundance. We found EPF, primarily of genus *Metarhizium*, in soils from all but one golf course. Fairways exhibited significantly lower EPF abundance than roughs, which are less intensively managed ($p = 0.018$). Golf courses with lower fungicide rates tended to have greater EPF abundance, and there was a significant negative relationship between fungicide rates and EPF abundance over all sites ($p = 0.034$). EPFs were not affected by soil characteristics including pH, moisture, clay, and carbon content. Future work will involve directly varying fungicide application rates on a single site, in order to reduce noise from site variation. For now, the data suggest that EPFs benefit from lower fungicide rates and reduced management intensity, though the benefits of EPFs must be balanced against managing fungal pathogens.

Identification of putant heat shock response phenotypes in *Arabidopsis thaliana* using the RootScope

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Abstract

When living organisms are exposed to stress, homeostasis is disrupted, which often leads to the misfolding and loss of function of proteins that are essential to vital processes. As plants are unable to avoid the many stressors present in their environment, they have developed and refined robust yet complex response networks. An abiotic environmental stressor is heat. Based on the current model of the heat shock response (HSR), detection of heat stress leads to titration of heat shock proteins (Hsps) away from inactive complexes with heat shock transcriptional factors (Hsfs). The released Hsfs can then activate expression of other Hsps that help restore misfolded proteins to their native structures (Hahn *et al.*, 2011). Yet, specific players involved in the HSR remain to be identified and characterized. In this investigation, a forward genetics approach was taken to discover the genes that underlie heat sensing and stress response regulation.

In this mutagenesis screen, EMS-mutagenized seedlings containing a *Hsp17.6*:GFP construct were subjected to a one-hour, non-lethal heat shock at 37°C. Spatial and temporal data on the roots of numerous seedlings were collected by the RootScope, an automated fluorescence microscope, allowing for analysis and visualization of the HSR as a kinetic curve.

Of the fifteen mutant pools screened during this project, one particular pool of seedlings displayed heat shock phenotypes in which *Hsp17.6* expression is significantly higher than observed in wild-type. In subsequent re-screens of the pool, seedlings with morphological defects also showed significantly higher *Hsp17.6* expression. To determine the genetic basis of the observed heat stress phenotypes, the identified seedlings from this pool have been transplanted to soil, so that future analysis of the offspring generation may reveal the heritability of the phenotypes.

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The influence of object neophobia on behavioral coping and hormonal stress responses in great tits (*Parus major*) selected for divergent personalities

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Novel object assays have been used extensively to explore avian behavior and it has been assumed that exposure to such novelty is mildly stressful—this assumption, however, is rarely tested. We did so here using captive adult great tits (*Parus major*) derived from the fourth generation of bidirectional artificial selection on spatial and object neophobia. The results confirmed this common assumption—we observed a moderate but significant elevation in plasma corticosterone following 10 min of exposure to a novel object in the home cage. The magnitude of the hormonal stress response was independent of selection line and not attributable to human observer effects. Immediately following exposure to the novel object, birds were placed in restraint for 30 min and bled for an additional corticosterone sample. We found that this second stressor induced a strong corticosterone elevation that was selection line dependent, with the fast exploration line exhibiting the stronger response. These hormone results were complimented by estimates of behavioral coping, including the measurement of abnormal repetitive behaviors (ARBs) and locomotor activity in response to the novel object. This component of the study allowed us to test the hypothesis that exploratory personality is genetically correlated with routine forming behaviors such as ARBs—a suite of traits that have been observed in other species—and then link this behavioral variation to the simultaneously measured variation in corticosterone responses. We discuss these results in the context of animal personality research as well as animal models of behavioral and physiological dysregulation.

Designing Efficient Algorithms for a Virtual Interactive Environment

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Abstract

Virtual interactive environments have more and more applications every day, from architectural models and psychological simulations to video games and entertainment media. These environments are often implemented using an overarching engine that contains a realtime working physics engine and is then supplemented by a programmed scripting language to design mechanics. This project seeks to design a virtual interactive environment from the ground up, building each desired mechanic from scratch.

In order to create this environment the desired mechanics must be defined and outlined. The mechanics that I will focus on include complex movements like navigating obstacles, collision detection, and failure state resetting. In this case, I chose to focus on the following complex mechanics: climbing ledges, climbing ropes and ladders, and a system where the user may fail and then re-spawn in a systematic user friendly way. A complex environment such as the one I created requires many more mechanics and scripts, but for the sake of brevity these will be eschewed.

To create this world I worked with the Unity Engine which utilizes the PhysX physics framework and is supplemented by the C# object oriented programming language. These two powerful technologies allowed for easily understood and repeatable design and implementation of the designed mechanics. The mechanics themselves were first algorithmically designed on paper using a combination of techniques including elements of graph theory, finite state machines, and general programming methods. Then each mechanic was implemented using C# to write scripts that work together to make the algorithm produce the desired mechanic. The algorithms were judged on efficiency on two standards: whether they achieve the desired outcome, and how consistently this outcome is achieved with low variability.

Ambivalence and emotional regulation in a gambling task: a behavioral and ERP study (preliminary findings)

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Ambivalence, or the state of holding positive and negative emotions in the mind simultaneously, can be uncomfortable and deplete cognitive resources. Finding methods for relieving ambivalence can be beneficial, allowing people to make the right decisions when they are feeling mixed emotions. In this study, participants played a gamble task designed to induce ambivalence. Participants could either win or lose a high or low amount, and winning or losing the low amount should elicit ambivalence. In the first block, they played with no instruction, and in the second and third blocks, they were asked to focus on either the positive or negative aspects of each trial (emotional instructions were counterbalanced). In the first block, the gamble task successfully induced ambivalence, $p = 0.031$. Focusing on the positive significantly reduced ambivalence, $p = 0.045$. Focusing on the negative also significantly reduced ambivalence, $p < 0.001$. However, emotional ratings for these trials appeared to be non-congruent. Due to the small sample size ($n = 11$), more data must be collected in order to draw stronger conclusions.

Memory Affects Individual Adaptive Learning

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Abstract

Individual beliefs within a changing environment require dynamic adaptive learning. Effective predictions of future events require memory recall of past experiences; -however, when the environment is uncertain, the past does not always predict the future and individuals must adapt rapidly (McGuire et al., 2014) New events and unexpected outcomes due to an unstable environment should influence and change an individual's current beliefs more so than expected ones. Optimal adaptive learning, within the experimental task used, has been shown to follow a Bayesian model with two distinct factors: Change-point Probability (CPP), the probability that the environment has changed outside of ordinary noise, and Relative Uncertainty (RU), the uncertainty of how stable the environment has been since the last change (Nassar et al., 2010). Our goals were to build upon and compare to previous data taken with this model while including a memory task to examine the relation between individual memory ability and individual adaptive learning, and to test whether memory correlation was different between young adults (age:18-35) and older adults (age:65-85).

The experiment contained a free recall task, the Wechsler Abbreviated Scale of Intelligence (WASI) IQ test, and a simple video game task. The free recall task presented preset word lists for subjects to memorize and verbally recall, and the video game consisted of a hidden helicopter that dropped coin bags for subjects to catch. The helicopter is set to randomly change locations across the screen and the coin bags always drop within a normal distribution around the central point of the helicopter. Subjects were limited to be between the ages of 18-35 and primarily consisted of University of Pennsylvania students. Preliminary data analysis shows similar learning rates and Bayesian model correlation for the video game task when compared to previous data. Memory data analysis suggests a positive correlation between recall percentage and increased performance on the video game task. Comparison between young and old adults showed similar correlation results between recall percentage and the Bayesian model factors, suggesting that, for both groups, memory ability is an influential factor in individual abilities to adapt within an unstable environment.

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Fine-tuning the CLARITY technique for unbiased 3D mapping of Alzheimer's disease pathology

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Abstract

On the cellular and molecular level, Alzheimer's Disease (AD) is characterized by the aggregation of β-amyloid plaques, formation of neurofibrillary tangles, increased glial activation, and eventually, neuronal death. These microscopic elements manifest in a patient as hippocampal memory loss and general cognitive decline. However, a specific link between pathology and cognitive symptoms remains elusive.

Recent development of protocols that clear brain tissue has allowed for greater insight into the pathology and progression of Alzheimer's disease (AD). A method of clearing, dubbed CLARITY, fixes the tissue so that structural components, important proteins, DNA, etc. remain intact but removes the majority of the lipids. Previous studies have shown that lipids are responsible for obscuring light during microscopy. Thus, CLARITY enables both better fluorescent staining as well as clearer imaging, revealing new aspects of disease pathology that are unable to be seen in un-cleared tissue.

CLARITY treatment of brain tissue has allowed for whole-brain and half-brain imaging on a mouse model, preserving many structural elements lost through slicing. By looking at a larger portion of the brain, it becomes apparent that there is a general order of amyloid plaque development. The mammillary bodies have been shown to be the first region to present pathology, followed by the septum and other subcortical structures. Other connections between glia and diseased proteins also become apparent. By imaging brains of mice at various ages or stages of the disease, we can have a better understanding of the pathways AD takes and how the various parts come together as the cognitive decline we see.

This summer, I worked with a graduate student, Rebecca Canter, under Professor Tsai's guidance to implement a modified CLARITY protocol to produce a whole-brain map of plaque load, tangle formation, myelin volume, and glia activity in a mouse model (FAD) at various stages of familial AD. We are also trying to confirm any of our findings in human tissue as well, as to make sure this is not a transgene effect from the mouse line. With any new technique comes skepticism so my main projects this summer involved designing and running the control experiments necessary at the different steps of CLARITY. We needed to make certain that the protocol itself was not creating any false data. In addition to those shorter experiments, I also assisted in the overall data collection.

Mapping and cloning of *csmd1*, a male sterile maize mutant

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Microarray experiment of wild-type and *csmd1* maize at two developmental stages

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Abstract

Male sterility has important implications in the production of hybrid seed, especially in self-fertilizing plants. Traditionally, the tassels of corn, an important cash crop, have been removed by hand, a necessary but arduous process. Here, we attempt to map and clone *csmd1*, a male sterile maize mutant with both pre-meiotic and post-meiotic defects. Pre-meiotically, *csmd1* plants exhibit excess callose around the meiocytes. Post-meiotically, *csmd1* microspores stop differentiating and collapse. *Csmd1* anthers have irregular epidermal cells that have increased divisions and reduced elongation as well as oval-shaped locules in the anthers. Despite these defects, *csmd1* plants continue through meiosis, sustain anther growth, and have morphologically near-normal tassels. Five families from a 1:1 *csmd1* background (134 plants) were first genotyped then screened for recombinants using two new polymorphic markers. Two potential recombinant plants were discovered, one of which was confirmed to have a sterile genotype but fertile phenotype. Future screening of the same families and their next generations is required to locate *csmd1*. Additionally, the anthers of 16 plants, 8 *csmd1* and 8 wild-type, were collected at 2.0 and 5.0mm for a microarray experiment. 20 anthers were collected for each 2.0mm plant sample and 10 anthers were collected for each 5.0mm sample. Further analysis of the microarray scans will elucidate factors critical for normal anther development in both haploid and diploid cells and fate determination in the male germ line.

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ESR Study of Membrane-Bound Alpha-Synuclein

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Abstract

α-synuclein is a human protein found primarily in the brain. Though its exact function is still under intense investigation, alpha-synuclein is suspected to play a role in SNARE complexes by facilitating vesicle fusion to the plasma membrane. Misfolded α-synuclein is known to aggregate into Lewy bodies, which are associated with neurotoxicity and Parkinson's disease. α-synuclein binding is sensitive to curvature and charge, and by using electron spin resonance (ESR) we hope to examine the changes in protein dynamics and binding when the charge and curvature of the substrate are changed. ESR measures the unpaired electron on a spin label attached to our protein.

We plan to examine the protein using site-directed spin labeling. By performing single site mutagenesis to change the desired residue to a cysteine, the spin label can be attached at any specific point along the protein. There is extensive literature on the C-terminus tail, a region of about forty residues at the end of α-synuclein that are highly negatively charged and disordered, but its function is still poorly understood. There are very few spin label studies on this region, and we hope our ESR study will contribute to the understanding of the C-terminus tail. In addition, we hope to contribute additional data on the dynamics of alpha-synuclein.

By the end of this summer research period we had isolated two variants of protein, and created mutated plasmid DNA for another four. We have purified wildtype and 12S protein, from which we can establish a baseline for future ESR experiments. We have WT, A89C, A90C, and T72C viable plasmid. This is groundwork for further work on alpha-synuclein, and we are looking forward to further research.

How forest fires burn; the impact of roads on wildfire progression

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Abstract

Previous research done by Narayanaraj and Wimberly was the first to look at the impact of roads on burn severity at the landscape level and utilized the idea that forest roads can act as physical barriers for fire suppression. The objectives of our study were to determine what factors, environmental and human, influence the burn severity of forest fires and to observe whether burn severity will increase or decreases in relation to distance to roads. Nine fires were analyzed in the Cascades Ecoregion in Oregon selected to reflect a range of landscape characteristics that are important for sampling burn severity locations under variable environmental conditions. All fires occurred between 2000 and 2013, a time period that encompasses a wide range of fire sizes, and during the months from July to September.

We collected, organized, and geo-processed geospatial data using GIS (Geographic Information System) in ESRI ArcMap. A sampling grid was created, points spaced every nine cells (270 meters), for each fire and layered over burn severity data calculated using the Relative Differenced Normalized Burn Ratio (RDNBR), distance to road, distance to stream, elevation, slope, Terrain Shape Index (TSI), Heat Load Index (HLI), tree cover, fuel types, and distance to wildland interface (WUI). We extracted values to the sample points, and then fit linear and spatial regression models using RdNBR as our dependent variable.

We found that as distance to road and WUI increased, there were varying significant effects (positive and negative) on burn severity from fire to fire. We also found that slope and TSI had consistently positive relationships. Further understanding and research can help predict severity patterns, allowing for better allocation of resources and help us understand the role roads play in forests.

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