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UNDERGRADUATE SYMPOSIA ABSTRACTS

Abstracts are listed alphabetically by the last name of the first author listed.

COMPARING EXPRESSION OF POTENTIAL IMMUNOGENIC PROTEINS IN HUMAN EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS.

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There is considerable research being conducted in the use of induced pluripotent stem (iPS) cells as autologous cellular therapies for the treatment of several diseases. One of the concerns surrounding this research is possible rejection of the transplanted cells. Zhao et.al showed that in mice, there was an immune mediated rejection of iPS, but not embryonic stem (ES) cells in a syngeneic transplant setting. This study implicated three tumor antigens in the immune response against iPS cells. However, Guha et.al found little immune response against differentiated and undifferentiated mouse iPS cells. These data might suggest that ES cells are more suitable for clinical applications in humans than iPS cells. To test whether human iPS cells might be rejected when used in cellular therapies, we measured expression of the three antigens, ZG16, HORMAD1 and Cyp3A in human iPS and ES cells as well as differentiated and undifferentiated cells from both stem cell types. We tested whether the differential expression of these markers could be used as an indicator of immune rejection by using PCR and flow cytometric analysis. The results indicated that there is little difference in the expression of these tumor antigens between human ES and iPS cells, and between differentiated and undifferentiated cells. This indicates that the expression of these markers would not likely cause a differential immune rejection or be a predictor of rejection in human iPS and ES cells. The results from this study also show that there are significant differences between mouse and human ES and iPS cells which should be explored further to understand how stem cells can be successfully used in autologous cellular therapies.

References

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PROMOTER ANALYSIS OF THE RAPI GENES

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The Rap1 genes, closely related to the Ras oncogenes, have been shown to have numerous functions, including qualities as tumor-suppressor genes. While previous research has provided clues to Rap1's roles in cells, nothing is known about the genes' "core" promoter regions—the minimum nucleotide sequence at or near the Transcription Start Site (TSS) that will initiate transcription. Previous research was able to locate putative promoter regions of the human Rap1A and Rap1B genes, and construct plasmid vectors containing these sequences upstream of a gene for green fluorescent protein (GFP). We transiently transfected these constructs into various human cell lines, lysed the cells, and purified their total RNA. Western blotting and fluorescent microscopy have been able to offer qualitative clues about the proficiency of the putative promoters, but Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) has now allowed us to make quantitative functional comparisons. Using a single promoter construct for calibration across experiments, we have successfully identified functioning promoter fragments for the Rap1A and Rap1B genes. Ever-increasing bioinformatics data has helped drive current research to isolate potential enhancer regions upstream of the core promoters, use sub-cloning techniques to insert these regions into our original promoter constructs, and analyze changes in GFP expression. Overall, by finding the "core" promoter and key regulatory sequences, this project is beginning to answer the question of how Rap1 gene expression is controlled in human cells.

SPURIOUS SYMPTOMS CAUSED BY INOCULUM CARRIERS USED IN PLANT DISEASE RESISTANCE EVALUATION

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Symptom development resulting from inoculation and infection with plant pathogens is necessary in order to evaluate plants for disease resistance. The standard technique for infecting soybean (*Glycine max*) with *Fusarium solani*, a root rot pathogen, involves inoculation of growth media with infested seed as an inoculum substrate carrying the pathogen. It has been reported that some uninfested substrates can have adverse effects upon shoot growth and root development.

These effects also include lesion-like necrosis similar to that caused by soybean root rot pathogens. The objective of our research is to determine if uninfested inoculum substrate affects soybean shoot or root growth and root symptom development. Soybean seeds were planted in media inoculated with seed of red sorghum, white sorghum, or sudan grass that had been infested with *Fusarium solani* or remained uninfested. After 14 days of growth, foliar and root necrosis scores, shoot and root dry weights, and stand counts were evaluated. When the effect of uninfested seed was compared with that of infested seed, the uninfested treatment caused more severe root rot symptoms and decreased shoot and root biomass by 20% and 15% respectively. Our results indicate that the uninfested inoculum substrate can cause both spurious growth reduction and symptom development that may mislead the researcher conducting soybean variety evaluations for disease resistance.

PREDICTING SUMMER SUCCESSION PATTERNS OF THREE DOMINANT GROUPS OF PHYTOPLANKTON IN A TEMPERATE DIMICTIC LAKE IN THE SUPERIOR NATIONAL FOREST

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Past research has established a common pattern of seasonal peaks of abundance of major divisions of phytoplankton in freshwater lakes. This common pattern was compared to direct observations of representative genera of three major divisions of phytoplankton in a mesotrophic lake in the Superior National Forest. Samples of phytoplankton were collected from a complete depth profile of Low Lake on five days throughout a 107 day sampling period during the summer of 2012. Abundance fluctuations were fitted to regression models for each representative genus at different depths. Peaks were observed for chlorophyta and diatoms following a cubic regression model, though the peaks occurred later in the summer than expected. Peaks of all cyanophyta followed no clear pattern except for *Microcystis* at the water's surface and *Aphanazomenon* at the thermocline (4m). The peak abundance of *Microcystis* occurred later than expected, while the peak of *Aphanazomenon* abundance occurred earlier. Deviations from the standard summer pattern of succession are accounted for by specific environmental requirements optimal for individual genera. These factors should be taken into account to adjust the common pattern of seasonal peaks to less eutrophic lakes and lakes with less temperature and nutrient fluctuations.

FUNCTIONAL ANALYSIS OF *Chromobacterium violaceum* PIGMENTATION GENES BY PRL27-TN5 TRANSPOSON MUTAGENESIS

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Functional genomics is an area of biology used to describe the relationship of an organism's genome to phenotype. An increasingly common method used to deduce the function of a gene employs transposon mutagenesis and bioinformatics. In this study, we isolated and identified *Chromobacterium violaceum* (hereafter, *Chromobacterium*) from water (phytotelmata) found in the leaves of the bog plant *Sarracenia purpurea* (northern pitcher plant). *Chromobacterium* was mutated with the transposon and kanamycin resistance-carrying plasmid pRL27-Tn5 through conjugation with a donor *Escherichia coli* strain EC100D-pir116. After isolating unpigmented mutant transformants via kanamycin resistance screening, genomic DNA was extracted and purified from each. Genomic DNA from each mutant isolate was then digested with the restriction enzyme *SacII* and circularized with a standard ligation protocol. The circularized DNA was then introduced into recipient *Escherichia coli* EC100D-pir116 by electroporation to isolate the transposon-carrying fragment of each *Chromobacterium* mutant. Each isolated transposon-carrying fragment was then amplified and sequenced. Sequences were compared to available genomic databases, and the mutated genes associated with pigmentation of *Chromobacterium* were identified.

BITTER TASTE RECEPTORS AND THEIR GENES: DO THEY INFLUENCE THE DIET AND LIFESTYLE CHOICES WE MAKE?

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The ability to taste bitter compounds has been well studied, and it has been found that while some individuals exhibit the ability to taste certain bitter compounds, others do not. It has been observed that individuals vary in their perceptions of the chemical phenylthiocarbamide (PTC), which is chemically similar to the substance found in *Brassicca* vegetables, such as broccoli and kale. The genetic basis for this observation has been attributed to single nucleotide polymorphisms in taste receptor gene *TAS2R38*. Questions this project intends to answer are: How does bitter taste perception influence dietary and lifestyle choices in the same group of subjects? And, are there genes other than *TAS2R38* gene that influence bitter tasting ability? Methods involved include extensive primary literature research,

Institutional Review Board approval, recruitment of human subjects, and procedural development and testing of the following techniques: DNA isolation and purification, Polymerase Chain Reaction, digestion by restriction enzymes, DNA analysis by agarose gel electrophoresis and gene sequencing. The polymorphisms in the taste receptor gene will then be compared to the diet and lifestyle choices questionnaire answered by the subjects. The project is anticipated to provide an insight to whether there is a genetic correlation between taste receptor genes and certain choices pertaining to diet and overall healthy lifestyles. All work carried out will be as per IRB approval.

ALTERING PLASMID PRL27 FOR USE IN KANAMYCIN RESISTANT ORGANISMS

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Plasmids have several typical components, including a gene to be translated into a target protein and an antibiotic resistance gene for isolation of organisms transformed by the desired vector. The plasmid pRL27 has both—a transposase-encoding gene for mutating genomic DNA of a host target organism and a kanamycin resistance gene. In our work, we planned to use the pRL27 plasmid as part of a random mutagenesis approach. However, one of our intended target organisms for this plasmid-mediated mutagenesis already has native genome-encoded resistance to kanamycin. Since other gene sequences on the plasmid are necessary for our random mutagenesis approach, the plasmid was altered to contain a different antibiotic resistance gene. Here, plasmid pRL27 was changed by inserting an ampicillin resistance gene and subsequently knocking out its kanamycin resistance gene. The resultant plasmid can be used in future applications with organisms which are naturally resistant to kanamycin.

GENERATING AND IDENTIFYING *Shewanella gopherii* EXTRACELLULAR Fe(III) REDUCTION MUTANTS

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Shewanella species are gram-negative bacteria found in aquatic environments throughout the world. They demonstrate unparalleled respiratory diversity through the process of extracellular respiration. The best-characterized pathway for extracellular respiration is the Mtr (metal-reducing) pathway in the model organism *Shewanella oneidensis* that allows this species to respire insoluble metal oxides. *Shewanella gopherii* appears to

reduce metals as *S. oneidensis* does; however, *S. oneidensis* can generate a current on a suspended carbon electrode, while *S. gopherii* cannot. Characterizing the electron transport chain of *S. gopherii* through identifying its genes essential for reducing insoluble Fe(III) may reveal a key dissimilarity in the extracellular electron transport pathways of *S. gopherii* and *S. oneidensis* that could explain these species' differing abilities to reduce carbon electrodes. Transposon mutagenesis was used to create a library of 5,500 *S. gopherii* mutants that were screened for abnormal Fe(III) reduction capabilities through a ferrozine assay. 61 inhibited and 9 enhanced Fe(III)-reduction mutants were identified. 37 of these 70 mutants had reduction capabilities 0-14% of that of the wild type, suggesting that approximately half of the genes affecting Fe(III) reduction are essential to this process. Selected transposon insertion mutants will be sequenced to identify the genes disrupted in the Fe(III) reduction pathway. Future studies could explore whether the inability of *S. gopherii* to generate a current on a carbon electrode is due solely to its non-motility. Characterizing *S. gopherii* may advance not only the understanding of the evolution of extracellular respiration, but also the biotechnological applications of *Shewanella* species.

DEVELOPING A CHARACTERIZATION METHOD FOR AUTOREACTIVE B CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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The symptoms caused by Systemic Lupus Erythematosus (SLE) are the result of autoimmune cells that bind and elicit an immune response against proteins that are necessary for maintaining healthy body function. A common target for the autoimmune cells of SLE patients is the Ro52 protein. Two peptides from the Ro52 protein, p107 and p277, were tested to determine their binding affinity to the immune cells in SLE patients. Then p107, which displayed better binding, was used to differentiate the surface markers on the different immune cells. This technique can be applied to SLE patients to characterize their autoimmune cells as a method to assess the severity of their disease.

POTENTIAL FOR ENVIRONMENTAL PHOSPHORUS LIMITATION ON THE MAINTENANCE OF LOW PLOIDY MORPHS OF THE NEW ZEALAND SNAIL, *Potamopyrgus antipodarum*

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Background/Questions/Methods: All else being equal, asexual females will produce twice as many daughters as sexual females, which make both male and female offspring. Because only females contribute directly to the rate of population growth, the production of males creates a two-fold cost of sexual reproduction that should theoretically result in the selective elimination of sex. Most research on the advantages of sex has focused on explicitly genetic mechanisms that offset male production costs. A complementary hypothesis posits that competitive interactions between sexual and asexual animal taxa may be mediated by the availability of dietary phosphorus (P). The role of P may be important when asexual animals have higher ploidy and per-mass P-rich nucleic acid content than sexual counterparts and environmental P availability limits biomass production. Here we evaluate aspects of the phosphorus hypothesis using the New Zealand freshwater snail *Potamopyrgus antipodarum*. This species has obligately sexual and obligately asexual individuals that co-occur in lakes across New Zealand. Asexual *P. antipodarum* are polyploid (triploid (3x) and >3x) and sexuals are diploid, and there is substantial across-lake variation in the relative frequency of sexual and asexual snails, and in the relative frequency of triploid and >3x asexuals.

Results/Conclusions: Consistent with the phosphorus hypothesis, we found in a lab study that individual growth rate decreased more under P scarcity for asexual tetraploids than asexual triploids. In a survey of 15 lakes across the South Island of New Zealand, we collected information on rates of biomass production in algae (the main food source of *P. antipodarum*), algal elemental composition, and elemental constraints on algal production. We also collected information on alkaline phosphatase expression in snails (an indicator of P limitation) and ploidy composition of local snail populations. Our results suggest that algal production in South Island lakes is strongly limited by nitrogen (N) availability. However, we also found evidence of NP co-limitation that varied in strength across lakes. We found no evidence for P limitation of algae or snail production in the absence of N additions. These results suggest that P limitation could influence competitive interactions within *P. antipodarum* populations to a variable degree across lakes. Our results also suggest that future work should investigate how N limitation and NP co-limitation differentially affect asexual and sexual individuals in this system.

THE EFFECTS OF DIFFERENT NITROGEN FERTILIZER RATES ON SOIL CHARACTERISTICS, PLANT PROPERTIES AND ECONOMIC RETURNS IN SOUTHEASTERN MINNESOTA CORNFIELDS

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Nitrogen fertilizers are essential to producing enough food for a growing human population. However, they can have negative environmental and economic effects, especially when applied in excess. We sought to examine these effects and optimize nitrogen application for no-till corn (*Zea mays* L.) fields in southeastern Minnesota. We examined two corn fields with different rotation histories: Field 1 was planted in the fourth year of continuous corn, and Field 2 was in its second year of corn following seven years of alfalfa. Different nitrogen levels were applied to each field, and soil characteristics, macroinvertebrates, plant properties, yield, and economic returns were assessed to evaluate the effects of these treatment levels. Soil nitrate concentrations were significantly greater under higher nitrogen treatments, suggesting that they could contribute to pollution through leaching or runoff. Other soil characteristics were not significantly affected by nitrogen treatment. Similarly, macroinvertebrate abundance and diversity did not differ by nitrogen treatment, although Field 2 had significantly more macroinvertebrates than Field 1, perhaps due to the prior alfalfa crop. Higher fertilizer levels increased corn height up to a threshold above which additional nitrogen did not contribute to plant growth, but nitrate concentrations in corn stalks showed no significant trends. Yield and economic returns did not differ significantly by nitrogen treatment, which indicates that farmers could reduce their nitrogen use without suffering financially. Field 2 had significantly greater yields, demonstrating the benefits of including alfalfa in rotation. This on-farm research helps optimize management practices on a localized scale.

A COMPARISON OF RECONSTRUCTED AND REMNANT PRAIRIES USING SMALL MAMMAL COMMUNITIES

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Native prairies have drastically decreased in size and distribution across the United States in the past 140 years. Reconstructions or restorations make up an increasing portion of prairie habitat in the United States today. This

study compares the structure of small mammal communities of a reconstructed prairie (Schottler Wildlife Management Area) to a remnant prairie (Jay C. Hormel Nature Center) to determine if reconstructions are successful in replicating a remnant prairie. The two sites are located six miles apart near Austin, MN. Trapping occurred over a span of five nights at each site to gather data on small mammal populations and biodiversity. Most commonly trapped mammals at the Schottler WMA site included meadow vole (*Microtus pennsylvanicus*) (n = 168), prairie vole (*Microtus ochrogaster*) (n = 116), and *Peromyscus* species (n = 22). Trapped mammals at the Hormel site included those found at the Schottler WMA (n = 52, n = 31, n = 4 respectively). Data analysis shows that the reconstructed prairie had a higher population size of meadow voles per acre and *Peromyscus* species (p = 0.045 and 0.039 respectively), and a comparison of the prairie vole populations suggested differences may be present as well (p = 0.061). The biodiversity of the remnant prairie was higher than the reconstructed prairie, though differences were not significant (p = 0.60). This study suggests that the Schottler WMA supports a greater number of small mammals than the remnant Hormel prairie, though it is unclear why the reconstruction is a better habitat for small mammals. More research is required to be sure that future reconstructions are successful.

THERMAL MONITORING OF KARST SPRINGS IN THE TWIN CITIES METROPOLITAN AREA

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Subsurface flow in karst aquifers depends primarily on the locations and geometry of preferential flow paths such as conduits and large fractures; one challenge in predicting an aquifer's response to changes such as contamination or pumping is that these exact flow paths are generally not well known. Thermal monitoring of springs is an inexpensive means to study flow geometry in aquifers; in particular, it can identify whether groundwater is in thermal equilibrium with an aquifer, where disequilibrium generally indicates shorter residence times and flow in larger spaces (conduits or fractures). We are currently monitoring three Platteville Limestone springs in the Twin Cities metropolitan area: Chalybeate Springs, Coldwater Spring, and St. Mary's Spring. Early results show that all three springs' water temperature tended to decrease from early January to early February, with Coldwater and Chalybeate decreasing smoothly and considerable fluctuation at St. Mary's Spring; the springs were also variable in absolute temperature and in the size of the decrease. Data from subsequent months should yield more evidence on whether water temperature changes are in phase with seasonal surface temperature,

and on the relationship between surface temperature and groundwater temperature at St. Mary's; in-phase relationships between surface temperature and groundwater temperature, as well as associations between surface and water temperature fluctuations, would indicate shorter residence times and greater conduit/fracture flow.

MONITORING AND EVALUATING BOX PAIRS ON THE ST. OLAF BLUEBIRD TRAIL

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In 1992, St. Olaf College established a bluebird trail on its natural lands in an effort to help restore the much-diminished eastern bluebird (*Sialia sialis*) population. Bluebird boxes are monitored annually and nest data has been recorded since the establishment of the trail. In the interest of increasing bluebird nest box occupancy, several years of nest box data were statistically analyzed to determine whether the bluebird trail boxes had significantly increased bluebird occupancy and whether the surrounding environment (site type) affected bluebird occupancy. Linear regression performed on fledgling proportion data revealed that the annual proportion of nest box fledglings that are bluebirds has significantly increased from 2005-2012, and a two-way ANOVA comparing site type to year showed no significant differences among years and site types. However, box pairs in prairie sites had more successful bluebird nests than box pairs in forest sites. Qualitative observations suggest that bluebirds nesting in boxes closer to trees experience higher predation and the boxes are primarily occupied by house wrens (*Troglodytes aedon*). Boxes in open prairie with tall vegetation mainly have tree swallows (*Tachycineta bicolor*), and research suggests that tall vegetation conflicts with bluebirds' insect-hunting strategies. Boxes located near soccer fields are primarily occupied by house wrens and tree swallows, and research suggests that this is because house wrens have a much higher tolerance of human disturbance than bluebirds. Suggestions were made to relocate low-bluebird-occupancy box pairs out of tall grass, away from forest edges, and away from areas of frequent human disturbance.

BROMELIAD DISTRIBUTION IN PEJIBAYE DE JIMENEZ, COSTA RICA

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Research exists on the distribution and habitat preferences of countless terrestrial plants. However, similar research concerning tropical epiphytes is less available.

Epiphytes live in varied and oftentimes harsh conditions, and inquiry into the distribution of this group provides a basis for understanding niche partitioning in tropical forests. Therefore, I conducted fieldwork to better understand the distribution of tree bromeliads on a group of trees, as well as the spatial distribution of bromeliads within tree canopies. The study was conducted in rural Pejibaye de Jiménez, Costa Rica from February 27 to April 20, 2012. I recorded the circumferences and diameters of the tropical madero negro *Gliricidia sepium* (Jacq.) Kunth ex Walp. Bromeliads living on the fence were counted and measured for their size. I photographed the canopies of 60 trees, taking one photograph for each of the four cardinal directions around the trunk of the tree. My results showed a strong positive relationship between tree size and bromeliad size. However, there was not a relationship between tree size and the number of bromeliads growing on each tree. I found that bromeliads preferred to grow on the east side of trees as compared with the west side. It is possible that precipitation brought by trade winds influences this trend, as water is the most limiting resource for tree bromeliads. My research provides information about a highly varied group of tropical plants, and raises further questions about the niche partitioning of species within the family Bromeliaceae.

ANTI-MALARIAL ACTIVITY OF NEW ARTEMISININ DERIVATIVES

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This project investigated the activity of four new artemisinin-based compounds against *Plasmodium falciparum* *in vitro*. The resistance of *P. falciparum* to many current anti-malarial medications impresses a need for new drugs. The tested compounds are the latest product of a twenty year collaboration between two laboratories (Chemistry and Clinical Pharmacology) at Johns Hopkins that have investigated the potency and chemical structure – antimalarial activity relationships of over 1000 antimalarial compounds. 96-well plate-based assays were conducted with serial dilutions of each compound (in quadruplicate), and parasites. Plates were incubated for 48 hours at 37 °C in 3%O₂/4%CO₂/93%N₂. [³H]hypoxanthine was then added, the cells were incubated for another 24 hours, and harvested. Incorporation of radiolabel into parasite nucleic acids was determined by scintillation counting. Dose response curves were plotted and EC₅₀ values were mathematically interpolated. The EC₅₀s indicate the potency of the compounds against malaria parasites. Antimalarial activity was correlated with chemical structure of the test compounds.

SULFURIC ACID NUCLEATION: A SYSTEMATIC STUDY OF THE EFFECT OF BASES

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Nucleation of particles composed of sulfuric acid, water, and nitrogen base molecules was studied using a continuous flow reactor. The particles formed from these vapors were detected with an ultrafine condensation particle counter, while vapors of sulfuric acid and nitrogen bases were detected by chemical ionization mass spectrometry. The effects of ammonia as well as several different amines were investigated. Enhancement of particle numbers when ammonia or amines were added indicates they have powerful effects on the nucleation properties of sulfuric acid vapor. Power dependencies for particle numbers on sulfuric acid and nitrogen bases will be presented. Particle detection efficiencies, the extent of particle growth, and cluster thermodynamics will be discussed with the help of computational fluid dynamics simulations of the flow and chemistry within the flow reactor.

AN ANALYSIS OF CYTOKINE GENE EXPRESSION IN ACUTE AND SUB-ACUTE MURINE MODELS OF VULVAR HYPERALGESIA

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Vulvodynia is a prevalent condition described as chronic vulvar pain. Recently, studies have shown that vulvodynia patients have higher levels of Interleukin -1 Beta (IL-1 β) and Tumor Necrosis Factor-Alpha (TNF- α) in their tissue biopsies compared to controls. Additionally, it has been shown that there is a relationship between the onset of vulvodynia, and a history of allergies and allergic reactions in women experiencing these symptoms. We have established an allergy-induced vulvar hyperalgesia model in mice, using sensitization and challenge with allergen oxazolone, and sought to examine the gene expression levels following oxazolone induced contact hypersensitivity of the labia. Our study found that mRNAs encoding proinflammatory cytokines IL-1 β , TNF- α , IL-6, IFN- γ , as well as chemokines CXCL1 and CXCL2, were highly upregulated at 6 and 24 hours post challenge. Additionally, oxazolone challenge resulted in increased mechanical hypernociception at 1, 3, 6, and 24 hours post challenge, as well as 24 hours and 5 days following three consecutive days of challenges in pre-sensitized ND4 mice. Furthermore we found that following 3 consecutive oxazolone challenges, these mRNAs were highly abundant 24 hours after discontinuing challenges, but resolved 5 days after the final challenge, even though hyperalgesia was still

discernible. These results suggest that pro-inflammatory cytokines contribute to vulvar hyperalgesia, and that this allergen-induced hyperalgesia is still present following resolution of inflammation.

SYNTHESIS OF AN EXTENDED ORGANIC LIGAND FOR COUPLING WITH A POLYOXOMETALATE

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The primary goal was the synthesis of a triol functionalized organic ligand for future coordination with a vanadium-based polyoxometalate (POM). Such a compound may result in catalytic properties and further understanding of metal-organic frameworks (MOF). The synthesis began with 1,3-dibromobenzene and was further extended. The first step of the organic synthesis involved a Sonagashira coupling of trimethylsilylacetylene with the 1,3-dibromobenzene to form 1,3-bis[(trimethylsilyl)ethynyl]benzene and was achieved with 18.85% yield. The second step involved deprotecting the ethynyl groups by removing the trimethylsilyl groups for further extension of the ligand. Both products were characterized via ¹H NMR spectroscopy. What is of particular interest with the potential synthesis of a 1,3-extended ligand is the potential for forming a cyclic network composed of six ligands bridging six POMs, thus allowing for solubility and full characterization.

PALEONTOLOGIST EXPERIENCE AT HELL CREEK FORMATION IN EASTERN MONTANA

Jasmine Hanson & Ron Nellermeoe (Advisor)
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This is a learning study on the topics of geology and paleontology in Late Cretaceous Hell Creek formation. This is an area where many Cretaceous dinosaur species and ancient fauna have been preserved from deposits over a span of time to approximately 65 million years ago in the Cretaceous period (Fastovsky et al., 1998). Articulated fossils are most commonly found in Hell Creek floodplains and water channel deposits from the Ancient Inland Seas (Fastovsky et al., 1998). This location also found evidence of gradual extinction of the Cretaceous period dinosaurs (Sloan et al., 1986). Field research in paleontology is an observational ongoing long term program at Concordia College in this area. On this annual trip students become skilled at interpreting the earth and its history by analysis of identifying sediments of this area including mudstone, sandstone, non-coalified and coalified (Fastovsky et al., 1998). The study conducted in the Summer of 2012 had several significant

findings; the discovery and restoration process these will be emphasized and followed throughout this paper. Fossil discoveries are marked using GIS mapping and excavated in the Hell Creek formation. Documented material were then secured and labeled for storage and transport to be restored as an ongoing process in Moorhead Minnesota. Structures were identified using texts, interpretations of past findings, and mentors help. They are then evaluated for evolutionary anatomical significance, to allow students to be a part of the bigger picture in geological, and faunal history.

CHARACTERIZATION OF A NOVEL SYNTHETIC PROTECTING GROUP: TRI-T-BUTOXYSILANE

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Tri-t-butoxysilane (TBOS) serves synthetic chemists as a means to increase chemoselectivity of chemical reactions involving multifunctional amine substituted reagents. TBOS acts by means of SN2 reaction, effectively hindering the amine group's reactivity due to the extreme steric and electronic hindrance characteristic to this protecting group. Once TBOS has bonded to the amine group its characteristic steric and electronic substrate shielding allows for a quick and easy purification by silica column. The final utility of TBOS is its excellent deprotection character; it's stable under aqueous conditions and can be cleaved by raising the pH.

COMPARISON OF SMALL MAMMAL COMMUNITIES ON NATIVE AND RESTORED PRAIRIES IN NORTHWESTERN MINNESOTA WITH SPECIAL EMPHASIS ON HABITAT CHARACTERISTICS

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Over the summer of 2012, we conducted small mammal trapping on Concordia College's Long Lake Field Station which has several recently restored prairie plots, as well as trapping preserved prairie sites. Our goal was to compare small mammal communities on the restored sites and well-established and preserved prairie sites. Trap grids were set and checked for three to four days. When a mammal was caught, species, mass, and sex were all recorded and marked. Dominant mammals were meadow voles (*Microtus pennsylvanicus*) and thirteen-lined ground squirrels (*Ictodomys tridecemlineatus*) at a majority of the prairie sites. Habitat data was gathered for gopher mound area, height of living

and dead vegetation, and litter depth. Regression analysis was used to investigate the relationship between habitat data and mammal diversity and evenness. We found a positive correlation between living vegetation and species diversity. We present capture results for comparison of these restored and preserved prairies.

CALCULATIONS OF RELATIVE ENHANCEMENT FACTORS FOR XANTHENE DYES USING SURFACE ENHANCED RAMAN SPECTROSCOPY

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Identification of pigment or dyes used in a work of art provides historians with interesting information about techniques and materials used during a period of time or by that artist. Art conservationists employ non-destructive analytical techniques for accurate identification of an artist's materials for restoration efforts. Raman spectroscopy provides a molecular fingerprint and can be used to identify many dyes and pigments. The use of conventional Raman spectroscopy for the study of dyes has two disadvantages; low sensitivity and competition with fluorescence processes producing background signals that mask the Raman signal. Surface-Enhanced Raman Spectroscopy (SERS) has been shown to be a valuable tool for the non-destructive analysis as it is able to overcome the aforementioned disadvantage of conventional Raman spectroscopy. The use of SERS by art conservationist has grown over the last decade and is applied to the hydroxyxanthene dyes in this work. Geranium Lake, an artist pigment known to contain hydroxyxanthene dyes, possesses a brilliant bluish red color and was used by Vincent Van Gogh at the end of his career. This study expands on previous work to fully characterize eosin, erythrosine, rose bengal, and phloxine, all halogenated hydroxyxanthene dyes, using SERS. We present a SERS detection limit study of eosin, erythrosine, rose bengal, phloxine and the dehalogenated dye fluorescein. The relative SERS enhancement factors of these dyes are reported, demonstrating the sensitivity of this technique.

PRODUCTIVITY AND SOIL CHARACTERISTIC PATTERNS IN A TALLGRASS PRAIRIE CHRONOSEQUENCE

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With more than 150 acres (60ha) of agricultural land restored to tallgrass prairie over a period of 15 year, the St. Olaf Natural Lands provide an excellent

opportunity to investigate changes in prairie ecosystems over time. The objectives of this study were to determine if plant production or soil characteristics change as prairies age, and to describe the effects prescribed fire has on these features. Seven St. Olaf prairies and a nearby remnant prairie were sampled. Aboveground net primary productivity (ANPP) tended to decrease with prairie age, which agrees with previous studies on these prairies. Fire also affected ANPP; previously unburned areas showed a 40-75% increase in ANPP after burning. No significant differences in species diversity or richness across prairies were observed, but a significant shift in plant species composition with age was demonstrated by higher percent grass biomass and lower percent forb biomass in older prairies. Physical soil characteristics changed with prairie age; soil organic matter and soil moisture decreased with prairie age while bulk density increased. Nitrogen levels were variable among prairies, and burning did not cause any significant differences in soils. Understanding these changes in restored grasslands will ultimately lead to better management and more successful restoration practices.

TRANSMISSION OF BARLEY/CEREAL YELLOW DWARF VIRUSES BY APHID VECTORS

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Barley and Cereal yellow dwarf viruses (B/CYDV) negatively impact the fitness of host plants and have a destabilizing effect on established ecosystems. Nitrogen and phosphorous input into ecosystems is increasing worldwide, which can strongly alter both plant and pathogen dynamics. Viruses rely on interactions between plant hosts, insect vectors and environmental factors in order to spread. Hence, relationships between virus transmission and both the level of and ratio between host nitrogen and phosphorous resources are difficult to establish, especially in natural systems, and remain poorly understood. We developed a method of inoculation of healthy plants using aphid vectors that previously fed on virus infected fresh plant leaves. While we originally tested an inoculation method in which aphids fed on a homogeneous virus inoculum obtained by grinding and clarifying infected plant tissues. This resulted in low aphid survival and low virus transmission rate in our lab conditions. We anticipate that the technique developed in this study will allow us further insights into the interactions between host nutrient levels and ratios, and virus transmission rate. The results obtained under laboratory conditions will allow us to make predictions on the broad connections between human nutrient loading and rates of viral infection.

SYNTHESIS OF LATE METAL IMIDES: SILYLAMIDES AS NITRENE DELIVERY AGENTS

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Coordination complexes containing multiple bonds between late transition metals and nitrogen are useful intermediates in a range of synthetic applications. We have investigated silylamide complexes of Co, Rh, and Ir supported by bis(phosphine) ligands as possible precursors to metal imides (M=NR). New complexes have been characterized by multinuclear NMR spectroscopy and X-ray crystallography, and current efforts are focused on probing their reactivity with silylium extraction agents for various synthetic applications.

INFLUENCE OF CA²⁺ AND MG²⁺ ON THE ANTIMICROBIAL PROPERTIES OF KOMBUCHA FERMENTS AGAINST BACTERIA AND YEAST

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Kombucha tea ferment is depicted to have a high source of nutrition, aiding in metabolic function and maintaining a symbiotic relationship with acid-loving bacteria and yeast. Understanding these relationships between diet, gut micro flora, and health is an increasingly important area of research. Recent studies have demonstrated that Kombucha tea provides variable antimicrobial activity against pathogenic microbes. With the increasing consumption of Kombucha tea, a beverage used for pro-biotic and other health benefits, it's important to explore the antimicrobial properties of Kombucha that may help maintain normal microbiota of gut. In this study, we tested Kombucha tea for antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and yeast using an agar diffusion method. Standard zone-of-inhibition assays were used to test the hypothesis that the variance in antimicrobial activity against *Saccharomyces aureus*, *Escherichia coli* and *Candida albicans* is due to the varying levels of dissolved minerals (calcium, magnesium, etc.) found in different water-types (well water, artesian water, city water, type-II water and distilled water). Results indicate that high-Ca²⁺, -Mg²⁺ content water correlates to the greatest zone-of-inhibition against *S. aureus*, with a 22% difference when compared to distilled water (no Ca²⁺ or Mg²⁺). Additionally a 5.5% linear difference was seen between each of the water-types, following a gradient trend. *E. coli* maintained a constant zone-of-inhibition

regardless of water-type, while *Candida* showed no zone-of-inhibition. These results indicates a direct relationship between Kombucha prepared with high-Ca²⁺, -Mg²⁺ content water and the antibacterial activity against *S. aureus*. These exciting observations are also suggestive of further study to determine how this relationship correlates with human colonic microbiota interactions and their microbial metabolic profiles. Our results highlight the improvement of the Kombucha fermentation by using a variety of cations at different concentrations. Strong antimicrobial potentials were found, particularly against *S. aureus*, which may be very useful to determine alternative approaches to synthetic antimicrobial drugs. Further research is needed to understand related active compounds and their mechanisms of action.

VALIDATING A NOVEL IMMORTALIZED HYPOTHALAMIC CELL LINE FOR STUDYING GENE EXPRESSION ALTERED IN DIET- INDUCED OBESITY

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Obesity, a major and growing health concern in the United States, is correlated to serious comorbidities such as diabetes mellitus (type II) and cardiovascular disease. Previous research from our lab has emphasized the importance of several hypothalamic neuropeptides important in energy expenditure, feeding, and activity that may be altered during the development of obesity. To better characterize interactions between these genes and neuropeptides within the brain, appropriate neuronal cell culture models must be identified. The primary objective of this study is to validate an immortalized adult-derived mouse hypothalamic cell line, CLU468, as a suitable model for studying gene expression altered in diet-induced obesity. Preliminary research on the expression of eleven genes important in energy expenditure, feeding, and activity was done using quantitative real-time RT-PCR. Total RNA was isolated from both the cell line and a positive control (mouse brain). Data from PCR amplification curves and melting peaks were used to determine which genes of interest were present in the cell line samples by comparison to the positive control. PCR products were visualized using agarose gel electrophoresis in which the expected bands for the genes of interest were revealed. Additionally, PCR products will be sequenced to validate specificity of gene amplification. Our data will be used to determine if the cell line CLU468 warrants further investigation for studies evaluating hypothalamic function, and whether this cell line may be

used as a suitable model for studying gene interactions in obesity-induced hypothalamic dysregulation.

PERILIPIN 2 LOCALIZATION TO THE SURFACE OF LIPID DROPLETS

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Perilipin 2 binds to the surface of lipid droplets in most eukaryotic cells. However, the conditions that allow perilipin 2 to distinguish the lipid droplet surface from other intracellular organelles remain undetermined. Here, we describe the development of an *in vitro* assay to identify features of the lipid droplet that mediate perilipin 2 binding. We show that protease treatment of isolated lipid droplets does not prevent perilipin 2 binding, and that perilipin 2 can bind directly to synthetic liposomes (which lack proteins or a neutral lipid core). Moreover, the binding of perilipin 2 to synthetic liposomes is influenced by the phospholipid composition of these vesicles. Analysis of perilipin 2 binding suggests that lipids that reduce the fluidity of the phospholipid monolayer, such as cholesterol or phospholipids with saturated acyl chains, decrease the binding of perilipin 2 to liposomes. Future experiments will further probe the characteristics of the lipid droplet surface that are required for perilipin 2 binding and determine if similar features of the lipid droplet surface mediate the binding of other lipid droplet-associated proteins.

THE EFFECT OF SHRUB ENCROACHMENT ON HARVESTER ANT AND LIZARD COMMUNITIES OF THE SEVILLETA NATIONAL WILDLIFE REFUGE

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The encroachment of woody shrubs on previously established grasslands is causing broad-scale habitat reorganization of southwest American ecosystems. As shrubs continue to expand their northern distribution boundaries, other plants and animals are being affected as well through proposed mechanisms of increased winter temperatures and the disappearance of grasses (Brown 1997; D'Odorico 2010). This increase in temperature could especially impact exothermic animals, including seed harvester ants and lizards that inhabited these sites before shrubs encroached. While numerous studies have shown the impact of shrub encroachment farther south where shrubs have already taken displaced grassland systems, little work has been done examining shrubland-grassland transition zones (Lalibaerte et al. 2004; Brown, Valone, and Curtin 1997). The Sevilleta National Wildlife Refuge presents an ideal location to conduct such research as it remains a primarily desert grassland system, but has

experienced the beginnings of shrub encroachment by the native creosotebush, *Larrea tridentata*.

This study compared the harvester ant and lizard communities of two sites on the SNWR, a grassland and a creosote ecotone, in order to predict possible future scenarios for these two animal groups in the face of shrub encroachment on the refuge. Twenty 40m x 40m plots were surveyed at each site, for a total of forty plots. In each plot the presence of harvester ant nests and individual lizards was noted. Ants were identified to species and lizards were identified by feeding guilds, active foragers or sit-and-wait predators. The number of nests of two commonly found harvester ant species, *Pogonomyrmex rugosus* and *Aphaenogaster cockerelli*, were calculated as densities per 100 meters squared. The same calculations were done for the total number of lizards found in each plot as well as the number of active foraging lizards and sit-and-wait lizards. After testing the differences in means for the densities of each group separated by habitat type with Kruskal-Wallis tests, no comparison yielded a statistically significant result at the 0.05 level. However, both the differences of *P. rugosus* nests and active foraging lizards across habitats displayed marginal significance. We hypothesize several factors contributing to the lack of significant differences between the habitat types. In the case of the ants, the species that were sampled are long-lived and can persist unaffected by a disturbance such as a change in vegetation type for decades. However as an extremely motile group the lack of a significant difference between the lizard communities of these two sites suggests that they are not affected by the encroachment of woody shrubs at this stage in transition.

AFTER THE SPRAY: INVESTIGATING THE FATE OF PARTICLES FROM CYCLODEXTRIN-CONTAINING AIR FRESHENERS

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The indoor environment contains many pollutants that can be damaging to health and offensive to human senses. A new generation of air fresheners contains beta-cyclodextrin (β -CD), a cyclic glucose oligomer that is reported by manufacturers to “eliminate” odors—rather than masking them with fragrances—by trapping offensive-smelling molecules in its core, rendering them scent-free. Aerosol Time-of-Flight Mass Spectrometry (ATOFMS) was used to analyze aerosolized mixtures of modified β -CD and a simulated indoor pollutant, N-(1-Naphthyl)ethylenediamine (NED), which have been shown to form a complex in solution. NED was selected as a model pollutant due to its UV-absorbing naphthalene (main constituent of mothballs) core and its protonatable amine moiety. A UV-absorbing benzoylsulfonate chromophore was synthetically attached

to β -CD to enhance ionization of the molecule in the ATOFMS and this modified β -CD (SulfBz- β -CD) was used in all subsequent experiments. Experiments were done to simulate real household use of cyclodextrin-containing air freshener in which SulfBz- β -CD was sprayed onto surfaces containing NED. These surfaces were agitated in order to simulate household activity that would resuspend settled odor-causing pollutants, cyclodextrin from the air freshener, and pollutant/cyclodextrin complexes. Markers that indicate complexation and resuspension of NED in SulfBz- β -CD-containing mixtures have been identified in the ATOFMS spectra, leading to the possibility of identifying the various fates of β -CD/pollutant complexes in real-world particles produced by consumer air freshener use in the home.

ECOLOGICAL STUDIES OF A PERENNIAL WOODLAND PLANT, *GEUM MACROPHYLLUM*, AND POSITIONAL CLONING OF A GENE FOR MERISTEM DEVELOPMENT IN CORN

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Large-leaved avens *Geum macrophyllum* is an herbaceous perennial found in moist woods and meadows, which produces spherical infructescences (seed heads) containing several hundred seeds. Each mature fruit (hereafter, seed) has a single elongated burr adapted to animal dispersal. In the population under study, we observed a 10-fold variation in seed mass in the *G. macrophyllum*. Our research is an attempt to understand the potential modes of selection maintaining the variation in seed mass. We examined the influence of overwintering conditions on germination and development, the influence of plant size on subsequent growth and competitive interactions, and variation in burr morphology within seed heads.

Maize (corn) is an important crop as well as a model organism for scientific investigation. In this study, we examined an inbred line of maize which had been exposed to chemical mutagenesis (ethylmethane sulfonate). A mutant phenotype with reduced meristem development was identified. The objective of our research is to identify the location of the gene for meristem development, which is known to be located on chromosome 1 within a region of several million base pairs. In this work the recently completed sequence of the entire maize genome allowed us to use several known markers associated with specific PCR primers to narrow down the location of the gene. Polymerase chain reaction and DNA sequencing to locate the gene for meristem development to a relatively small region. Further work will examine the mutant phenotype for closely linked genes changed in the mutagenesis event,

which can be sequenced and compared to the sequence of the wild type inbred line to determine the nature of the mutation.

A NEW INSTRUMENT FOR ANALYSIS OF SINGLE AEROSOL PARTICLES: THE COUPLING OF ATOFMS AND LIBS

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Knowledge of aerosol particle composition is crucial in order to gain an understanding of health and environmental effects of atmospheric particulate matter, and requires measurements that can provide detailed information about chemical components. An Aerosol Time-of-Flight Mass Spectrometer (ATOFMS) is used for the semi-quantitative analysis of the composition of individual atmospheric particles upon laser-induced desorption and ionization. Integration of the spectroscopic technique of Laser-Induced Breakdown Spectroscopy (LIBS), in which light emitted by the elemental components of the particle upon irradiation with a laser is collected, should increase the quantitative data provided by this instrument. Due to the similarities between both techniques, integration of the two should be straightforward and requires only minor additions to the ATOFMS. This presentation will focus on the advantage of coupling these two techniques as well as the inherent physical and electronic challenges, including: plasma generation (excitation laser pulse energy), plasma dynamics in an electric field and vacuum conditions, emission detection (collection angle and distance from the light source), and software program design and implementation. These challenges are being systematically addressed and are presented here as evidence of the range of issues that arise in producing a new single-particle analysis instrument.

DNA BARCODING: DEVELOPING A PROTOCOL TO DETERMINE AVIAN PREDATION PREFERENCE IN THE GOLDENROD GALLFLY SYSTEM

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Interspecific community interactions of predation and parasitism can be challenging to track in complex systems. In the case of the goldenrod gallfly system, it is unclear whether two bird predators, the downy woodpecker (*Picoides pubescens*) and black-capped chickadee (*Poecile atricapillus*) have a preference for which larva they predate. We hypothesize that birds

prefer larger goldenrod gallfly larva (*Eurosta solidaginis*) over either of the parasitoid species, the wasp (*Eurytoma gigantea*) or the beetle (*Mordellistina convicta*). However, when analyzing galls attacked by birds, it is not yet possible to decipher which parasite species the bird attacked. We are developing a protocol to use DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COI) gene in order to determine species preference. Our protocol includes DNA extraction from the larvae and from a scraping of the empty gall chamber, then amplification and sequencing of the COI gene. There is currently a known COI sequence for *Eurosta solidaginis*, but we seek to find the sequence for *Eurytoma gigantea* and *Mordellistina convicta*.

THE TRADE-OFF BETWEEN PHOTOSYNTHESIS AND PHOTO PROTECTION

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Every plant must balance its photochemistry between performing photosynthesis, protecting itself from toxins produced by the photosynthetic process, and fluorescing. It is commonly believed that when a plant has a high level of photosynthesis it must have a trade off with photoprotection and expels less energy breaking down and recycling compounds such as hydrogen peroxide produced in the light-harvesting phase of photosynthesis. Plants that follow this method of balance will use less energy to create and maintain antioxidants to participate in photoprotection cycles such as hydrogen peroxidase recycling. This can be measured by looking at the rate of antioxidant activity from leaf samples.

In this experiment the gas exchange data of three common garden crops, *Solanum lycopersicum* (tomato), *Phaseolus vulgaris* (green bean), and *Solanum tuberosum* (potato) were compared with two antioxidants, glutathione reductase and ascorbate peroxidase, that comprise of the H₂O₂ recycling cycle along with the amount of chlorophyll a and b, the grams of protein per gram fresh weight, and a comparison of fresh weight and dry weight. This data was compared between the three different plants, two of which had a high level of photosynthesis, and other with a relatively low level. An interesting result of this experiment was that the antioxidant glutathione reductase of the hydrogen peroxide recycling cycle did not conform to the energy trade off when it was compared with the three species. This is especially interesting since the other antioxidant, ascorbate peroxidase, did in fact follow the trade off.

DNA BARCODING: A COLLABORATIVE RESEARCH PROJECT BETWEEN THE BIOTECHNOLOGY PROGRAM AND THE URBAN FARM COLLECTIVE, LED BY A PSEO BIOTECHNOLOGY STUDENT

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DNA barcoding involves identifying species using short, standardized gene region(s). Why do we need DNA barcoding? Although there are millions of known species, many still are yet to be identified. Interbreeding capabilities, morphological variations and ecological context, and genetic similarities add to the challenge of species identification using traditional taxonomy methods. Through DNA barcoding, it is possible to identify the origin of species or the individual source of consumer products (meat, vegetables, etc). At MCTC we are mapping the various cultivars planted by the Urban Farm Collective group. Barcoding will provide insight about their origins and perhaps new information. The project involves identification, documentation, and collection of specimens, DNA extraction, ‘barcode’ amplification and comparison with a barcode database using bioinformatics. The choice of barcoding region is important: ideally it will be a single DNA locus which distinguishes species, but is similar enough to be compared. The chloroplast genes *rbcL* and *matK* are utilized for universal plant barcodes. This project enables the implementation of various new competencies in multiple science courses and STEM workshops for high school students, to introduce biotechnology, botany and gardening opportunities for practical inquiry. It has proven that advanced techniques can be conducted by freshmen and PSEO students. We thank the Dolan DNA Learning Center, Cold Spring Harbor Laboratories, New York.

SYNTHESIS OF AMIDES USING A CATALYZED CONTINUOUS FLOW PROCESS

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The result of research efforts focused on the development of a more efficient, economical, and environmentally friendly method of amide synthesis has led to a novel catalytic method for the conversion of nitriles and amines into amide products. A lab scale continuous flow reactor system fitted with a titanium dioxide packed catalyst column was successfully used to convert several amines into amide products using acetonitrile and water. A method for the successful isolation of amide products was developed and the reactor

conditions optimized. The conversion rates of investigated amines demonstrate improved efficiency and comparable yields to cited literature values.

ACTIVITY PATTERNS IN THE GLOBUS PALLIDUS, THALAMUS AND SUB-THALAMIC NUCLEUS IN AKINETIC-RIGID AND TREMOR PREDOMINANT PARKINSONIAN PATIENTS

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Introduction: Parkinson's disease (PD) is characterized by a loss of dopamine producing cells in a brainstem nucleus called the substantia nigra pars compacta. The loss of these cells impacts the function of several brain regions, especially the basal ganglia structures, which are intimately involved in voluntary movement control as well as cognition and other functions. Tremor in the extremities, stiffness of joints, gait and balance problems are some of the cardinal motor symptoms of PD. However, the exact combination of symptoms (both motor and non-motor) expressed in a patient and its severity varies from person to person. Imaging studies have shown differences in the dopamine uptake patterns in the basal ganglia nuclei between the various parkinsonian phenotypes.

Objective: This study aims to quantify the physiological changes in the activity patterns of neurons in the globus pallidus and subthalamic nucleus and to contrast the changes between various PD phenotypes. The STN and pallidum are of specific interest because deep brain stimulation (DBS) therapy or lesioning in these structures has shown to be effective in minimizing many of the motor symptoms of PD.

Methods: Patient's enrolled for DBS or lesion surgery to alleviate parkinsonian symptoms were identified from a movement disorders database. Patients for whom both the complete OFF medication unified Parkinson's disease rating score (UPDRS) and electrophysiology data from the globus pallidus or STN were available were included in this study. Patients were sorted into the tremor dominant or akinetic rigid group based on their UPDR score. The average scores relating to tremor symptoms were divided by the average rigidity and posture scores to create a ratio. Patients were then put into groups according to their ratio score. The times of occurrence of action potentials from single neurons were identified from the electrophysiology records. These timestamps were used to calculate the average discharge rate of the cell and to identify and quantify the presence of bursting and oscillatory activity.

Results: A total of 61 patients have been identified from the database. 23% (n=14) patients were

characterized as tremor-dominant and 77% (n=47) as akinetic-rigid. A total of 40 electrophysiology recordings from the pallidum and 27 from the subthalamic nucleus (STN) are available for the tremor- dominant group. For the akinetic-rigid group 305 and 145 cells are available from the pallidum and STN respectively. We are currently in the process of quantifying and comparing the neuronal activity patterns.

Discussion: The Underlying mechanisms that result in PD are still unclear. It is also unknown why deep brain stimulation has the therapeutic effect that it does. An understanding of the changes in basal ganglia structures during PD and specifically the differences between the different subsets of PD will enable us to further our understanding of how PD develops. Measuring the different firing patterns may also help us understand the physiological mechanism behind DBS benefits and help physicians target the best structures to maximize the therapeutic effects of DBS.

CONFIRMATION OF THE KEY REGULATORY SITES IN THE PROMOTER AND 3'-UTR OF ZEB-1

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One of the key hallmarks of cancer is the multistep process of invasion and metastasis. The relationship between these two processes is important for an understanding of the biology and mechanisms of cancer. One of the mechanisms for a metastatic cancer is through epithelial mesenchymal transition (EMT). In EMT, epithelial cells depolarize and acquire a mesenchymal phenotype. Previous research has shown the ZEB1 gene to be a contributor to tumor progression in metastatic types of cancer. The ZEB1 gene, which is expressed in all cell types, is over expressed in metastatic cancers. This research investigated the regulatory regions of the ZEB1 gene. The purpose of this research was to investigate regulatory regions of the ZEB1 gene, the promoter and 3'-UTR, in primary and metastatic breast cancer. Characterization of these regulatory regions will help to determine what mutations exist between the primary and metastatic tumors and affect the binding on transcription factors and miRNA.

IRON DEFICIENCY DURING BRAIN DEVELOPMENT INDUCES EXPRESSION OF GENES INVOLVED IN NEOVASCULOGENESIS

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Anemia is a condition in which blood oxygen carrying capacity is reduced due to low hemoglobin level. According to the World Health Organization, more than one billion people worldwide are affected by anemia. Anemia can lead to tissue hypoxia, a health problem in which there is an inadequate oxygen supply in the body. The primary cause of anemia is iron deficiency, which results in a nutritional disorder called iron-deficiency anemia (IDA). IDA has its most profound effect on the developing fetus/infant leading to defects in brain development and poor cognitive outcomes. In the brain, hypoxia induces a compensatory increase in blood vessel outgrowth. Here, we hypothesize that iron deficiency leads to brain hypoxia and therefore induces new blood vessel growth in the neonatal brain. To test our hypothesis, we measured the mRNA levels of genes associated with hypoxia-mediated neovascularization. Pregnant rats were rendered iron deficient (FeD) from early gestation through postnatal day 10, 12 and 15 (P10, P12 and P15) and mRNA expression of several genes expressed specifically in brain endothelial cells were assessed by qPCR. FeD increased the expression of endothelial cell marker genes, *glucose transporter 1* (Glut1), *von Willebrand factor* (Vwf) and neovascularization associated genes, *vascular endothelial growth factor* (Vegf), Vegf receptor (Flk1), *chemokine (C-X-C motif) ligand 12* (Cxcl12) and *angiopoietin 2* (Ang2), in the whole brain of P12 rats. In the hippocampus and cerebral cortex of P10 and P15 rats, FeD also increased Glut1, Vwf, Vegfa, Flk1, Cxcl12 and Ang2 expression compared to controls. Finally, fluorescent immunohistochemistry, using a Glut1 primary antibody, demonstrated that FeD leads to a 42 and 57 percent increase in vessel density in the hippocampus and cerebral cortex, respectively. These results suggest that FeD induces blood vessel growth in the developing neonatal brain. Since delivery of nutrients to the brain is critically important for normal development, these findings open a new avenue of research on the impact of FeD on the developing brain.

RAP1A AND RAP1B GENE EXPRESSION IN HUMAN CELLS: CELL TYPE- AND CELL CYCLE-SPECIFIC EXPRESSION

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Rap genes are of interest due to their involvement in a multitude of proposed signaling pathways including those affecting cell-to-matrix adhesion and apoptosis. A clear understanding of the roles of these genes has been hampered by what appears to be cell-type-specific differences in their expression. Expression of Rap1A and Rap1B genes in human cell lines was studied using real time reverse transcription polymerase chain reaction (quantitative RT-PCR). Human

fibroblastic cells were used to prepare a pooled RNA sample to use as a reference within each sample run. Human cell lines were cultured in the absence of growth factors until growth arrest, to relate gene expression to the cell cycle. A breast epithelial carcinoma line, MCF-7, a relatively normal breast epithelial line, MCF-10A, and a prostate carcinoma cell line, LnCap, were cell lines of particular interest, but other human cell lines were studied as well. Average Rap1A expression was greater than Rap1B in all cell lines. For most lines, we saw no distinct expression patterns for either gene, as cells were growth arrested. However, in breast epithelial cells, Rap1A expression appears to be diminished in the absence of growth factors, while Rap1B expression increases with growth arrest. Further experiments are in process to compare baseline Rap1A and Rap1B expression for cell lines of interest. This project has set up a biological model of the expression of native Rap1A and Rap1B under different growth conditions. From here we can compare core promoter and enhancer constructs engineered into the same human cell lines to see if they exhibit similar expression patterns. This will allow us to identify the DNA sequences that control expression of these genes.

FOLLOWING MIQE GUIDELINES FOR REAL-TIME QUANTITATIVE PCR

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Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) is a molecular technique that allows the quantification of minute amounts of nucleic acid material. It has gained popularity over the past decade because of its known sensitivity, specificity, and reproducibility. qPCR differs from traditional PCR in that quantitative measurements are taken after each amplification step. Despite its great potential, qPCR is rarely utilized effectively due to numerous stages where error can be introduced. Areas of error include poor RNA quality before reverse transcription, improper primer design, and inadequate selection of reference genes. Because of this, minimum guidelines have been established (MIQE) to ensure that data obtained by RT-qPCR are legitimate and reproducible. Following these guidelines, it has been determined that the expression of Zinc finger E-box Binding factor 1 (ZEB1), a putative metastasis biomarker, increases by greater than 50 % in a metastatic breast tumor relative to the corresponding normal breast tissue.

STUDYING BRAIN ACTIVITY DURING PERCEPTION: A COMPARISON OF CORRELATIONS ACROSS STATES OF PERCEPTION

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Visual stimuli can be processed by the brain without reaching a state of consciousness. A previous experiment in our laboratory demonstrated that subjects shown an annulus made up of randomly-moving dots sometimes perceive a rotary motion of the dots, and at other times perceive no motion. This experiment revealed that patterns of brain activity were more stable during perception. The hypothesis for this study was that this stable state is accompanied by, and perhaps mediated by, an increase in connectivity between areas of the brain. This theory was tested by measuring, via magnetoencephalography (MEG), the amount of correlated brain activity during periods when subjects perceived rotary motion and periods when they did not. It was found that the correlations between activity of MEG sensors was not significantly higher during states of perception. Additionally, inhibitory feedback from the front of the brain to the early visual areas during perception was found.

BIOGEOGRAPHY OF MITE HARVESTMEN (ARACHNIDA: OPILIONES: CYPHOPHTHALMI) IN EASTERN AUSTRALIA

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Cyphophthalmi, commonly known as mite harvestmen, are a globally-distributed lineage of small arachnids that inhabit leaf-litter habitats. *Austropurcellia* Juberthie 1988 is a genus of mite harvestmen known from numerous localities in Eastern Australia, with its distribution centered in the Wet Tropics. We describe new species of *Austropurcellia* from museum lots; each new species is known from only a single to a few collections and few specimens. The new species greatly expand the range of the genus to almost the entire east coast of Queensland and clarify a cryptic species complex in the vicinity of Daintree National Park in the far north. We present a new distribution map of the genus and discuss the importance of the Burdekin Gap and Black Mountain Corridor to its current distribution. In addition, we integrate morphological data with currently available phylogenetic data to present as complete a picture as possible. We have reached a solid understanding of the biogeography and morphological variation of mite harvestmen in Queensland based on currently known samples, but that understanding would be greatly augmented with the addition of more genetic data from

many of these new species and additional sampling in southeastern Australia, extending throughout Queensland but also into New South Wales.

QUALITATIVE EFFICACY OF BACTERIOPHAGES AGAINST *Staphylococcus epidermidis*

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Antibiotic resistance is becoming an increasing threat to the human population, and an alternative form of treatment is needed. Bacteriophage therapy may be an effective alternative to the use of antibiotics to treat bacterial infections--especially in cases of antibiotic-resistant strains. In our experiment, we isolated strains of *Staphylococcus epidermidis* from four environmental sources: bovine mastitis, primary effluent, activated sludge, and residential water. Out of dozens of candidates screened, only a select few were positively identified as *S. epidermidis* by means of differential and selective testing (at least one from each environmental source). Attempts to isolate and concentrate phage from the respective environmental sources were made, however, we were unable to enrich the phages to a high enough concentration for the use in efficacy trials. Phages specific to *S. epidermidis* were obtained from Dr. Vijay Aswani of the Marshfield Clinic in Wisconsin and tested against the isolates to examine the efficacy of bacteriophages against isolated *S. epidermidis* strains. Qualitative efficacy was determined as one of the acquired phages (P5E) was effective against an isolated vancomycin-resistant strain of *S. epidermidis* (AS6-C1-L).

IDENTIFICATION OF MIDGUT BACTERIAL FLORA OF THE PITCHER PLANT MOSQUITO (*Wyeomyia smithii*) LARVAE BY 16S RRNA, RECA, AND GYRB GENE SEQUENCE ANALYSIS

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North Hennepin Community College

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The purple pitcher plant, *Sarracenia purpurea* L., is a mixotrophic hydrophyte native to North America bogs and fens. The modified leaves of this plant collect rainwater which captures arthropods as a supplemental source of nutrients. Although this system traps and kills a wide variety of insects for the plant, *Wyeomyia smithii*, the pitcher plant mosquito, reproduces exclusively within pitcher plant fluid (phytotelmata).

The bacterial community of pitcher plant phytotelmata has been extensively characterized, and

continues to be studied as a model of microhabitat ecology. However, the impact of *Wyeomyia smithii* on the microbial flora of this environment has not been established. In an effort to better understand the relationship between the phytotelmata prokaryote flora and *Wyeomyia smithii* larvae, we attempted to isolate and identify the dominant, culturable bacterial species found in the midgut contents of *Wyeomyia smithii* larvae from pitcher plants collected from a central Minnesota bog. In this study, 16S rRNA, *recA*, and *gyrB* genes were amplified from the fifteen most common midgut isolates. Amplicons from all three genes were then sequenced and compared to genomic databases for identity matches.

INVESTIGATION OF THE GROWTH MECHANISM OF TIN WHISKERS

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Increased environmental regulation of lead has led to increased use of tin as a replacement in solders for circuit boards. From the copper-tin interface tin whiskers can grow and potentially cause electronic malfunctions. This focus of this work is on the dynamics of the copper-tin boundary layer interfacial forces. Several different layering techniques used in practice include electroplating, hot-melting (soldering), and mechanical force. Electroplating was found to be most successful at reconciling the two layers with a bath containing stannous methanesulfonate as a tin source, vanillin as a brightener and Triton X-100 as a wetting agent.

A SPATIAL SURVEY OF METHANOGENESIS IN MINNESOTA STREAMS

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Recent research in biogeochemistry has been focused on greenhouse gases (GHG). Though CO₂ is regarded as the dominant GHG, methane is also prevalent, with a global warming potential of 25 times greater than that of CO₂ on a 100-year time horizon (IPCC, 2007). While methanogenesis is widely explored in the context of wetlands, information regarding methanogenesis in streams is sparse (Ortiz-Llorente et al., 2012). Agricultural runoffs load nutrients into streams and can potentially lead to increased sedimentation, creating a favorable anoxic environment for methanogenesis (Harrison et al., 2012; Karakashev et al., 2005). The purpose of this study was to survey methane concentrations and water quality in streams across Minnesota. From this survey, we wish to discern stream characteristics that are associated with high methane

concentration.

Data was collected during the fall from 30 sites for dissolved methane concentration, physical stream characteristics, and dissolved nutrients. Gas samples were analyzed using gas chromatography. Methane production was modeled using multiple linear regression as a function of stream characteristics. While there was variation in methane concentrations, the highest stream methane concentration detected was 457.500 ppm, with a mean of 50.950ppm. Methane concentration was most strongly associated with sediment type, flow rate, and dissolved oxygen. The strong association between fine sediment and high methane concentration implies that more effort should be dedicated to understanding methane production in streams, especially in an agricultural context.

ROLE OF ENVIRONMENT AND COMPETITION ON AMMOPHILA INVASION OF WEST COAST DUNES

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Invasive species are species that have adverse effects on habitats and bioregions where they are present in an economic, environmental, or ecological manner. Lately, the introduction and spread of the invasive grass species *Ammophila arenaria* and *Ammophila breviligulata* has posed serious economic issues as well as ecological issues along the Pacific Coast in the US, and this invasion has been coined the “*Ammophila* problem”. Differing growth patterns and changing ecosystem dynamics have been noted since the *Ammophila* problem first began, and dramatic changes in dune morphology as well as biodiversity on the dunes are the most drastic changes associated with the invasion. Coastal communities have taken great strides to control the invasion by removing large amounts of *Ammophila* grasses in an attempt to restore the dunes to their original condition and this leaves large areas of high disturbance on both the front and back of the dunes. We were curious as to how dune position, competition, and species factors on the fitness measures of the grass species *Ammophila arenaria*, *Ammophila breviligulata*, and *Elymus mollis*. These conditions were varied over a four-month period spanning from May to August of 2012 under natural outdoor conditions along the northern Oregon coast. Competition reduction factored with species significantly increased the total biomass of *A. arenaria* as well as *E. mollis* whereas *A. breviligulata* was exhibited no significant change in biomass ($P = 0.0427$ for *A. arenaria*; $P = 0.0402$ for *E. mollis*). In addition, the aboveground biomass was significantly increased all three species as a result of competition removal ($P = 0.0217$). The environmental effect was assessed by examining fitness measures of the grasses on the foredune and on the

backdune, and results suggested that environment had no significant effect on either of the three grass's total biomass ($P = 0.398$). The lack of effect due to the environment is speculated to be a result of the diverse competitors in the backdune and foredune. The implications of accelerated biomass production in *A. arenaria* and *E. mollis* are discussed in relation to the *Ammophila* removal efforts that are taking place along the Oregon coast, and predictions are made regarding the future of the coastal dune biodiversity.

DEVELOPMENT OF TOOLS TO STUDY AND REGULATE GENE EXPRESSION IN *Daphnia magna*

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The crustacean *Daphnia pulex* has recently had its genome sequenced, which revealed over 30,000 genes. A closely related species that is the focus of much study in environmental toxicology is *Daphnia magna*, but one problem is that methods for gene characterization are not well developed in either organism. One method of characterizing genes is the use of RNA interference (RNAi), which uses short segments of RNA and the organism's cellular machinery to reduce the expression of specific genes. Using homology searches and PCR, known genes in the dopamine pathway from the fruit fly *D. melanogaster* and *D. pulex* were used to clone gene fragments from *D. magna*. In addition partial fragments of the *distalless* gene, which has been implicated in appendage formation in other organisms, and has been shown to control *Daphnia magna* 1st and 2nd antennae lengths, were also isolated and used for RNAi. Here I report on the successful isolation of various dopamine neuron specific gene fragments. These gene fragments were for *in vitro* synthesis of dsRNA, which was used for RNAi. Early stage *D. magna* embryos were extracted from pregnant females and injected with these various dsRNA molecules. This work has led to the successful cloning of genes from *Daphnia magna* and the beta testing of a micro-injection method as a way of introducing dsRNA to *D. magna* embryos.

CHARACTERIZATION OF GLYCOSAMINOGLYCANS AND THEIR INTERACTIONS WITH TAT PEPTIDE TO INCREASE DRUG SPECIFICITY

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The interactions between cell-penetrating compounds (CPCs), positively charged molecules that can cross cell membranes, and glycosaminoglycans (GAGs)

are not well understood, but could be exploited for drug design and development. The goal of this study was to compare the binding interactions and thermodynamics between trans-activating transcription factor (TAT) peptide, and four negatively charged GAGs using isothermal titration calorimetry (ITC). GAGs are harvested from biological sources, making them widely polydispersed. A colorimetric assay employing 2,3-dimethylmethylene blue was designed to quantify the density of negative charge on each GAG. Two-dimensional nuclear magnetic resonance (NMR) spectroscopy and high-performance liquid chromatography (HPLC) were used to support the results of the colorimetric assays. GAG characterization has led to the conclusion that TAT affinity is dependent upon GAG charge density and hydroxyl stereochemistry. Understanding the preferential affinity of CPCs to certain GAGs could aid in cellular delivery of CPC-conjugated drugs by improving drug targeting and specificity.

COMPARISON OF *Peromyscus leucopus* AND *P. maniculatus* DENSITIES ON VARIOUS PRAIRIES AND FOREST SITES THROUGH THE USE OF CELLULOSE ACETATE ELECTROPHORESIS ON ALLOZYMES OF SALIVARY AMYLASE.

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Over the summer of 2012, we conducted small mammal trapping on Concordia College's Long Lake Field Station, which has several recently restored prairie plots, as well as trapping preserved prairie sites such as Bluestem Prairie (Clay Co.), Neal Wildlife Management Area (Norman Co.), and forested sites at Minnesota State University Moorhead (MSUM) Regional Science Center (Clay Co.). Our goal was to compare *Peromyscus leucopus* and *P. maniculatus* densities on the forest and prairie sites. Data analysis was conducted with the use of Cellulose Acetate Electrophoresis on allozymes of salivary amylase in order to differentiate between *Peromyscus leucopus* and *P. maniculatus*. At this point in the research there is a lower prevalence of *P. maniculatus* with 54 samples identified compared to *P. leucopus* with 83 samples identified. Data indicated a higher prevalence of *Peromyscus maniculatus* over *P. leucopus* in prairie sites and the opposite in forested sites. These results may indicate ecological replacement of *Peromyscus maniculatus* by *P. leucopus* in forest sites in Minnesota.

THE SIGNIFICANCE OF THE PROTEIN PHOSPHATASE 1H (PPM1H) GENE IN COLORECTAL CANCER (CRC)

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A transposon based mutagenesis system was employed to identify genes that are involved in the initiation of colorectal cancer (CRC) in mice. This screen identified dozens of genes that could be involved in CRC development, including genes known to initiate CRC (e.g. APC) as well as novel genes. One gene identified, Ppm1h gene is a tumor suppressor that had not previously been identified as a CRC associated gene. This research investigates the role and significance of the Ppm1h gene in human CRC using short hairpin RNA fragments (shRNA) to knock down Ppm1h gene expression in the CRC cell line, DLD-1. Cells transfected with empty vector plasmids were used as a negative control. Cell death in DLD-1 CRC cells was induced with 5-Fluorouracil (5FU), an anti-metabolite, at different doses. Apoptosis was measured using Annexin V-FITC, which binds to phosphatidylserine, and fluorescently tags cells in the early stages of apoptosis. Cells were simultaneously stained with Propidium Iodide (PI) to select for necrotic cells. Results obtained were inconclusive; however there may have been a some resistance to apoptosis in cells in which the Ppm1h gene expression was silenced. This was overall consistent with the hypothesis that suggests that Ppm1h is a tumor suppressor. Mutations in this gene can cause resistance to cell signals that initiate apoptosis in CRC cells among other signaling pathways. Further research in other cell lines is yet to be done to confirm these observations.

HUMAN AIRWAY EPITHELIAL SECRETIONS INHIBIT THE FORMATION OF

***Pseudomonas aeruginosa* BIOFILMS**

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Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen that rarely causes respiratory disease except in individuals with specific risk factors, such as cystic fibrosis (CF). The ability of *P. aeruginosa* to readily form biofilms in vivo is associated with airway colonization in susceptible individuals. Thus, we hypothesized that non-CF airway epithelial cells secrete one or more compounds that inhibit *P. aeruginosa* biofilm formation. To test this, we modified and optimized a static biofilm assay and tested the ability of apical Calu-3 cell secretions to inhibit biofilm formation by the *P. aeruginosa* strain, PA14. PA14 formed robust biofilms after 18-24 hours, and biofilm formation was significantly inhibited in the presence of Calu-3 secretions. Preliminary experiments using secretions from a Calu-3 cell line not expressing the CF transmembrane regulator (CFTR) suggest that CF-like secretions do not inhibit PA14

biofilm formation. Thus, normal airway epithelial cells, but not CF-like epithelial cells, appear to secrete one or more compounds that inhibit *P. aeruginosa* biofilm formation.

This project was funded by the Augsburg College Undergraduate Research and Graduate Opportunities (URGO) Office, the Augsburg Sundquist Scholars Program, the Augsburg McNair Scholars Program, and the Augsburg Department of Biology.

**These two authors contributed equally to the project.*

CARDIAC RESPONSE TO VOLUNTARY EXERCISE IN DYSTROPHIC MICE

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There is an ongoing debate over whether or not exercise might benefit boys with Duchenne Muscular Dystrophy (DMD). The disease is characterized by a failure to produce the protein dystrophin, which inevitably leads to muscle deterioration. Because of this, treatment often focuses on maintaining mobility. However, the weakening of cardiac muscle is often the cause of death in patients with DMD. Previous research has shown that exercise actually benefits dystrophic skeletal muscle but less is known about the heart's response. This study tests the hypothesis that voluntary, low-intensity exercise causes no additional damage (such as fibrosis, deleterious hypertrophy, or other signs of cardiomyopathy) to dystrophin-deficient cardiac muscle. To investigate this, 4 week old mdx mice (the mouse model for DMD which lacks dystrophin) were assigned cages with (RUN, n=9) or without (SED, n=5) a running wheel for 8 weeks. Mice with access to wheels ran 2.0 to 7.4 km/24-hours. After the 8 week period, mice were sacrificed and hearts were examined histologically and biochemically for collagen content. Real-time PCR was used to measure changes in gene expression associated with fibrosis and heart failure. No data obtained has shown that voluntary exercise increases fibrosis or heart damage. However, further investigation of heart functionality is required after finding an upregulation of Myosin Heavy Chain Beta (an early sign of heart failure) in the RUN mice compared to the SED mice ($p=0.014$).

SEPARATION AND DETECTION OF HYDROXYL RADICAL OXIDIZED (TERT-BUTOXYCABONYL)-TRYPTOPHAN USING REVERSE PHASE HPLC-UV

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The free radical oxidation of amino acids by reactive oxygen species (ROS) is related to protein misfolding and aggregation leading to many pathological conditions. However, simple methods for the separation and detection of oxidized amino acids in biological samples are not widely available. In this study, we used amine protected tryptophan as our model amino acid. Tryptophan was oxidized by a free radical hydroxyl group, generated from the Fenton reaction. The structure of the oxidized compound was determined using proton and carbon nuclear magnetic resonance spectroscopy, and infra-red spectroscopy. The oxidation mixture was separated by high pressure liquid chromatograph (HPLC) with UV detection at 220 nm and 280 nm. An HPLC gradient method was developed and optimized for separation of oxidation product from non-oxidized tryptophan in less than 35 minutes.

COMPARING NITRATE CONCENTRATIONS IN GROUNDWATER FLOWING FROM NON-TILL AND CONVENTIONAL FARM FIELDS INTO RICE CREEK

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Eutrophication, caused by high concentrations of nitrate, is a concern in much of the agricultural waterways around the world. In this study, the concentration of nitrate in groundwater flowing into Rice Creek from a non-till farm field through both prairie and woodland buffers was compared to groundwater flowing from a conventional farm field through a woodland buffer located across the stream. It was hypothesized that at equal distances from the stream the groundwater from the non-till field would have a smaller concentration of nitrate than that of the groundwater from the conventional farm field. Water samples were collected from groundwater wells on either side of the stream four times over the course of a month. The concentration of nitrate from each of the wells was determined using flow injection analysis. It was concluded that at an equal distance on either side of the stream the groundwater from the non-till field contained a smaller concentration of nitrate than that of the groundwater from the conventional field.

THE ROLE OF NEUROTRANSMISSION IN STRIATAL NEURON DEVELOPMENT

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The striatum is a forebrain region buried beneath the cortex and is involved in regulating movement, motivation and reward. Abnormal striatal function is associated with Parkinson's and Huntington's disease and

addiction. The striatum contains several neuron types, but primarily (~95%) Medium Spiny Neurons (MSNs). During development MSNs undergo major changes in electrophysiology and morphology. These changes are associated with normal maturation and are critical for normal function of the MSNs

Striatal MSNs receive a variety of neurotransmitter inputs, and themselves produce one of the adult nervous system's principle inhibitory neurotransmitters, GABA (which is similar to the substance GHB). Neurotransmitters are not only responsible for cell-cell communication: they can also modulate neural plasticity and serve as growth signals. Indeed, we are born with almost twice as many brain-cells as we need, and in the developing brain, the presence or absence of neurotransmission can determine whether a neuron lives or dies.

In this study we sought to determine whether neurotransmitters such as GABA mediate morphological changes, and consequently function, of MSNs during development. Cultured MSNs were chronically treated with neurotransmitter antagonists, and microscopy was used to assay MSN morphology at several time-points. Interestingly, blocking either glutamate or GABA altered neuronal morphology, but the effects were distinct from each other. Comparison with normal development suggests that the morphological changes mediated by GABA activity proceed those mediated by glutamate. Current experiments focus on determining the intracellular signaling pathways involved.

DEVELOPMENT OF A METHOD TO QUANTIFY SUGARS, ALCOHOLS, AND ORGANIC ACIDS IN A FERMENTATION MIXTURE UTILIZING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV-DETECTION

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The primary goal of this research project is to develop a method employing high-performance liquid chromatography (HPLC) such that it can be adapted for implementation in a teaching laboratory. Quantification of key compounds of interest in a fermentation mixture by HPLC was chosen primarily because of its applicability to analytical, organic, and biochemistry concepts and for its relevance to biofuels and food/beverage industries. While refractive index detection (RID) is commonly utilized to monitor carbohydrate, alcohol, and organic acid content in fermentation mixtures, it is an objective of this project to utilize more commonly available UV-visible detection to quantify these analytes. In specifying the use of UV-detection for this project, a derivatization measure will need to be introduced in order to detect sugars as

carbohydrates lack a strong UV-absorbing group in their chemical structure.

HIGHLY CYTOKINERGIC SPE-7 ANTI-DINITROPHENYL-IMMUNOGLOBULIN E, BUT NOT ϵ 26, CAUSES ANTIGEN-DEPENDENT THERMAL AND MECHANICAL HYPERALGESIA

Elena Tonc, Madison Mack, Tijana Martinov, Abigail Wetzell, Estela Shabani, Camilla Engblom, Evelyn Balsells, and Dr. Devavani Chatterjea (Advisor)

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SPE-7 and ϵ 26 are cytokinergic anti-dinitrophenyl IgE antibodies that initiate production of inflammatory cytokines independent of antigen-stimulation *in vitro*. However, their *in vivo* effects have not been well characterized in mouse models. We tested these IgE clones in our models of thermal and mechanical hyperalgesia induced by passive antibody sensitization and antigen challenge. SPE-7 caused significant hyperalgesic responses for at least 6 hours following antigen challenge after sensitization, while ϵ 26 caused only slight thermal and mechanical hyperalgesia at 1 hr that resolved after 3 hours following antigen challenge subsequent to sensitization. Responses to antigen challenge for both clones were accompanied by equivalent levels of histamine release and neutrophil influx and comparable increases in levels of inflammatory cytokines (TNF- α , IL-1 β , IL-6, and CXCL1) in the paw tissue when compared to sham-sensitized or vehicle challenged groups. Neither clones stimulated antigen-independent cytokine production in the mouse hind paw. Therefore, we found that mast cells may be critical initiators of allergen-evoked hyperalgesia in mice, but these responses may vary by clone of antibody involved in the response. Therefore a consideration of the nature and repertoire of IgE antibodies produced during an allergic response is essential to elucidating their pathophysiological implications for various health outcomes including pain.

CELLULAR DYNAMICS OF THE DRAINING LYMPH NODES IN MURINE MODELS OF ALLERGEN-INDUCED VULVAR PAIN

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The role the allergic immune response plays in facilitating pain remains largely unexplored, particularly for chronic pain disorders where active inflammation may no longer be present. An example of such a condition is vulvodinia, a chronic vulvar pain condition with an unknown etiology, but associated with a history of allergies.

To investigate the role allergic events play in the transition to prolonged states of pain (mechanical hyperalgesia), our lab adapted the oxazolone induced contact hypersensitivity (CHS) model to the mouse vulva. This study investigates the cellular dynamics of the vulvar draining lymph nodes to identify key cellular events in this transition. 24 hours following three consecutive challenges, active inflammatory infiltrate is observed in the tissue and, in the draining lymph nodes, a significant increase in T and B cell numbers along with CD44+ cells was found. In contrast, following ten consecutive challenges, only a significant increase in B cells was seen in the draining lymph nodes 24 hours after the last challenge. Additionally, CD44 expression was only significant in non-T cells. Interestingly at this timepoint, perhaps due to an earlier inflammatory response in this more persistent model of allergen-induced hyperalgesia, a significant inflammatory infiltrate is not found in the tissue. This suggests B cells and CD44+ cells, particularly CD44+ expressed on non-T cells, are important components of the shift to persisting hyperalgesia that warrant further investigation.

THE EFFECTS OF VEGETATION ON PREDATION OF BIRD NESTS ON THE FOREST FLOOR

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Bird abundance can be directly influenced by rates of predation. The Katharine Ordway Natural History Study Area oak forest in Dakota County has a very low abundance of ground nesting bird species. This is hypothesized to be due to the extremely high rates of predation that was noted in an earlier study at the site. The primary objectives of this study were to determine whether there are any microhabitats that might provide refuge for ground nesting birds in the forest and whether vegetation abundance, structure, and diversity may contribute to the risk of nest predation in certain areas of the forest. In two separate studies, we placed 140 ground nests in the forest containing one quail egg and one clay egg. In the first study 100 nests were placed regularly on a 200*200m patch of the forest containing a variety of vegetation coverage. In the second study 40 nests were divided into 20 nests placed in high Garlic Mustard, *Alliaria petiolata*, (GM) density plots and 20 nests placed in low GM (NGM) density plots to determine whether this abundant nonnative plant may influence the risk of nest predation. Overall predation rates were very high with a two week nest survival probability of 0.069. High vegetation coverage seemed to play a significant role in decreasing nest predation rates, although not low enough to permit nesting success of ground nesting birds. GM

does not seem to influence the risk of nest predation. Evidence points to raccoons and mice as primary predators.

EFFECTS OF PHYSICAL CO-CULTURE ON COMMUNICATION BETWEEN MAST CELLS AND ASTROCYTES

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Mast cells are tissue-bound immune system cells that play important roles in allergic response and inflammation. In the central nervous system, the interactions between mast cells and neuronal cells such as astrocytes can have important implications for inflammatory diseases. This study aimed to investigate the effects of physical co-culture between mast cells and astrocytes on cell-cell communication using immortalized model cell lines to explore their role in immune responses. The secretory granules of mast cells contain an array of inflammatory mediators such as serotonin and β -N-acetylhexosaminidase. After appropriate stimulation, secretion of serotonin via regulated exocytosis in mast cells was quantified using HPLC, and β -N-acetylhexosaminidase secretion was quantified using an absorbance assay. For both the release of serotonin and β -N-acetylhexosaminidase, results showed the activation of mast cells cocultured with astrocytes was significantly restrained when compared to mast cells cultured alone. This observation is currently in comparison with chemical co-culture effects on mast cell and astrocyte function and highlights the importance of mast cells participation in inflammation.

THE DEVELOPMENT OF ORGANIC CHEMISTRY LABORATORIES FOR THE PICOSPINTM-45 PROTON NMR SPECTROMETER

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Analytical tools, such as the picoSpinTM-45 proton NMR spectrometer, have proven to be a valuable teaching method in the chemistry department at Augsburg College. Many current lab exercises, as well as new experiments, were carried out and investigated in order to expand on our current organic chemistry laboratory curriculum with the utilization of the picoSpinTM-45. ¹H NMR spectra of many samples were then obtained in order to determine the most efficient sampling technique and to assess how students can best benefit from this hands-on approach to learning.

HUMAN AIRWAY SECRETIONS INHIBIT *Pseudomonas aeruginosa* ACTIVITY: EFFECTS ON SURVIVAL, GROWTH, AND FLAGELLAR MOTILITY

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Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen that is a leading cause of morbidity and mortality in patients with cystic fibrosis (CF). The relative selectivity of *P. aeruginosa* colonization in CF patients suggests that the CF airway, in contrast to normal airways, is particularly hospitable to *P. aeruginosa*. One likely possibility for this difference is that normal but not CF airways secrete compounds that attenuate the virulence of *P. aeruginosa* and prevent colonization. In this study, we examined the ability of normal airway epithelial secretions to inhibit two properties of *P. aeruginosa* associated with colonization: proliferation and flagellar motility. For these initial experiments, we used apical secretions from polarized Calu-3 cell monolayers and the *P. aeruginosa* strain PA14. Treatment of PA14 cells with Calu-3 secretions dramatically reduced both average swimming speed (30 vs. 15 μ m/sec) and the number of motile bacteria (56% vs. 42%). Inhibition of PA14 growth was not seen after 24-hours of incubation with Calu-3 secretions, in contrast to previous reports. Our preliminary attempts to identify the active component(s) in Calu-3 secretions suggest that one or more proteins >50 kDa are responsible. We conclude that normal airway epithelial secretions directly inhibit certain *P. aeruginosa* functions associated with airway colonization.

This project was funded by the Augsburg College Undergraduate Research and Graduate Opportunities (URGO) Office and Sundquist Scholars Program.

**These two authors contributed equally to the project.*

FORMATION OF WATER SOLUBLE POLYOXOMETALATE-ORGANIC LIGAND FRAMEWORKS

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This project looked at making a soluble polyoxometalate (POM)-organic ligand framework using A-type Keggin sandwich POMs of the form $[M_3(PW_9O_{34})_2]^{17-}$. Previous work by our group has used 4,4'-bipyridine as the bridging ligand between POMs, resulting in insoluble networks. In an attempt to increase solubility in water, alternate ligands were explored. Biphenyl-4,4'-dicarboxylic acid and the 2,2'-hydroxyl

derivative have been implemented. Due to the bidentate nature of the carboxylates, cerium cations as the “sandwiched” metals in the POM were also incorporated to allow for increased coordination number, instead of the traditional first-row transition metal cations. The building blocks (ligand and POM) and resulting networked materials were characterized by FTIR and NMR spectroscopies.

HUMAN AIRWAY SECRETIONS INHIBIT *Pseudomonas aeruginosa* ACTIVITY: EFFECTS ON SURVIVAL, GROWTH, AND FLAGELLAR MOTILITY

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Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen that is a leading cause of morbidity and mortality in patients with cystic fibrosis (CF). The relative selectivity of *P. aeruginosa* colonization in CF patients suggests that the CF airway, in contrast to normal airways, is particularly hospitable to *P. aeruginosa*. One likely possibility for this difference is that normal but not CF airways secrete compounds that attenuate the virulence of *P. aeruginosa* and prevent colonization. In this study, we examined the ability of normal airway epithelial secretions to inhibit two properties of *P. aeruginosa* associated with colonization: proliferation and flagellar motility. For these initial experiments, we used apical secretions from polarized Calu-3 cell monolayers and the *P. aeruginosa* strain PA14. Treatment of PA14 cells with Calu-3 secretions dramatically reduced both average swimming speed (30 vs. 15 $\mu\text{m}/\text{sec}$) and the number of motile bacteria (56% vs. 42%). Inhibition of PA14 growth was not seen after 24-hours of incubation with Calu-3 secretions, in contrast to previous reports. Our preliminary attempts to identify the active component(s) in Calu-3 secretions suggest that one or more proteins >50 kDa are responsible. We conclude that normal airway epithelial secretions directly inhibit certain *P. aeruginosa* functions associated with airway colonization.

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**These two authors contributed equally to the project.*

PERCEPTION OF CAUSALITY

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Humans perceive causal relationships in their everyday experiences, often making judgments based on

these perceptions. Our laboratory is interested in studying brain activity during causal perception, but before we can implement such an experiment, we must first determine suitable stimulus parameters for such an experiment. We explored the effects of two parameters, probability and timing, on subjects' perception of causality. Subjects viewed a display of moving and colliding spheres and reported whether or not the collisions of the spheres caused them change color. In one experiment, we varied the time between sphere collision and color change; the color change could occur at various intervals either before or after the collisions. We found an inverse relationship between the distance in time between events and perception of causality, as well as an asymmetric effect whereby subjects were much more likely to assign causality when the collisions occurred before the color. In the second experiment, we varied the fraction of colliding spheres that resulted in a color change. We found a correlation between the fraction of collisions producing a color change and causal perception. Interestingly, subjects fell into two distinct groups, based on their tendency to assign causality to the stimuli.

MUTAGENIC EFFECT OF FINASTERIDE (PROSCAR ©) ON HUMAN CULTURED PERIPHERAL BLOOD LYMPHOCYTES USING SISTER CHROMATID EXCHANGE

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Proscar ©(Finasteride) is used to treat benign prostatic hypertrophy (BPH) by inhibiting the enzyme 5-alpha-reductase, an enzyme necessary to convert testosterone into 5-alpha-dihydrotestosterone (DHT). DHT is a major contributor to BPH. Finasteride is a drug that has been listed as a hazardous unclassified drug by the Center for Disease Control (CDC). There have been conflicting reports on the mutagenic potential of Finasteride. Finasteride was tested at clinical doses (2.5 mg/day, 5 mg/day, and 10mg/day) on human peripheral blood lymphocytes using the Sister Chromatid Exchange (SCE) Technique to help clarify the potential mutagenic activity of the drug on human cells. Early results show an increase in SCEs in treatment cultures compared to controls, which suggests Finasteride has a mutagenic effect on human PB lymphocytes.

FROG FUNGUS: THE LOCAL BATTLE AGAINST CHYTRIDIOMYCOSIS

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Populations of anurans worldwide are declining due to several factors. These include habitat loss, climate

change, environmental pollution, and disease. In Burnsville, Minnesota we report a population of anurans infected by the parasitic fungus *Batrachochytrium dendrobatidis*, a disease referred to as chytridiomycosis. In this study we sought to determine if the disease was introduced to the area by a decorative koi pond or if the infected frogs found in the pond were a symptom of a general infection of anurans in the area. Throughout the summer and fall of 2012 we collected swabbed samples from anurans in the Murphy-Hanrehan park region in order to determine the infection rates. We found that *B.d.* infection was widespread throughout the park region including several different aquatic environments and across several different species. The infection rate was significantly higher in smaller, man-made aquatic environments than in larger natural environments. Our data suggests the decorative koi ponds were not the source of the infection; that the proximity of individuals in an aquatic environment is correlated with infection rates; and that small man-made aquatic environments increase *B.d.* infection rates on anurans in those environments.

HUMAN AIRWAY EPITHELIAL SECRETIONS INHIBIT THE FORMATION OF

***Pseudomonas aeruginosa* BIOFILMS**

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Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen that rarely causes respiratory disease except in individuals with specific risk factors, such as cystic fibrosis (CF). The ability of *P. aeruginosa* to readily form biofilms in vivo is associated with airway colonization in susceptible individuals. Thus, we hypothesized that non-CF airway epithelial cells secrete one or more compounds that inhibit *P. aeruginosa* biofilm formation. To

test this, we modified and optimized a static biofilm assay and tested the ability of apical Calu-3 cell secretions to inhibit biofilm formation by the *P. aeruginosa* strain, PA14. PA14 formed robust biofilms after 18-24 hours, and biofilm formation was significantly inhibited in the presence of Calu-3 secretions. Preliminary experiments using secretions from a Calu-3 cell line not expressing the CF transmembrane regulator (CFTR) suggest that CF-like secretions do not inhibit PA14 biofilm formation. Thus, normal airway epithelial cells, but not CF-like epithelial cells, appear to secrete one or more compounds that inhibit *P. aeruginosa* biofilm formation.

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