



Ontario Biology Day

March 12-13, 2011 @ Wilfrid Laurier University

Abstracts

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Poster Session: Saturday, March 12th in the Science Courtyard from 5:30pm to 7:00pm.

List of poster presentations:

Poster B-1:

Evaluation of the Effect of a Constructed Highway on Mangrove Species Composition Using GIS and Landsat TM Satellite Data

Amber Brant (Department of Biology, Trent University)*

Geospatial applications like GIS and remote sensing are being used more frequently to answer ecological questions in large-scale scopes. Mangroves are tropical forests that are found in estuaries, along riverbanks and in shallow lagoons. Like most forest communities, mangroves are organized into species-based distributional patterns. A highway was constructed through a mangrove forest in the Atasta Lagoon, Campeche, Mexico in 1986. The working hypothesis here is that the species composition of the mangrove forest is altered by the construction and abandonment of a highway. It was predicted that one of the four species found in the forest will be found in a higher relative abundance along the highway, and as a result, Simpson's biodiversity will be lower along the highway.

Empirical field sampling was used to supplement remote sensing applications in the study. Stratified random field plots were sampled in situ for total abundance of each of the four species types: *Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia germinans* and *Conocarpus erectus* L. Simpson's biodiversity index (1-D) was determined for each field plot. The field samples were used to calibrate a Landsat TM satellite image with digital reflectance values represented by spectral vegetation indices. The Enhanced Vegetation Index (EVI) was used as a reliable predictor of Simpson's biodiversity index for the area ($R^2=0.64$, $p<0.01$). A biodiversity map was produced and compared with distance from highway using GIS methods. Results demonstrate that spectral vegetation indices can be used to predict Simpson's biodiversity, based on measured reflectance from the forest canopy. Results are pending for the effect of the highway on biodiversity and/or abundance of one common species.

Poster B-2:

Maternal and paternal effects on hatching success in Chinook salmon

Breanne Reibl* & Yolanda Morbey

Department of Biology, University of Western Ontario.

Hatching success of salmon embryos is an important factor influencing fitness. The purpose of this study was to quantify maternal and paternal (genetic) effects on hatching success in Chinook salmon. Specific maternal traits that we examined were fertilization success, egg diameter, chorion thickness, and proximate composition. Eggs and milt were collected in the fall from Chinook salmon in the Sydenham River in Owen Sound, Ontario. Some eggs were analyzed for proximate composition and some were fertilized *in vitro* in a half sibling mating design. Ten embryos per family were collected after 24 hours of incubation to assess fertilization success and chorion thickness. The remaining embryos in each family were allowed to grow until they hatched. Hatching success represents the proportion of fertilized eggs that successfully hatched. Analysis of variance showed that most maternal traits did not affect hatching success. However, fatter eggs increased hatching success. In contrast, we found strong paternal effects on hatching success (the sire explained 91% of the variance in hatching success). This was a surprising result, and suggests that there may have been unaccounted for non-genetic, paternal effects, perhaps relating to sperm quality.

Poster A-1:

The effects of temperature on the infectivity of viral hemorrhagic septicemia virus type IVb on fish cell lines with different and similar thermal requirements.

Chan Kwun Hung^{1*}, John Pham¹, John Lumsden², Brian Dixon¹, Niels Bols¹

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Viral Hemorrhagic Scepticemia Virus (VHSV) is the causal agent of viral hemorrhagic septicemia, a disease that kills susceptible fish and causes economic losses in aquaculture. We investigated the impact of temperature on the infectivity of a Great Lakes strain of VHSV, genotype IVb, on cell lines from rainbow trout (RTgill-W1), fathead minnow (EPC) and Zebrafish (ZEB2J). Past studies have shown VHSV genotypes I and IVa to survive temperatures ranging from 4°C to 30°C, yet the infectivity of the virus at elevated temperatures remains largely unexplored. In order to test the temperature dependency of VHSV IVb, fish cells were exposed to 16 times diluted viral particle containing EPC cell lysate, whose 50% tissue culture infective dose (TCID50) was determined by the endpoint dilution assay, and incubated at 26 and 30°C. On days 1, 3, 5 and 7 post-dosing, cultures were monitored visually and mRNA samples were collected and the presence of viral transcripts sought through Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). At high temperatures the virus is able to enter cells, but not cause cytopathic effects (CPE). We hypothesized this to be due to the denaturation of viral proteins necessary for the virus life cycle to be completed.

Poster A-2:

The Role of the Transcription Factor Kaiso in the Development of Ovarian Cancer

Lowell De Villena *

Department of Biology, McMaster University

Ovarian cancer is one of the most common gynaecologic malignancies, and is currently the fifth leading cause of cancer death among women in the Western world. Among the multiple types of ovarian cancers, approximately 90% of ovarian tumours arise from the ovarian surface epithelium. Epithelial cells are characterized by strong cell-cell adhesion mediated through the adherens junction. The adherens junction is composed of the transmembrane epithelial-cadherin (E-cadherin) and its cytosolic adaptor proteins (β -, α -, and p120-catenin). E-cadherin forms homotypic interactions with E-cadherin on adjacent cells while the catenins either anchor E-cadherin (via its cytosolic domain) to the actin cytoskeleton or regulate cadherin stability and turnover. The catenins also have nuclear roles and regulate gene expression. For example, p120^{ctn} also interacts with the transcription repressor Kaiso and relieves Kaiso-mediated repression of target genes implicated in development and tumourigenesis (e.g. *matrilysin*, *cyclin D1*). This phenomenon is usually correlated with an upregulation of N-cadherin, a characteristic of epithelial-mesenchymal transition (EMT) and an essential step in ovarian tumour invasion.

Preliminary experiments from our lab recently revealed that Kaiso was misexpressed in 50% of ovarian tumours compared to normal ovarian tissue. Additionally, Kaiso was expressed in several ovarian carcinoma cell lines. We hypothesize that Kaiso modulates cellular function in ovarian epithelial carcinomas. Due to the opposing Kaiso and cadherin expression of OVCA 420 compared to the various ovarian tumour cell lines, an inducible Kaiso-overexpressing ovarian carcinoma cell line will be generated to assess its functional role in cellular processes associated with cadherin-switching under controlled conditions. This inducible Kaiso-overexpressing OVCA 420 cell line will be used to assess the effect of Kaiso on various tumourigenic processes such as cell migration and invasion. The completion of these functional experiments should provide insight on the intracellular role of Kaiso in ovarian carcinoma progression.

Poster B-3:

The Effects of Species Richness and Endophyte Infection on Invasion

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Characteristics of the invader and community can influence the likelihood of an invasion occurring. Invasion by exotic species may be deterred by high resident community diversity. However, equally diverse communities may not be equally resistant to invasion. The dominance of species in communities structured by selection prevents complete resource use, leaving resources open for invaders. Invasion should then be higher in communities structured by selection relative to communities structured by complementarity. The association with the endophyte *Neotyphodium coenophialum* increases the competitive ability of the invasive host *Festuca arundinacea*, and can enhance its ability to invade established communities. Endophyte infected hosts should then be better invaders relative to uninfected hosts. Therefore, we predict invasion to be highest in communities with endophyte infected invaders that are also structured by selection.

To determine whether endophyte infection influenced invasion success we created experimental tall grass prairie communities and mimicked natural invasion with propagules of infected and uninfected *F. arundinacea*. To determine whether community invasibility was influenced by selection or complementarity we altered the composition and species richness of the experimental communities subject to invasion and calculated selection and complementarity values for each community. Understanding how the properties of diverse communities like selection and complementarity affect invasion can provide novel perspectives on the way biodiversity affects ecosystem functioning.

Poster A-3:

The Virulence Properties of Mycobacterium tuberculosis SapM.

Anna Li*, Johanna DeLongchamp, Mazen T. Saleh

Department of Biology, Laurentian University.

Mycobacterium tuberculosis is a facultative intracellular pathogen that causes human Tuberculosis. It has the capacity to evade and survive the immune defence mechanisms of the host, in particular the survival within the hostile environment of the macrophages. Upon entry into a host macrophage, the mycobacterium prevents phagosome maturation, which is an essential process that would normally destroy the pathogen. Thus, to rationally develop more effective chemotherapeutic agents, it is important to understand the *M. tuberculosis* – host interaction, specifically the genes that are potentially involved in virulence. The survival mechanism of *M. tuberculosis* is still unclear but secreted proteins are proposed to be involved in intracellular survival. It has been proposed that the secreted acid phosphatase, SapM, is linked to the survival of the pathogen. In this study, *Mycoplasma capricolum* transfected with pMT85 plasmid containing SapM gene will be used to demonstrate virulence properties of SapM. The virulence will be tested by infecting macrophages with transfected *M. capricolum*. By better understanding the pathogenesis of infection, new strategies for prevention and treatment of Tuberculosis can be developed in the future.

Poster B-4:

Does adult colouration increase genetic mating success in subadult male Purple Martins?

Cordelie DuBois*, Bridget Stutchbury, Scott Tarof

Biology Department, York University.

In the Purple Martin (*Progne subis*), a migratory songbird, one-year-old males have a distinctive, female-like, subadult plumage in contrast to older males that have full iridescent purple colouration. The effect of this distinctive subadult plumage on genetic mating success of one-year-old males was tested both for within-pair and extra-pair young by measuring the area of purple on a male and comparing it to his known paternity. The amount of purple the breast and bib area ranged from zero to 1432.54 mm among males, indicating a wide variety of different plumage patterns within the age class. Bib and breast measurements were significantly positively correlated. There was no significant effect of variation in purple colouration on proportion of within-pair young in a male's own nest, number of extra-pair offspring he sired, or total reproductive success. Purple colouration does not, therefore, appear to be a predictor of genetic mating success among one-year-old males. The dramatic colour variation among young males could function in individual recognition, or may be random variation upon which selection is not acting

Poster C-1:

The effects of neurosteroids progesterone and allopregnanolone on social learning in female ovariectomized mice

Kelsy Ervin*, Riccardo Dore, Elena Choleris

Department of Psychology, University of Guelph

The social transmission of food preferences (STFP) in rodents involves a naïve “observer” animal developing a food preference for a novel food that it smells on a “demonstrator” animal’s breath, allowing the observer to avoid the costs of trial-and-error learning. Performance on this task in female mice is related to levels of gonadal hormones such as progesterone acting through receptors in the brain. In addition to progesterone receptors, progesterone is converted to allopregnanolone (AP), a positive modulator of the GABA_A receptor. Both progesterone and AP affect cognitive functioning, and the effects of progesterone could be mediated by AP. We therefore tested the effects of progesterone and AP on STFP, injected before social interaction (1 hour before for progesterone, 20 minutes for AP) or immediately after the interaction. Mice injected with the highest doses of progesterone prior to learning showed improved performance on the task; those injected with the same dose after learning had impaired performance. AP-treated mice had a prolonged preference for the demonstrated food at the lowest dose when injected both before and after learning. Higher doses impaired performance when administered before learning but improved performance when given after learning. Given these results, the different effects of the two drugs on this task suggest that progesterone affects performance not only through its conversion to AP, but through other neural mechanisms (e.g. neurotransmitter systems, protein synthesis). We are currently investigating whether and how progesterone and allopregnanolone affected behaviour during social interaction.

Poster B-5:

The Effects of Two Dietary Supplements on the Longevity and Life History of the Cricket *Acheta domesticus*

Warren Fingrut*

Department of Biology, McMaster University

The cricket *A. domesticus* has recently emerged as a new model of aging, one which allows researchers to achieve fast results with large sample sizes. Further, *A. domesticus* is omnivorous and follows a hemimetabolous life cycle in which nymphs eat the same foods as adults, making it ideal for the study of dietary supplementation. In a recent study, amino acid supplementation consisting principally of the branched-chain amino acids (BCAAs) leucine, valine, and isoleucine was shown to have an antiaging role in male mice. Separately, studies have shown that Royal Jelly (RJ), a principal food of the honeybee queen, has antioxidant properties and displays insulin-like activity *in vivo*.

The objective of this study was to reproduce the reported mammalian longevity extension by BCAA supplementation in the cricket model of aging. Additional goals included investigating and characterizing the effects of BCAA and RJ supplementation on *A. domesticus* life history.

In this study, age-matched cohorts of *A. domesticus* were fed either control, BCAA supplemented, or RJ supplemented diets. Cricket growth rate, maturation date, survivorship, activity level, and longevity were compared between groups. Maturation status was indicated by wing morphology and mature genitalia, and activity was assessed via open field test.

Both the BCAA supplemented and RJ supplemented cohorts displayed significantly extended longevity, improved survivorship, delayed maturation, lengthened female growth period, and increased activity compared to controls. Importantly, this study provides support for the recent finding that BCAA supplementation improves longevity.

Poster B-6:

Functional traits underlie adaptive drought-induced parental effects in the annual grass *Avena barbata*

Rachel M. Germain*, Christina M. Caruso, and Hafiz Maherali

Integrative Biology, University of Guelph

Adaptive parental effects are gaining recognition as potentially widespread strategies for preparing offspring for survival in predictable environments. However, our current understanding of how adaptive parental effects evolve has been limited by the failure to identify both when parental effects are in fact adaptive and what functional traits underlie their expression. Here, we address these issues using a reciprocal transplant experiment in a greenhouse to study drought-induced parental effects in the annual grass *Avena barbata*. Throughout development, offspring from dry-grown parents outperformed those from wet-grown parents through aboveground biomass and seed set. Interestingly, we found no interaction between parent and offspring environments on offspring performance, indicating that parental effects were adaptive but without tradeoffs that typically lead to adaptive matching. Seed nitrogen content, but not seed weight, was correlated with germination success and radicle length, but this relationship was stronger for the offspring of parents grown in wet than dry environments. Overall, our results suggest that the evolution of parental effects is less contingent on environmental similarity across generations than previously thought, while identifying a possible evolutionary pathway where selection can act on parental effects through specific functional traits.

Poster B-7:

Phylogeography of *Caenorhabditis remanei*: population structure and incipient species.

Yong suk Jeon*, Alivia Dey and Asher D. Cutter

Department of Ecology and Evolutionary Biology, University of Toronto.

Nematodes in the genus *Caenorhabditis* is widely used model organisms with both gonochoristic (male-female) and androdioecious (male-hermaphroditic) species. Nucleotide diversity within a species can be affected by four key evolutionary forces: genetic drift, natural selection, mutation, and migration. *C. remanei* is gonochoristic species that inhabits around the globe, making it suitable species for phylogeographical study. Previously, only a single population of *Caenorhabditis remanei* has been investigated for nucleotide polymorphism; showing approximately 20 fold greater genetic diversity than hermaphroditic relatives (Cutter 2006). Here, we examine how migration between populations might affect the distribution of genetic diversity throughout the species. We first gathered approximately 59 strains of *C. remanei* from Asia, North America, and Europe. We quantified nucleotide variation for 15 loci each ~700 bp long (linked to X chromosome). After DNA of individual strains was sequenced, numbers of statistical analysis were carried out to investigate neutrality of selection, level of nucleotide diversity, and population structure. Test of neutrality imply complex German has highly skewed allele frequency distribution. It suggests complex population demography: either population contraction or recent admixture of multi-population. In terms of population structure, there was strong level of correlation between genetic distance and geographical distance (Spearman's Correlation: $\rho = 0.75$, $p < 0.0001^*$). In fact, Chinese sample is strongly genetically differentiated: this may represent distinct species.

Poster C-2:

Changes in Adolescent Locomotor Activity Following Immune Activation Persist into Adulthood in Male Long-Evans Rats

Eric Johnson^{1*} (*Department of Biology, ¹University of Western Ontario*)

Klaus-Peter Ossenkopp² (*Department of Psychology, ²University of Western Ontario*)

The purpose of the study was to investigate the long lasting behavioural and physiological effects of immune activation during adolescence and young adulthood on rats. Adolescent male Long Evans rats (*Rattus norvegicus*) were injected repeatedly with either lipopolysaccharide (LPS) from gram-negative *Escherichia Coli* or a saline control (NaCl), and then tested in an open field for locomotor activity. Injections began at 39 days of age (P39), and were conducted weekly for 5 weeks. Adult locomotor activity was then examined weekly, for 5 weeks beginning at P95. Locomotor variables that were examined include, total distance travelled, movement time, and vertical movements. The time spent in the periphery was analyzed during adolescent and adulthood tests as a measure of thigmotaxis. Animal mass was measured 1 day after receiving injections, and 1 day after locomotor testing during adulthood. Animals appear to have developed tolerance to the locomotor and weight-loss effects of LPS. Adult behaviour may suggest that chronic adolescent immunological stress leads to lasting behavioural effects that persist well after the effects of the drug.

Poster B-8:

Spatial variability in granivory determines the strength of stochastic community assembly.

Laura Johnson^{1*}, Rachel Germain¹, Andrew MacDougall¹, Karl Cottenie¹, Elizabeth Gillis²
(*Department of Integrative Biology, University of Guelph¹; Department of Resource Management, Vancouver Island University²*)

High diversity in many biological communities is not well explained by trait-based deterministic models, in part because stochastic processes also influence community assembly. Testing how deterministic and stochastic processes combine to regulate diversity, however, has been limited by the spatial complexity of these interactions. Here, we demonstrate that spatial variability and the impacts of granivory result in fine-scale switching between deterministically and stochastically regulated plant community assembly in an otherwise environmentally homogenous 18-hectare tallgrass prairie. In field edges, where granivory is high, traits affecting seed palatability deterministically create homogeneous subsets of less palatable species (low α and β diversity). As granivory impacts decline in more open areas, stochastic priority effects increasingly determine assembly (high α and β diversity). The net effect across the study area is a heterogeneous and species-rich array of co-occurring species, whose occurrences can be explained by cryptic but predictable fine-scale differences in the strength of limiting factors.

Poster C-3:

Estrogenic activity of Hamilton Harbour sediments, quantified using a recombinant yeast screen

Sujane Kandasamy*, Joanna Y. Wilson
Biology Department, McMaster University.

Endocrine Disrupting Chemicals (EDCs) have been reported to cause widespread reproductive anomalies in fish populations. Discharge from waste-water treatment plants, combined sewer outflows, and industry all contribute to the intersex gonads and feminization of male fish noted throughout contaminated sites in the Hamilton Harbour region. In wildlife populations, high levels of estrogens, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls have been observed to cause feminization of the male reproductive duct, gonadal intersex, and the induced expression of vitellogenin in male fish.

Six sites (Sherman Inlet, Ottawa Street Slip, Pier 27, Burlington Wastewater, LaSalle Marina, Desjardin Canal) within Hamilton Harbour were sampled for possible sediment-bound EDCs. The Yeast Estrogen Screen bioassay was used to test the estrogenicity of the sampled extracts, in order to elucidate if a build-up of sediment EDCs was a contributing factor in the feminization of the round goby. This quantification could help determine which specific compounds are responsible for endocrine disruption in the Hamilton Harbour region. It is hypothesized that the samples that contain the greatest accumulation of estrogenic EDCs will correspond to the sites with the highest prevalence of feminization in round goby. The YES bioassay was optimized for healthy yeast growth conditions; however, β -Galactosidase levels were only observed to increase for higher-end 17β -estradiol levels (10^{-6} to 10^{-1} M). Future research should address the negative implications of EDCs on reproductive functions, so that remediation efforts and disposal policies can be modified for the Hamilton Harbour region.

Poster A-4:

Olfactory dysfunction in lupus-prone MRL mice

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McMaster University*

Systemic lupus erythematosus (SLE) is an autoimmune disorder where the immune system attacks the body's own organs, including brain. In addition to brain atrophy, neurological, and psychiatric manifestations, it has been recently reported that SLE patients may develop olfactory deficits of unknown origin. To further study this, we use two substrains of lupus-prone MRL mice that differ in disease onset times. The MRL/lpr mice show signs of SLE-like disease around 7 weeks of age, while MRL +/+ mice develop similar signs much later in life. We currently compare their performance in olfactory preference tests at 7, 12, and 17 weeks of age. Each mouse was exposed to a filter paper with a control stimulus (water), attractant (peanut butter), neutral scent (vanilla), and repellent (peppermint) at 0.01%, 1%, 10%, and 50% dilutions. Throughout the test periods, both groups showed expected behaviors: increased concentrations of the peanut butter scent resulted in increased exploration, increased concentrations of the vanilla scent resulted in insignificant response, and increased concentrations of the peppermint scent resulted in overall avoidance. However, group differences were significant at the lowest scent concentrations. Starting at 12 weeks of age, autoimmune MRL/lpr mice were not as responsive to the peanut butter scent and the peppermint scent as the control MRL +/+ group. These results suggest that spontaneous development of SLE-like disease in MRL/lpr mice is accompanied by hyposmia (i.e., impaired olfactory sensitivity). As such, the results support the hypothesis that onset of systemic autoimmunity affects the olfactory system and related cellular mechanisms.

Poster A-5:

Serotonin receptor type 7 regulation of NMDA receptor subunit phosphorylation in the hippocampus

Jerry Li^{1*}, Seyedeh Maryam Vasefi¹, and Michael A. Beazely²

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Serotonin (5-HT) receptor type 7 is one of the subtypes of 5-HT receptors of the nervous system including the hippocampus. Upon receptor activation, this G-protein coupled receptor (GPCR) induces a cascade of intracellular signalling pathways. One consequence of receptor activation is the activation of intracellular kinases and the phosphorylation of additional proteins. We hypothesize that the N-methyl-D-aspartate (NMDA) receptor is one such target. 5-HT7 receptor activation increases NMDA receptor subunit phosphorylation on serines 896 and 897 of NR1 whereas NR2A phosphorylation is significantly reduced. With respect to NR2B, tyrosine 1472 phosphorylation is increased whereas tyrosine 1336 phosphorylation remains unchanged. Co-incubation with the 5-HT7 receptor antagonists, SB 258719 and SB 269970, partially or completely reversed the effects of the 5-HT7 receptor agonist, LP 12, on NMDA receptor subunit phosphorylation. Long-term treatment of hippocampal cultures does not significantly change NMDA receptor subunit phosphorylation, although both NR1 and NR2B receptor expression is significantly reduced after 24 h LP 12 treatment. This is the first investigation of the ability of 5-HT7 receptors to regulate NMDA receptor phosphorylation. Our findings suggest that not only do 5-HT7 receptors indeed signal to NMDA receptors, they may also selectively target specific NMDA receptor subunits.

Poster A-6:

A molecular analysis of the *pin1* gene and its effect on phosphorylating the CTD domain of RNA polymerase II

Zahraa Majeed*, Jim Karagiannis

Department of Biology, University of Western Ontario

The conversion of phosphoserine/threonine proline motifs between cis and trans conformations modulates the activity of a variety of cellular regulatory networks. The conversion between the cis and trans forms is catalyzed by enzymes referred to as peptidyl-prolyl cis/trans isomerases (PPIases). By controlling the cis/trans state of their targets, these enzymes can modulate substrate protein activity, stability and/or phosphorylation status. One of this class of enzymes most important targets is the carboxy-terminal domain (CTD) of the largest subunit of the RNA polymerase II. The RNA pol II CTD is composed of tandem heptapeptide repeats (YSPTSPS) whose phosphorylation pattern determines a “CTD code” that modulates the recruitment of accessory factors affecting transcription. To determine the effect of cis/trans isomerization on CTD phosphorylation in the fission yeast, *Schizosaccharomyces pombe*, the dosage of the *S. pombe* PPIase, Pin1p, will be modulated and assayed for its effect on cellular growth and CTD phosphorylation status.

Poster C-4:

A pilot study on the hypothalamic-pituitary-adrenal (HPA) axis in subjects with mild allergic asthma

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The stress hormone cortisol is secreted during HPA axis activation. If the HPA axis is dysregulated, there is an increased likelihood for the development of inflammatory diseases. Individuals with mild allergic asthma have the potential to experience inflammatory symptoms following exposure to a sensitizing antigen, but it is unknown whether this is associated with the dysregulation of the HPA axis. This study hypothesizes that dysregulated HPA axis activity is associated with the development of mild allergic asthma. Salivary samples were collected from mild allergic asthmatics (n=10) and age and gender-matched normal control subjects (n=10) upon awakening, and assayed for cortisol using commercially bought ELISA kits. The awakening cortisol levels between the study groups were compared using Mann-Whitney and Wilcoxin statistical tests. Mild allergic asthmatics had significantly different cortisol levels of 7.5 nmol/L ($\sigma=6.2$ nmol/L) compared to normal controls with baseline cortisol levels of 14.6 nmol/L ($\sigma=5.4$ nmol/L) ($p<0.05$). Data shows that mild allergic asthmatics had attenuated cortisol levels compared to normal individuals. Since the HPA axis is associated with cortisol levels, dysregulated HPA axis activity can be associated to mild allergic asthma. Speculating the mechanism of the relationship between the HPA axis and asthma, inflammatory mediators (IL-1, IL-6 and TNF- α) associated with the HPA axis and enzymes (11 β -hydroxysteroid dehydrogenase) dysregulating cortisol can be studied. These factors are hypothesized to be overexpressed in asthmatic individuals, which could explain, in part, how lower cortisol levels may impart risk of developing allergen-induced late-phase responses in subjects with mild allergic asthma.

Poster A-7:

Cross amplification of microsatellite primers among seven *Littorina* species.

Stephanie Pedersen*, Dr. Elizabeth Boulding, and David Hunt

Department of Integrative Biology, University of Guelph

Littorina plena, a species of marine gastropods with free swimming larvae display high rates of dispersal across intertidal zones along the western coast of North America. Six polymorphic microsatellites were isolated from *L. plena* and screened against six related *Littorina* species: *L. scutulata*, *L. subrotundata*, *L. sitkana*, *L. saxatilis*, *L. obtusata*, and *L. littorea*. Through the analysis of microsatellite loci in *L. plena*, it is the main objective to isolate and design primers for 8 - 10 microsatellite loci which not only display high levels of variation, but that also have low frequencies of null alleles. Microsatellite regions were sequenced from extracted DNA of live *L. plena*. Primers were designed and screened for polymorphism using polyacrylamide electrophoresis. It was hypothesized that primers designed for *L. plena* will amplify at low frequencies for *L. subrotundata*, *L. saxatilis*, *L. sitkana*, *L. obtusata*, and *L. littorea*, and more frequently in *L. scutulata* due to differing phylogenetic distances. Amplification was seen across all species even those not closely related to *L. plena*. The potential for the study to determine genetic diversity in a population will be beneficial for work in conservation, as well as studies in gene flow of dispersing populations. Loci which amplified throughout the genus can be used for future microsatellite analyses in non *L. plena* species, and is an indicator of conserved genetic sequences.

Poster A-8:

Circulating RNA in Human Plasma

Kripa Raman* and Dr Guillaume Paré

Department of Biology; McMaster University

Microvesicles (MVs) are membrane bound sacs released by healthy and diseased cells, which contain mRNA, miRNA and proteins. Previously, circulating RNA was thought to be an experimental artifact due to the high concentration of ribonuclease in blood. But, sequestration of endogenous RNA within MVs, offers protection against ribonuclease degradation. The use of this circulating plasma RNA as a noninvasive molecular diagnostic tool is accumulating interest. Differential RNA concentrations have been observed in cancers, thereby putatively suggesting a method of early diagnosis. But, prior to clinical use microvesicle RNA of healthy individuals must be further studied. Current research has not identified a cell types which are significantly represented by circulating RNA nor have these RNA transcripts been quantified. Due to the high degradation of mRNA transcripts, the Affymetrix Human Exon array was utilized for quantification. Preliminary findings indicate high significance towards the nebulin and mitochondrial RNA transcripts. It may be speculated that skeletal muscles secrete a larger proportion of MVs. The identification of circulating mtRNA is also a novel finding. The RNA transcript diversity observed in plasma along with the potential for use as a biomarker, are strong stimulators to conduct further research.

Poster C-5:

Temperate foliose lichen soredial development and growth is enhanced by reduced temperature conditions

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Lichens offer a unique system for the study of development as fungal and photosynthetic partners form a single entity with distinct morphology. However, past efforts to culture lichen propagules in laboratory conditions have been largely unsuccessful. Previous field experiments utilizing *Parmelia sulcata* propagules suggest that optimal growth occurs during the winter months. This study investigates factors influencing early development of asexual propagules (soredia) in temperate foliose lichens with the aim of determining conditions where growth occurs consistently under both field and laboratory conditions. We hypothesized that low temperature conditions support early soredial development. Soredia of the lichen *Parmelia sulcata* were seeded onto 128 plastic coverslips and randomly assigned to either a small temperature controlled chamber cooled to approximately 7°C or a chamber at 23°C with similar relative humidity and light exposure. The cold chamber admits light through a (34cm x 21cm) clear plastic window. We quantified developmental success on each coverslip by counting the number of colonies that had formed early attachment hyphae after ten weeks. The low temperature treatment yielded a significantly greater number of developing colonies per coverslip and a greater proportion of successful coverslips (0.89 ± 0.04 s.e. vs 0.50 ± 0.06 s.e.). In a complementary field experiment coverslips seeded with soredia were placed outdoors in August and December. The field results thus far, confirm earlier findings that soredia favour winter conditions for growth. To determine the environmental factors most important for development initiation, comparisons between temperature, light and humidity data gathered under both laboratory and field conditions will be examined.

Poster B-9:

Continental patterns of local disappearance of North American breeding songbirds as a function of climate

François Thériault* and David J. Currie

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In this study, we investigated whether temperature and precipitation affect the disappearance risk of breeding songbirds at a local scale, across North America. We compiled data from the North American Breeding Bird Survey (BBS), an extensive dataset detailing the annual counts of bird species on 4905 different 40km routes scattered throughout the continent, since 1967. A local disappearance was defined as the absence of a given species on a route where it had been present the previous year. Each route was characterized in terms of its average mean annual temperature and average annual precipitation. Optimal temperature and precipitation were also computed for the 206 passerine species included in our study by overlaying distribution and climatic maps. The temperature and precipitation at which a species occurred most frequently were deemed its climatic optima. Preliminary results show no significant effect of either temperature or precipitation on the “observed disappearances : potential disappearances” ratio, suggesting no North American climate (within the area covered by the BBS) is inherently less suitable for songbird species in general. However, difference between local and optimal climate – expressed as a percentage of the maximal observed difference between both values for each individual species, to facilitate comparisons among species – had a significant effect on local disappearances. For North American songbird populations in general, species are more likely to disappear in locales with increasing divergence from optimal temperature and precipitation. Second-order polynomial regressions statistically account for 95% - 79% of the variance in local disappearance probability. Remarkably, climate-dependent disappearance probability never exceeds 18%. Either climate does not set range boundaries, or the limits are sharper than we could detect with this technique.

Poster A-9:

Isolation of an Antibiotic Adjuvant Targeting the Outer Membrane.

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Gram-negative bacterial infections are a growing concern as they confer resistance to multiple antibiotics. The outer membrane (OM) of these organisms provides a barrier that prevents small drug molecules from entering the cell. This results in multi-drug resistance against organisms such as opportunistic pathogens *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. A strategy to overcome the membrane barrier using existing drugs is to examine the activity of hydrophobic antibiotics effective only for Gram-positive organisms in combination with outer membrane permeabilizers. Screening a *Streptomyces* supernatant library in the absence and presence of novobiocin against *E. coli*, a model Gram-negative organism revealed a number of *Streptomyces* isolates that produce compounds that synergize with novobiocin to inhibit *E. coli* growth. A hit from the library was chosen for investigation of the natural product that presents antibiotic adjuvant activity and that may be targeting the OM. The purpose of this research is to isolate the adjuvant molecule from the supernatant of *Streptomyces* species PS#13 through optimization of the synergy assay protocol and a series of chemical purification methods. The characterization of the adjuvant molecule will present a new avenue for discovering agents to treat Gram-negative infections.

Poster B-10:

Recreational boaters as a vector of spread for aquatic invasive species in the Lake Simcoe region

Kristina Wantola*

Department of Biology, York University

The hypothesis that recreational boaters may introduce non-indigenous aquatic biota into Canada's inland lakes via in water transport had never been directly tested, hence, we investigated whether recreational boaters are a vector of spread for invasive species through the Trent-Severn Waterway into Lake Simcoe. We surveyed boater habits and vessel fouling at the Couchiching and Gamebridge locks, over a 10 day period in August and September of 2011. We also collected water samples from any location on the boats containing standing water, later identified and counted all Crustacea and molluscs in the samples. A total of 312 zooplankton individuals were collected from the boats, including 15 different species among which were 9, apparently healthy *Dreissena veligers*. Most of the boats originated in Lake Simcoe and 88% of the animals found were from these vessels. The average number of veligers per boat was 0.14 and the probability of finding a veliger was 1.58%. Neither the starting location (P from a linear regression = 0.634), nor an overall fouling index (p = 0.504) were useful predictors of the log-transformed number of animals found, but the volume of water that was collected was correlated with animals collected (p = 0.0481). The longest distance reported for a trip was 718.59 Km, and, on average, boaters would visit 2-3 or more than 6 different waterways in a season. Recreational boaters appear to be a vector of spread of both native, and at least one invasive species in the Lake Simcoe region, although the lake itself now likely serves more of a source than a sink of invaders.

Poster B-11:

Crouching as a Sleep-Like State in Crayfish

Shawn Hudes & Joffre Mercier

Brock University

Crayfish are among the few invertebrate organisms in which a sleep state has been detected. Preliminary 24-hour video recordings of crayfish (*Procambarus clarkii*) behaviour failed to detect the previously described position associated with sleep in the crayfish, that of the crayfish lying on one side. The experiment set out to test whether the crouch position (with a curled tail posture) for crayfish could be considered a sleep state by continuously video-recording solitary crayfish behaviour in a tank for 24 hours before, and for 48 hours after (each with a 12:12 Light:Dark cycle), undergoing a sleep deprivation procedure and determining whether the crayfish engaged in more crouching afterwards. It was hypothesized that there would be an increase in crouching afterwards in order to make up for a sleep debt. The results of video analysis did not support crouching as a sleep-like state. Furthermore, crouching occurred during both the light and dark phases, and there was a trend suggesting that crayfish crouch more during the light phase, but this was not statistically significant. Variability in crouch times tended to increase between individuals in the 1st day after sleep deprivation as well as the 2nd day (but less pronounced) after sleep deprivation, suggesting large differences in how crayfish respond to stressors. A non-significant trend suggested that the light cycle might influence the response to the deprivation procedure. Future work needs to be done to clarify whether crayfish sleep, the significance of crouching, and responses to psychological stressors.

Poster B-12:

Influence of forest roads on small diurnal mammal activity

Amy Flasko*

Biology Department, Trent University - Supervisors Dr. Erica Nol and Dr. Jim Schaefer

Many studies have shown the negative effects of habitat loss and fragmentation on various species across the globe. However, little is known about the effects of smaller, unmaintained or secondary roads frequently found in areas under protection, such as conservation areas. The activity levels of small, diurnal mammals (primarily *Tamias striatus* and *Tamiasciurus hudsonicus*) from a large regional forest in southern Ontario were investigated to determine the effects of forest roads. Live traps, sand planks and a continuous census were used along 100 m transect lines set up perpendicular to forest roads in deciduous and coniferous forest types. It was predicted that a higher density and increased activity of small, diurnal mammals would be found further away from forest roads, and that the activity of small, diurnal mammals would vary between forest types; although both forest types would exhibit avoidance of forest roads. Logistic regression and Fisher's exact test were used to quantify results and determine significance. Results showed no effect of road edge in either forest stand, however there was a significant difference in mammal activity between forest types (Fisher's exact test, $p = 0.0003$). With the difficulties experienced in data collection, more research is required to conclusively accept results of this study.

Poster C-6:

The effect of alcohol on coherent motion perception

Lauren Whitney^{1*} & Dr. Brian Timney²

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Motion perception plays an integral role in daily life. An individual's interaction with their environment is reliant largely upon an accurate visual perception of their surroundings. Motion is a multidimensional entity varying in both simple, such as real and illusory motion, and complex forms. The focus in this study is coherent motion, a type of complex motion processing. Coherent motion describes a motion display, typically in the form of a random dot array, in which some percentage of dots are moving in a single direction while others are moving at random. To test an individual's acuity to coherent motion, psychophysical techniques are designed in which the observer must distinguish between two subsequent displays, one demonstrating random and one demonstrating coherent motion in differing velocities and test directions. A threshold can then be generated which describes the percentage of coherence (i.e. the percentage of dots moving in one direction) that must be presented for the observer to clearly discriminate coherent from random motion. The goal in this study is to incorporate these psychophysical techniques to evaluate subjects' acuity to coherent motion in both sober and intoxicated states. Because of the popularity of alcohol consumption and the implicit importance of accurate motion perception, it is of great interest to examine how aspects of motion perception are affected by alcohol. It is hypothesized that, when intoxicated, the subject's ability to distinguish coherent motion will be impaired in comparison to their results in the sober state.

Poster C-7:

Response of phosphoenolpyruvate carboxylase to cadmium toxicity in *Arabidopsis thaliana*

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University of Western Ontario

The presence of cadmium can negatively impact plant growth on a molecular and physiological level. To limit toxic exposure, plants exude metal-chelating agents such as organic acids into the rhizosphere and on the intracellular level in order to sequester cadmium. In order to quantify the plants response to cadmium toxicity, one can measure the production of phosphoenolpyruvate carboxylase (PEPc) which is an important intermediate within the tricarboxylic acid cycle for the production of organic acids. PEPc is regulated by genes encoding *AtPPC1-4*. Previous work on stress responses has shown that *AtPPC1* reacts to phosphorus deficiency however no published work has linked cadmium toxicity with *AtPPC* isoenzymes. Results indicate that there was an up regulation in gene expression by the first three *AtPPC* genes in both shoot and root samples between treatments lacking and containing cadmium.

Poster B-13:

Influence of forest roads on small diurnal mammal activity

Amy Flasko*, Erica Nol and Jim Schaefer

Biology Department, Trent University

Many studies have shown the negative effects of habitat loss and fragmentation on various species across the globe. However, little is known about the effects of smaller, unmaintained or secondary roads frequently found in areas under protection, such as conservation areas. The activity levels of small, diurnal mammals (primarily *Tamias striatus* and *Tamiasciurus hudsonicus*) from a large regional forest in southern Ontario were investigated to determine the effects of forest roads. Live traps, sand planks and a continuous census were used along 100 m transect lines set up perpendicular to forest roads in deciduous and coniferous forest types. It was predicted that a higher density and increased activity of small, diurnal mammals would be found further away from forest roads, and that the activity of small, diurnal mammals would vary between forest types; although both forest types would exhibit avoidance of forest roads. Logistic regression and Fisher's exact test were used to quantify results and determine significance. Results showed no effect of road edge in either forest stand, however there was a significant difference in mammal activity between forest types (Fisher's exact test, $p = 0.0003$). With the difficulties experienced in data collection, more research is required to conclusively accept results of this study.

Abstracts for Oral Presentations, Ontario Biology Day 2011

Alterations in the composition of fluid secreted by the Malpighian tubules of the mosquito, *Aedes aegypti* in response to rearing salinity.

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Department of Biology, York University

Insects are capable of maintaining their state of hydration with changing environmental conditions because they have evolved a distinct mechanism to keep a balance between the water gained from the environment and the water lost. Terrestrial insects face dehydration, whereas freshwater aquatic insects such as the mosquito, *Aedes aegypti* larvae face dilution. The mechanism involves endocrine factors that target specific ion-regulatory organs such as the Malpighian Tubules (MTs), to regulate haemolymph composition.

It is important to understand the different factors governing a state of balance within this organism to understand how they tolerate varying salinity. The purpose of this research is to identify regulatory mechanisms that the larvae possess to permit survival in different salinity. The Malpighian tubules secrete KCl and NaCl in varying ratios depending on the environmental salinity that they are exposed to. The K⁺ concentration of secreted fluid from Malpighian tubules of larvae exposed to 30% seawater (SW), 120mM NaCl or freshwater (FW), was measured using a modified Ramsay assay with ion-selective electrodes. It was determined that K⁺ concentrations were dependent on the rearing media of mosquito larvae. Malpighian tubule secretions obtained from FW reared larvae contained higher K⁺ concentration (50.78±4.18 mmol/L) compared with larvae reared in 30% SW (25.65±2.49 mmol/L) or 120mM NaCl (22.64±1.43 mmol/L). The results suggest that Na⁺/K⁺ concentration ratio in the secreted fluid is determined by Na⁺ levels in the rearing media and not necessarily the osmolarity of the media.

The sudden emergence of fertility in aged sterile hybrids of two recently diverged species of *Drosophila*.

Josh Alpern* & Amanda Moehring

Department of Biology, University of Western Ontario

Speciation is the process wherein a single ancestral species splits into two genetically isolated species as outlined by the Biological Species Concept (BSC). The BSC defines a species as a breeding population that is reproductively isolated from other breeding populations. One example of this reproductive isolation is the production of hybrids which are sterile. Hybrid sterility ultimately prevents gene flow between diverging populations, which results in the establishment of two separate species. Haldane's Rule states that if sterility is only present in one sex, it will be present in the heterogametic sex (XY males or ZW females). In *Drosophila pseudoobscura* there are two subspecies that follow Haldane's rule by producing hybrid sterile males, yet when the F₁ male progeny are allowed to age, they regain fertility. In previous unpublished work, it was found that the hybrid sperm do not fully divide during the process of spermatogenesis. I have been able to observe that when fertility is acquired in the hybrids, the inability to segregate occurs in fewer sperm. Furthermore, the presence of undivided sperm was observed in several other interspecies hybrids, verifying the universal nature of this phenomenon. The results show that the inability of heterogametic chromosomes to segregate during meiosis is a possible cause of the formation of reproductive barriers ultimately resulting in speciation.

Limiting R0: Selection for Epidemic Frequency in Spatially Structured Host Populations.

Tallulah Andrews*, Jonathan Dushoff

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The basic reproductive ratio (R0) of pathogens is limited in nature. Explicit trade-offs of life-history traits are the most common mechanism to explain this limit. However, it has recently been shown that under certain conditions spatial patterns of infection can give rise to 'emergent trade-offs' and relatively stable R0. By replicating the model and exploring variants of it, we investigated the selection mechanism proposed to explain these emergent trade-offs. The stability of the emergent trade-offs was examined by relaxing many of the assumptions involved in the original finding. When infectious period was held constant, transmission rate evolved toward maximal values indicating selection for epidemic frequency was the mechanism responsible for the emergent trade-off. Results were robust to substantial relaxation of assumptions but may be dependent on the regular patterns which emerge from the homogenous spatial structure of hosts. Rabies has both a significant spatial structure of hosts and an unusually low R0. As a result it is a prime candidate for selection for epidemic frequency in nature.

Neurochemical processes in the *C. elegans* dopamine neuron system

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The use of *C. elegans* as a drug screening tool presents an exciting prospect for new drug discovery. Conventional studies have employed mammalian models that effectively approximate the human response to candidate treatments. However, such studies are expensive and far too time consuming. Although In-vitro designs have been explored extensively, they are of poor relevance to the human condition. Not only is *C. elegans* high throughput screening compatible, it also presents a reliable approximation of the human response to drug treatments (Moy et al., 2009). Prior studies have demonstrated its sensitivity to neuroactive compounds, indicating the great potential this model holds for research in the field of neuropharmacology (Nass et al, 2003). Furthermore, metabolic processes for dopamine have been shown to be conserved between humans and *C. elegans* (Wienshenker et al., 1995). The objective of this study was to characterize compound A induced degeneration of the *C. elegans* dopamine neuron system and to document the neuroprotective influence of Compound B. It was hypothesized that the worms would demonstrate behavioral sensitization that is consistent with dopamine receptor supersensitivity induced by Compound A treatment (Kita et al., 2003 & Bhargava et al., 1980). This behavioral sensitization was accepted as a visible manifestation of underlying neurochemical damage (Kita et al., 2003). Compound B was expected to reduce this supersensitivity as it has been shown to do in murine models. Behavioral analysis of the worms via electrotaxis studies has been performed in order to test the aforementioned hypotheses. Preliminary results have revealed that behavioral modification occurred in response to treatment with compound A, while further experimentation is necessary to ascertain the influence of compound B.

Factors affecting lion (*Panthera leo*) distributions within the Welgevonden Reserve, South Africa

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Lions are of conservation concern as their ranges have been reduced due to anthropogenic mediated habitat loss. Having knowledge about lion spatial distributions and interactions with prey, habitat types and other lion prides are important considerations in conservation management. Lions have been hypothesized to seek habitats with higher prey abundances. Alternatively there is also evidence that lions prefer environments that allow for concealment and ambush strategies. Overall, Lions are considered territorial, and neighboring prides should show little overlap in their territories. We consider these hypotheses in Welgevonden, a small, actively managed reserve in South Africa. Vehicle transects were performed over a ten-week period, during which the GPS coordinates of herbivores were recorded. We also obtained access to herbivore GPS coordinates from ten years of annual helicopter transect data, and four years of lion GPS collar data. The spatial usage of lions was compared to the positions of the seven most common prey in Welgevonden and to eight habitat types using multiple regression. Overlap of lion homeranges was compared between prides. We found that 67% of the variation in lion distributions is accounted for by a positive relationship to prey abundance, and negative relationships to less accessible habitat types. Homeranges of lions in northern and southern prides had almost no overlap. These results suggest that territoriality is the main determinant of lion distributions. Within each homerange, the results support the prey availability hypothesis. Future inquiry within Welgevonden should examine herbivore habitat choice over varying lion populations.

Cloning, Expression, Purification and Crystallization of the Catalytic Domains of Yeast De-ubiquitinating Enzymes

Leila Ashurov*, Sara Chavoshi and Vivian Saridakis *Department of Biology, York University, 4700 Keele Street, Toronto, ON, M3J 1P3*

De-ubiquitinating enzymes (DUBs) are specialized proteases, which cleave ubiquitin-protein bonds. They have many crucial roles in the cell such as rescuing ubiquitin tagged proteins from destruction by the 26S proteasome. There are 17 DUB-encoding genes in the genome of the yeast *Saccharomyces cerevisiae*, sixteen of which belong to a class called Ubiquitin-Specific Processing Proteases (UBPs). Little is known about UBP regulation, proteins they interact with and their substrates.

Currently, we are preparing expression cassettes for the catalytic domains of yeast UBP proteins to enable the expression and purification from *E. coli*. Once purified protein is obtained, it can be used for crystallization trials. Protein crystallization is an essential in protein structure determination, which can be used to study their regulation. Thus far, positive clones for the catalytic domains of UBP3, UBP5, UBP7, and UBP11 have been obtained. Expression studies indicated that UBP3 and UBP5 were insoluble and a soluble form of these proteins is required for purification and subsequent setting up of crystal trails. There is an ongoing effort to clone different fragments of the catalytic domains of the UBP's, in hope of obtaining soluble proteins.

Is there metabolic suppression in the brain mitochondria of hibernating thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*?

Claudia Augustine*, James F. Staples

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The objective of this study is to determine whether the metabolism of brain mitochondria is suppressed in torpid and interbout euthermic phases of hibernation in ground squirrels, *Ictidomys tridecemlineatus*. The hibernation season consists of torpor bouts, when the animal's metabolism is reduced, with periodic arousals leading to phases called interbout euthermia, when the animal's metabolic rate and body temperature rise to a summer-active level. In torpid phases, mitochondrial metabolic suppression is tissue specific, with a 70% reduction of respiration rates in liver, but not in skeletal muscle. In liver, this metabolic suppression is reversed during interbout euthermia. We measured mitochondrial oxygen consumption rates of saponin-permeabilized brain tissue at 37°C using an Oroboros oxygraph polarographic O₂ electrode. We also compared the effects of different energetic substrates on respiration rates. Preliminary data indicate that non-phosphorylating and state 3 (ADP phosphorylating) respiration rates do not differ between torpor and interbout euthermia. However, different substrates were consumed at different rates, with succinate supporting the highest rate of oxygen consumption in comparison to glutamate, pyruvate and β-hydroxybutyrate.

Optimum developmental temperature as an indicator of adaptation of ten populations of *Drosophila simulans* (Diptera: Drosophilidae)

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When a population is exposed to a new environment, the population can either adapt through genes that are specifically beneficial in that one environment, or through genes that allow the population to be “plastic” and survive in a wider range of environments. Life history traits, such as survivorship, can serve as indicators of how well-adapted a population is to its environment. Optimum temperatures have been investigated for many species, but most studies have looked only at single populations rather than investigating the adaptability of a species as a whole, and no studies have examined whether a species is more likely to locally or plastically adapt. We investigated whether *Drosophila simulans* have locally or plastically adapted to their environment by comparing the optimum fitness temperature of eleven populations from around the World. The relative fitness of eggs, larvae, and adult flies at six temperatures were used to determine how well adapted *D. simulans* are to their native environments. Differences in fitness were observed for some life stages, but not for others. Detailed results and their correlation to a local vs. plastic response will be discussed.

The evolution of sexual dichromatism in *Trogonidae*

Samantha S. Baker*

Department of Biological Sciences, University of Windsor

A difference in integumentary colours between males and females of a particular species, or sexual dichromatism, is common in many taxa of birds. In most cases of sexual dichromatism males display exaggerated sexual ornaments (i.e. bright plumage) whereas female usually do not possess these counterparts and are usually cryptic in colour. In the family *Trogonidae*, their plumage is colour varies throughout the order as well as between males and females of the same species. Trogons are typically sexually dichromatic, but dichromatism varies in this order. There are species that are strongly dichromatic and others that are more similar in chromatism, which makes them an excellent study species. To gain a better understanding of sexual dichromatism within this family, an investigation of the correlation of plumage dichromatism is being conducted in comparison to environmental influences and life histories of the species. Data on dichromatism will be evaluated in two different ways. A matrix will be constructed based on traits that are present or absent in males and females as well as obtaining environmental and life history data which both will be attained through published literature. A collection of reflectance spectrometry will also be obtained from museum specimens. Exploring the ecological factors and the life histories that contribute to sexual dichromatism will create a better understanding of plumage colouration within species as well as among different species in the Trogon family and will create a better understanding of how sexual dichromatism can correlate to the environment Trogons live in.

The Influence of Settlement Expansion on Dissolved Oxygen in Lake Naivasha, Kenya.

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On February 16th 2010, Lake Naivasha, Kenya experienced a major fish kill, which had serious consequences for the communities surrounding the lake. Although community members held the expanding floriculture and agricultural industries responsible, morphological examination of the fish suggested death by suffocation due to depleted concentrations of dissolved oxygen [DO]. The substantial increase in the Naivasha population has resulted in the proliferation of informal settlements, which lack sewage infrastructure. We hypothesize that the increased loading of organic matter from these settlements – which is swept in to the lake during heavy rains – is resulting in episodic drops in [DO] below fish survival requirements. A 30-year time series (1980 – 2010) quantifying the spatial distribution and expansion of human settlements within the basin was constructed using Landsat MSS and TM satellite imagery. Pixel-based maximum likelihood supervised classification methods based on 2007 and 2010 ground-truth observations were used to classify the images. A time series of [DO] in Lake Naivasha was compiled for the same period from the literature and ongoing monitoring programs revealing a highly variable [DO] (12.7 – 1.90 mg/L). Minimum [DO] during onset of the rainy season (Dec-Jan) was below the fish survival threshold. Simple and multiple regression analyses were used to determine the relationship between the magnitude of human settlements within the basin and the decline of [DO] in the lake. By providing objective evidence for the cause of the fish kill, this study can be used to develop effective management strategies to ensure the sustainability of this indispensable freshwater ecosystem.

Chronic waterborne and dietary exposure nickel to round gobies: sub-cellular analysis

Upasana Banerjee*, Erin M. Leonard and Dr. Chris M. Wood

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Objectives:

- i. To determine time-dependent Ni bioaccumulation in various organs from a joint waterborne and dietary exposure.
- ii. Assess the sub-cellular distribution of Ni in the gills, gut, liver and muscles.
- iii. Attempt to relate toxicity endpoints such as death and essential ion disruption to the non-detoxified sub-cellular fractions of the metal

Methods: Approximately 200 fish were collected from Hamilton Harbour and exposed to different dietary and waterborne Ni concentrations for 30 days. Fish were taken out on certain time points and dissected to obtain kidney, gills, liver, gut and carcass. Sub-cellular fractionation was conducted on gills to know Ni accumulation in different cell fractions. Digested organs/fractions were analyzed by the Graphite Furnace Atomic Absorption Spectrophotometer.

Results: Round gobies in 50 $\mu\text{mol Ni/L}$ treatment had the highest mortality on day 10, whereas, 10 $\mu\text{mol Ni/L}$ treatment had the highest mortality on day 30. Fish in the control tank showed 100% survival rate. It was also found that Ni bioaccumulation in kidney increases overtime. On the other hand, Ni has acute effect on gills but this organ develops a mechanism to reduce bioaccumulation chronically. Through sub-cellular fractionation on gills, it was found that Ni concentration in organelle fraction increased overtime which caused percent Ni in metal sensitive fraction (MSF) to increase and percent Ni in biologically detoxified metal fraction (BDM) to decrease. Overall, mortality might be related to Ni bioaccumulation in kidney and increase in percent Ni in MSF.

Chronic waterborne and dietary Ni exposure to round gobies: sub-cellular analysis

Upasana Banerjee*, Erin M. Leonard and Dr. Chris M. Wood

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Time-dependent Ni bioaccumulation and sub-cellular Ni distribution is determined in various organs from a joint waterborne and dietary exposure. Relation between non detoxified sub-cellular fractions of the metal and toxicity endpoints such as death and metal ion disruption is also identified. For the experiment, fish were collected from Hamilton Harbour and exposed to different dietary and waterborne Ni concentrations for 30 days. Fish were sampled on days 0, 2, 4, 10, 20, 30 and dissected to obtain kidney, gills, liver, gut, muscle and carcass. Sub-cellular fractionation was conducted on gills to determine percentage of biologically detoxified metal (BDM) versus metal sensitive fraction (MSF). Digested organs/fractions were analyzed by the Graphite Furnace Atomic Absorption Spectroscopy. It was found that round gobies have approximately double sensitivity to acute Ni exposure compared to other freshwater teleosts such as rainbow trout. Round gobies in the highest 50 $\mu\text{mol Ni/L}$ did not survive past day 10, whereas, there was an 8% survival in the 10 $\mu\text{mol Ni/L}$ treatment by day 30. Fish in the control tank had 100% survival. Ni bioaccumulation in kidney increases overtime, where a significant increase is correlated with the highest mortality in the 50 $\mu\text{mol Ni/L}$ treatment. Additionally, acute Ni exposure led to a significant increase in gill Ni accumulation, which was restored back to background levels by day 30, suggesting a regulatory mechanism in the gills. Sub-cellular fractionation of the gills showed significant Ni accumulation in the organelles (org) overtime which caused a ~13% percent increase of Ni in the MSF and a ~10% decrease in the BDM. Overall, mortality might be related to Ni bioaccumulation in kidney and an increase in MSF of the gills.

Effects of amiRNA silencing of methylthioadenosine nucleosidase expression in *Arabidopsis thaliana*

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In the Yang cycle, methionine is recycled by hydrolysis of 5'-methylthioadenosine (MTA) to methylthioribose and adenine. Methylthioribose is ultimately converted into methionine and from there it is converted to S-adenosyl-L-methionine (SAM). There are two MTN coding genes in the *Arabidopsis* genome, designated MTN1 and MTN2. Knocking down the expression of both genes using T-DNA inserts results in an increase in MTA levels and a complex phenotype including yellowing of young leaves, delayed first true leaf development, delayed bolting, male and female sterility, thickened vasculature and twisting of the roots. Because of the strong phenotype observed in the *mtn1-1 mtn2-1* double mutant, and the lethality of other allele mutant combinations we have used artificial microRNAs to study the phenotype of plants with a more moderate decrease in MTN activity. My research focuses on quantifying the MTN enzyme activity in these plants along with documenting their development and pollen viability.

The role of calmodulin (CaM) in regulating the nucleoside transporter hENT1

Alex Bicket*

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Equilibrative nucleoside transporters (ENTs) are integral membrane proteins which are ubiquitously expressed in human cells and are responsible for the transport of adenosine and other nucleosides. Little is known about ENT regulation or the proteins involved in modulating their function. Recently, calmodulin (CaM) was identified as a protein that interacts with ENT1 in a biochemical assay. The goal of my study is to provide evidence that CaM interacts with hENT1 in a physiologically relevant system and to identify the role of CaM in regulating ENT1 function. To determine if CaM associates with hENT1 in the cell, I will investigate colocalization of both hENT1 and CaM in COS-7 cells using GFP-tagged hENT1 transfected into COS-7 and fluorescent antibodies against CaM. Confocal microscopy will be used to determine if the proteins colocalize in the cell. To determine if CaM is involved in trafficking of hENT1 in the cell, ³H labeled S-(4-nitrobenzyl)-6-thioinosine (NBTI) binding assays will be conducted. NBTI is a tight binding inhibitor of hENT1 and can be used to determine the amount of hENT1 present at the cell membrane. Comparing the hENT1 levels on COS-7 cells with the presence and absence of Ca²⁺ will determine the role of CaM on trafficking since CaM is dependent on Ca²⁺ concentrations in the cell. This study will provide new insights which will enhance our understanding of how hENT1 is regulated and how it functions in the cell.

Developmental and evolutionary analysis of *Frost* expression during cold recovery in *Drosophila*

Xin Y. Bing*, Jian Zhang, Heath A. MacMillan, and Brent J. Sinclair

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Frost (Fst) is a candidate gene associated with recovery from cold exposure in adult *D. melanogaster*. To assess whether *Fst* is part of the response to cold at all developmental stages, we tested whether *Fst* was upregulated in all life stages, and compared the levels of induced and constitutive expression among stages. Ten different developmental stages of *D. melanogaster* (of both sexes in adult flies) were exposed to 0°C for 2h, and allowed to recovery at 25°C for 1h, while control groups were kept at room temperature. *Fst* was not significantly upregulated during recovery from chilling in the egg, 1st, 2nd and wandering larval instars, and 20-day old adults of both sexes relative to untreated individuals of the same developmental stage. By contrast, *Fst* was significantly upregulated in the 3rd instar larvae, prepupae, and pupae relative to control, although basal expression of *Fst* was highest in these instars relative to other developmental stages. Of note, 2 and 5-day old adults of both sexes demonstrated the highest expression of *Fst* relative to control, consistent with previous findings.

Expression of Fatty Acid ω -Hydroxylase Genes in Soybean Hairy Roots

Avi Biswas* (*Department of Biology, University of Western Ontario*), Supervisor: Dr. Mark A. Bernards (*Department of Biology, University of Western Ontario*).

Root rot caused by the plant pathogen *Phytophthora sojae* is a major cause of lost soybean yield worldwide. Preformed suberin in soybean roots has been shown to act as an innate, physical barrier to *P. sojae* infection. ω -Hydroxy fatty acids, which constitute part of the poly(aliphatic) domain of suberin, are of particular interest as their quantity in preformed suberin correlates with greater partial resistance to *P. sojae*. Since soybean is difficult to genetically modify through traditional *Agrobacterium tumefaciens*-based genetic transformation systems, and *P. sojae* is a root pathogen, we are developing a hairy root model system to study this host-pathogen interaction. The hairy root phenotype is linked to infection with *A. rhizogenes*, which can also be used to genetically transform plant tissue. As part of this model system development, we have used hairy roots derived from both resistant (Conrad) and susceptible (OX760-6) soybean cultivars to determine the expression profiles of six putative soybean fatty acid ω -hydroxylase (FA ω H) genes previously identified in our group. This study aims to determine which FA ω H genes contribute to soybean root suberin formation, as a first step in understanding the genetic regulation of the development of this aspect of soybean resistance to *P. sojae* infection.

Genetic influences on brain size in the Chinook salmon (*Oncorhynchus tshawytscha*)

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In recent studies it has been of particular interest to investigate possible influencing factors as an explanation for variability within brain structure, size, and function of various species. Various factors such as habitat complexity, home range size, wild or hatchery decent and reproductive status have been under recent exploration across several fish species. In this study, we examine possible genetic influences on brain size in the Chinook salmon (*Oncorhynchus tshawytscha*) across several family crosses. To conduct this research, brain mass and brain volume measurements were obtained and compared against offspring of multiple family crosses. Results thus far have shown that there is no significant genetic influence on total brain mass and brain volume across families of hatchery decent. However, the investigation of whether differences arise between several brain structures such as the bulbus olfactorius, telencephalon, optic tectum, and cerebellum is underway. This research will be one of the limited studies analyzing a possible genetic heritable link to overall brain structure and size with specificity to the bulbus olfactorius, telencephalon, optic tectum, and cerebellum. The determination of brain structure inheritance in fish could potentially aid researchers in understanding brain function at an intraspecific level, develop insight into natural levels of variation, and examine microevolutionary and macroevolutionary patterns.

Investigation of germicidal UV impacts on the marine flagellates, *Tetraselmis apiculata* and *Heterosigma akashiwo*.

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Dispersion of phytoplankton via marine ballast water has the potential to become a significant environmental problem. Introduction of invasive phytoplankton species to an area can have negative ecological and economic effects. One of the current purification treatments for ballast water involves the addition of bleach to the water that can adversely impact water quality and aquatic life upon discharge. Effects of germicidal UV irradiation as an alternative treatment for purifying ballast water have been investigated on a series of green phytoplankton and harmful algal bloom isolates: *Tetraselmis apiculata* and *Heterosigma akashiwo*, respectively. A number assessment using the flow cytometer aided in determining cell numbers and the Clark-type O₂ electrode was employed to determine photosystem damage of UV treated isolates. UV damage to the photosystem has been assessed by evaluating photosynthetic efficiencies of UV treated cells with increasing light irradiances. It was thought that cell viability to UV exposure is related to the density of the cells within a sample and the amount of time that cells are subjected to germicidal UV irradiation. The viability of *Tetraselmis apiculata* was strongly impacted by UV treatments. In contrast, the coastal harmful algal bloom species, *Heterosigma akashiwo*, was insensitive to the range of UV exposures. Variation in the UV effects will influence the efficiency of UV treatment.

Hematopoietic Stem Cell Expansion and Transplantation & Spy1 Expression in Induced Pluripotency

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The effectiveness of bone marrow transplantation (BMT) in the treatment of leukemia is limited by the number of hematopoietic stem cells (HSCs) that are able to “home” into the bone marrow niche. It is expected that increasing the HSC population extracted from the donor prior to transplantation will enhance engraftment and reconstitution of the recipient’s hematopoietic system. Furthermore, finding alternative sources for HSCs, such as manipulating adult somatic cells into induced pluripotent stem cells (iPS) and directing them towards the hematopoietic lineage, may also improve BMT. Both cases require overriding senescent barriers normally in place as a protective mechanism. Senescence is controlled by a balance of intrinsic cell cycle regulators, proteins commonly misregulated in cancer. Hence, safe expansion of HSCs will require appropriate regulation of the cell cycle. Speedy (Spy1) is a novel cell cycle regulator shown to be implicated in stem cell fate in the breast, brain, and the hematopoietic system. I hypothesize that HSCs safely expanded for a brief time *in vitro* will correlate with optimal reconstitution *in vivo*, and that the expression of Spy1 levels will correspond with increased stemness in iPS. The genomic signatures of HSCs expanded across a time course will be assessed using qRT-PCR. To study stemness, mouse embryonic fibroblasts are infected to manipulate levels of Spy1, and induced to become pluripotent with the FUV-OSKM plasmid. Results to date support that Spy1 expression correlates with genes used to induce pluripotency, including Oct4, c-Myc, and Sox2. Ultimately, safely expanding HSCs could greatly enhance the treatment of blood diseases.

The Effect of Nucleoredoxin Overexpression on the Wnt- β -Catenin Pathway and Epithelial-Mesenchymal Transitions.

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The earliest epithelial-mesenchymal transition (EMT) in mammalian embryogenesis occurs when cells of the inner cell mass become mesenchymal and migrate outwards to form extraembryonic endoderm. This event is recapitulated *in vitro* using the mouse F9 cell model. F9 cells differentiate into primitive endoderm following activation of the Wnt- β -catenin pathway, exposure to exogenous reactive oxygen species (ROS) or treatment with retinoic acid (RA). Previous work has shown that Wnt6 is up-regulated in F9 cells in response to RA treatment and that the β -catenin pathway is positively regulated by ROS. Nucleoredoxin (NRX), a redox-sensitive negative regulator of the β -catenin signaling pathway, has been shown to inhibit embryonic development in *Xenopus*. I hypothesize that NRX negatively regulates Wnt- β -catenin signaling and maintains F9 cells in a less differentiated state. Therefore, overexpression of NRX in the presence of RA should inhibit the EMT and the differentiation of F9 cells into extraembryonic endoderm. NRX expression in F9 cells has been confirmed at both the mRNA and protein level. Overexpression studies remain to be undertaken and will further elucidate the role of NRX in the Wnt- β -catenin pathway.

Quantification of lipid content, soluble triglyceride concentration, and nicotinamide co-factors through periods of starvation and recovery in *Drosophila melanogaster*.

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Lipid metabolism is a complex metabolic network that can be influenced by different stresses. Both starvation stress and starvation-recovery responses involve a shift in metabolic flux which causes changes in various metabolite concentrations. In this research, I address the effects of starvation and starvation-recovery on the concentrations of soluble triglyceride, total lipid, and nicotinamide co-factors in *Drosophila melanogaster*. These metabolites were quantified because they indicate the flux and general state of the metabolic network. The relative levels of nicotinamide co-factors indicate if the network is in a net oxidative or reductive state. The lipid content indicates the amount of remaining energy stores and triglyceride concentration denotes the level of available energy precursors at any one point. In previous studies triglycerides were commonly measured with the assumption that they mirror lipid content. My results show no significant differences in triglyceride concentrations for both starvation, and starvation-recovery periods. While lipid content showed significant decreases in both starvation and starvation-recovery periods. Nicotinamide results are forth coming. Taken altogether these results suggest a relatively stable pool of triglycerides due to the balance between lipid mobilization and triglyceride breakdown and further suggest that triglyceride concentration may not always accurately reflect lipid content. On the other hand, lipid stores are mobilized or replenished based on the metabolic need. Due to the suggestion of this dynamic system further research into triglyceride cycling is the next step forward.

The Role of Spy1 Localization in the DNA Damage Response

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The DNA damage response (DDR) is an important protective mechanism that elicits cellular responses such as cell cycle arrest, DNA repair, senescence, and apoptosis. These responses exist to ensure that cells replicate without genetic errors and repair damaged DNA, as well as to initiate apoptosis if the damage is severe. Deregulation of these checkpoint pathways leads to genomic instability and subsequent oncogenesis. Spy1 is a unique cell cycle regulator that is found at elevated levels in several human cancers. It has been shown to promote cell viability and prevent apoptosis by overriding the DDR. Spy1 is expressed and degraded in a cell cycle dependent manner and can activate cyclin-dependent kinases. Data supports that Spy1 is a nuclear protein that can be shuttled to the cytoplasm under specific conditions. However, when, why and how this occurs is unknown. We hypothesize that sub-cellular localization of Spy1 plays a key role in stability of the protein following DNA damage. A mammalian GFP-tagged human Spy1A expression plasmid was used to characterize Spy1 localization via fluorescent microscopy following DNA damage. In addition to monitoring changes in localization, we sought to determine whether Spy1 nuclear export was dependent upon the exportin CRM1. To this end, GFP-Spy1 was treated with leptomycin B, a cytotoxin that binds to CRM1 and inhibits nuclear export. This work seeks to determine when and how Spy1 cellular localization is regulated following DDR. This is important since misregulation of Spy1 permits deregulation of the overall cell cycle and therefore contributes to tumorigenesis.

A Multigenerational Study of Reproductive Impacts of Pharmaceutical Drug Exposure in Zebrafish (*Danio rerio*).

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Over the last thirty years, pharmaceuticals and personal care products (PPCPs) have been documented to exist in our surface waters at ngL^{-1} to μgL^{-1} concentrations. This is largely due to increasing human consumption of PPCPs and inadequate removal by sewage and wastewater treatment plants. Exposing marine species to these biologically active compounds may pose ecotoxicological threats, although little research has been able to identify the physiological effects of chronic PPCP exposure. The present research consists of a multigenerational assessment of the reproductive impacts of PPCP exposure in zebrafish (*Danio rerio*). Fish were exposed for 6-weeks to environmentally-relevant concentrations of representative compounds from commonly-found classes of drugs in wastewater effluent: Analgesics/anti-pyretics (acetaminophen), lipid regulators (gemfibrozil), anti-epileptics (carbamazepine) and anti-depressants (venlafaxine). Fish will be exposed to single compounds, a mixture of these compounds as well as wastewater effluent. Embryos collected during the last two weeks of these exposures will be reared to sexual maturity, and these F1 fish will undergo pairwise assessments of breeding success. Fish exposed to $10 \mu\text{gL}^{-1}$ of aqueous acetaminophen and venlafaxine showed significant declines in viable embryo production compared to controls. From a previous zebrafish exposure to 25% (by volume) wastewater effluent, F1 zebrafish showed significantly higher mating success than controls in pairwise breeding trials, although viable clutch sizes were not significantly different. Overall, these results suggest that impacts to fish reproduction exist in wastewater-impacted environments. Whether or not there is a multigenerational impact from parental PPCP exposure remains an issue requiring deeper investigation.

Crouching as a Sleep-Like State in Crayfish

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Crayfish are among the few invertebrate organisms in which a sleep state has been detected. Preliminary 24-hour video recordings of crayfish (*Procambarus clarkii*) behaviour failed to detect the previously described position associated with sleep in the crayfish, that of the crayfish lying on one side. The experiment set out to test whether the crouch position (with a curled tail posture) for crayfish could be considered a sleep state by continuously video-recording solitary crayfish behaviour in a tank for 24 hours before, and for 48 hours after (each with a 12:12 Light:Dark cycle), undergoing a sleep deprivation procedure and determining whether the crayfish engaged in more crouching afterwards. It was hypothesized that there would be an increase in crouching afterwards in order to make up for a sleep debt. The results of video analysis did not support crouching as a sleep-like state. Furthermore, crouching occurred during both the light and dark phases, and there was a trend suggesting that crayfish crouch more during the light phase, but this was not statistically significant. Variability in crouch times tended to increase between individuals in the 1st day after sleep deprivation as well as the 2nd day (but less pronounced) after sleep deprivation, suggesting large differences in how crayfish respond to stressors. A non-significant trend suggested that the light cycle might influence the response to the deprivation procedure. Future work needs to be done to clarify whether crayfish sleep, the significance of crouching, and responses to psychological stressors.

Effects of gall density on the mortality rate of inhabitants associated with the gall of *Diplolepis rosaefolii* (Hymenoptera: Cynipidae) on two host roses.

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Galls induced by *Diplolepis rosaefolii* (Cockerell) occur in clusters of varying density on the leaflets of *Rosa blanda* and *Rosa acicularis* in central Ontario. The impact of gall density on the success of the inducer, along with members of the component community of inquilines and parasitoids was investigated. Galls on *R. acicularis* had a higher percentage of inquilines and a lower percentage of inducer species than did galls on *R. blanda* which had that a higher percentage of inducer species and a lower percentage of inquiline species. The percentage of parasitoids on both host plants were similar. This may indicate a negative inquiline driven effect on inducer success on *Rosa acicularis* and positive inducer driven effect on inducer success on *Rosa blanda*.

The effects of two nodulation inhibitors on nodule number in the *Pisum sativum* (L.) mutant E151

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Leguminous species have served as model organisms for plants with reduced needs for chemical pollutants because they can grow with limited fertilizer application. One well-known model legume is pea (*Pisum sativum* L.) and many root symbiosis mutants exist for this species. These mutants are used to study the development and regulation of two beneficial symbioses, nodulation and mycorrhization. E151 (*sym15*) is such a nodulation mutant that develops a low number of nodules and exhibits delayed nodulation, the phenotype of which is root-controlled. The mutant is hypothesized to be faulty in a systemic control system (also known as AON for autoregulation of nodulation), where a root-derived signal is perceived by the shoot, triggering another signal that is ultimately sensed by younger roots which then inhibit the formation of nodules. AON-deficient mutants, from various leguminous plant species, which form a large number of nodules have been described as having differential sensitivity to either ethylene or nitrate, both of which are known inhibitors of nodulation in pea. I have determined E151 sensitivity to these nodulation inhibitors. The mutant exhibits decreased nodule numbers when grown in their presence. As such, E151 characterized as a low nodulator, may belong to a new class of AON-deficient mutants which are sensitive to both ethylene and nitrate. E151 may become an important tool to understand in greater depth the AON mechanism. Understanding autoregulation of root symbioses will be a critical step in elucidating the physiology behind the formation, regulation and maintenance of this mutualistic symbiosis.

Adaptations of the native pumpkinseed sunfish (*Lepomis gibbosus*) to the invasive zebra mussel (*Dreossema polymorpha*)

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Biodiversity is a function of the interactions between a variety of organisms in a community. One of the leading causes of biodiversity loss is the introduction of non-native species. Zebra mussels (*Dreossema polymorpha*) are a well known invasive species abundant in many North American lakes following their introduction into the Great Lakes in the late 1980s. This experiment was designed to assess if a native fish, the pumpkinseed sunfish (*Lepomis gibbosus*), has adapted to the availability of zebra mussels as a new food resource. We sampled sunfish from Lake Opinicon, a lake that has a large zebra mussel population due to its connection with the Rideau Canal system. It was hypothesized that pumpkinseed sunfish are incorporating zebra mussels into their diet, as this sunfish species is known to be a shell crusher and has been observed consuming zebra mussels in laboratory conditions. Pumpkinseed sunfish were sampled by angling in littoral areas around the lake. Short-term and long-term diets were analyzed using stomach contents and stable isotopes, respectively. As hypothesized, pumpkinseed sunfish are consuming zebra mussels, yet surprisingly they have exhibited a greater diet shift than originally expected. These findings provide evidence that native pumpkinseed sunfish are adapting to zebra mussels and given the opportunity they may become a biological control for this invasive species.

The effects of heat stress on Trinidadian guppy's (*Poecilia reticulata*) sperm quality and reproductive success

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Global warming and its potential effects on biodiversity have become an accepted phenomenon among nearly all scientists. Some species may have to adjust their life history characteristics to increasing temperatures in order to survive in warmer environments. If increased temperatures negatively effects species' reproductive cycle it will also lead to negative effects on species' population. In this study, Trinidadian guppies (*Poecilia reticulata*) were used to test the hypothesis that increased temperatures will decrease sperm density, tail length, head length and male reproductive success. 15 males and 15 females were reared in 25°C water to stimulate current natural conditions, and 15 males and 15 females were reared in 30°C water to reflect the effects of global warming. Every female was placed in a tank, with water set to the temperature they were reared in. Two males, each reared in either temperature, were added to the tank for a 24-hour copulation period after which the females were left in the tanks to give birth. Sperm was then extracted from each male and sperm density, tail length and head length were all measured using a dissection microscope and a Z1 microscope. Sperm from males reared in 25°C water was significantly longer and denser than sperm from males reared in 30°C water. Fin clips were then collected from all females, males and offspring in order to conduct paternity tests, which remain to be determined. These findings suggest that increased water temperatures negatively influence sperm quality and male reproductive success.

Plant hormonal response to nickel and cadmium heavy metal stress

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Plants are sessile and are therefore greatly susceptible to their surroundings. Human activity, such as mining, burning fossil fuels, and application of sludge to agricultural land, increase the amount of heavy metals in the soil. In turn, this manifests as an abiotic stress in the form of high concentrations of heavy metals within the plant. Therefore, an internal signal to orchestrate the appropriate growth response to this stress is essential and there is a strong possibility this is achieved through plant hormones. However, there are very few studies characterizing the hormonal responses to the heavy metal stress. This study will use the grass *Deschampsia cespitosa* (tufted hair grass) to profile two hormone classes – the growth inhibiting abscisic acid (ABA) and growth promoting cytokinins (CK) - in response to the build-up of the toxic metals, cadmium and nickel. I hypothesized that hormones mediate plant response to the abiotic heavy metal stress. I predicted that there would be an increase in ABA levels and a decrease in CK levels in both the roots and the shoots of *Deschampsia cespitosa*, as it is exposed to sub-lethal levels of Cd and Ni. ABA and 18 different CKs were then extracted, purified by solid phase extraction and quantities measured by electrospray ionization, liquid chromatography-tandem mass spectrometry (LC-(ESI) MS/MS). The patterns of these hormone profiles will be discussed.

Migration Patterns of the White-throated Sparrow (*Zonotrichia albicollis*): A Stable Isotope Approach

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Differential migration occurs when certain individuals or populations within a species migrate further than others, creating a separation on the wintering grounds. Such a migration pattern has been documented in the white-throated sparrow (*Zonotrichia albicollis*) with females and tan-striped individuals migrating farther south than males and white-striped individuals, respectively. The goal of this study was to determine which factors were the most influential on the wintering latitudes of white-throated sparrows. To do so, we used stable-isotope analysis of crown feathers obtained from birds breeding in Algonquin Provincial Park between 2006 and 2010. The effects of sex, morph, age class, climate, and experience on wintering latitudes were examined. Sex and morph were the only two significant factors affecting this parameter: males wintered farther north than females, and tan-striped individuals wintered farther north than white-striped individuals. While we confirmed the former pattern of separation, the latter differs from that reported in the literature. This pattern difference will give a new insight into the migration patterns of this widely studied species. Furthermore, the comparison of the results from this study with those of previous ones provides ample support for leapfrog migration.

The Effects of PACAP-38 on Muscle Contractions in *Drosophila* Larvae

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide involved in regulating a variety of physiological processes. Previous studies demonstrate that one PACAP form, PACAP-38, elicits its effects through modulation of ion channels. In *Drosophila* larval muscles, PACAP-38 has been shown to increase intracellular calcium concentration via influx through voltage-gated ion channels. The current study examined effects of PACAP-38 on muscle contractions in *Drosophila* larvae. Considering its effects on calcium channels, it was predicted that exogenous PACAP-38 would induce concentration-dependent contractions in larval body wall muscles. A possible role of PACAP-38 as a co-transmitter was also considered by observing whether the peptide potentiated glutamate-induced contractions. At concentrations ranging from 0.1 nM to 1 μ M, PACAP-38 did not induce contractions; however, concentrations of 0.1 and 1 nM appear to potentiate glutamate-induced contractions. PACAP-6-38, an antagonist at the PAC-1 receptor specific for PACAP-38, was applied to observe whether the peptide was being released constitutively onto the muscle fibers. Preliminary trials do not suggest constitutive release of PACAP-38.

The molecular basis of *Xenopus laevis* gene expression in the thyroid and lung

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Understanding how the cells in an embryo become differentiated is a central issue in developmental biology. Characteristics of *Xenopus laevis* development can be exploited to allow insight into the development of a body pattern and eventual organ morphogenesis of an embryo. This investigation explores the ability of retinoic acid to induce presumptive thyroid tissue to become lung tissue as a means of discovering new genes involved in the development of these organs. Treatment with retinoic acid at stage 24 causes the loss of expression of *pax2*, an important thyroid transcription factor, while causing expression of lung surfactant protein genes in the thyroid. We have demonstrated that expression of *hex*, another important thyroid transcription factor, is also lost as a result of treatment with retinoic acid. This further suggests that this transition is a change in fate for that tissue. We have now used explants of presumptive thyroid tissue to generate high quality RNA that will be used to compare global gene expression of normal thyroid tissue to that of thyroid tissue that has been converted to lung by retinoic acid. This data will be used to discover new genes that are associated with the normal development of these organ systems. Genes that are significantly different between these samples will be further characterized by whole mount in situ hybridization to determine their expression pattern in the embryo. Information gathered in this investigation will help describe the molecular pathways involved in the formation of the thyroid and lung.

Testing AON characteristics of E151, a low-nodulating mutant of *Pisum sativum*, with a series of approach-graft experiments.

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Approach-grafting was used on *Pisum sativum* to identify certain physiological characteristics of the auto-regulatory system controlling legume nodulation, known as AON. This involved the excision of tissue from the shoot of two juvenile plants, exposing phloem tissue in each organism. These wounds were then bound together, joining their respective vasculatures. Inoculation of one plant (the “sensor”) with rhizobia bacteria resulted in nodulation and the subsequent expression of chemical signals involved with AON. These chemicals circulated through both members of the graft. Depending on the delay between the inoculation of the sensor and the other root system (the “receptor”), nodulation was inhibited in the receptor root system to varying degrees due to these AON signals initiated by the sensor. Two series of grafting experiments were conducted. WT/WT isographs were used in three trials, where sensor-receptor delay was at 3, 5 and 7 days respectively. Following this, grafts incorporated the low-nodulating pea mutant E151-*sym15*. Proposed to be AON-deficient, previous work has identified the action of SYM-15 to be in the roots. If involved in AON signalling, *sym15* would likely function in either synthesis of the initial AON signal (which would become apparent if E151-*sym15* were used as the sensor) or receiving of the final inhibitory signal (observable if E151-*sym15* were the receptor). Trials of E151-sensor/WT-receptor and WT-sensor/E151-receptor were conducted and compared to WT isographs. Deviations from the WT isograph observed with E151-*sym15* as the receptor indicated *sym15* is involved with the reception of the final chemical signal in the AON pathway.

Carbonic Anhydrase in the Anal Papillae of the Larval Mosquito, *Aedes aegypti*

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The ability of mosquito larvae to withstand osmotic and ionic perturbations in their aquatic environment has been attributed to the physiological transport processes that take place across the anal papillae and other osmoregulatory organs. The present study focuses on the mechanism by which anal papillae take up ions, particularly Na^+ and Cl^- , from the external medium in order to maintain proper haemolymph ion levels. Early observations suggested that Na^+ and Cl^- influx across the anal papillae epithelium are coupled with H^+ and HCO_3^- efflux, respectively. In order to understand whether carbonic anhydrase (CA) is providing H^+ and HCO_3^- as counter ions for Na^+ and Cl^- , tissue specific RT-PCR was carried out to localize the distribution of CA in larval mosquitoes, *Aedes aegypti*. Results confirmed the presence of CA in the whole body, midgut, hindgut, malpighian tubules, and anal papillae. A real time, quantitative PCR study to determine the level of expression in each tissue is underway. The Scanning Ion selective Electrode Technique (SIET) was employed to observe whether H^+ and Cl^- fluxes across the anal papillae of *Aedes aegypti* were CA dependent. Using SIET microelectrodes, H^+ and Cl^- ion fluxes across the anal papillae epithelium were measured in mosquito larvae treated with the CA or chloride transport inhibitors Methazolamide, SITs, and DIDs. Results thus far have shown that when CA inhibitors were applied, H^+ flux was significantly reduced suggesting that carbonic anhydrase activity may be a central element in ionic regulation in the anal papillae.

Identification of Basidiomycota isolated from bats, using DNA sequence analysis and septal pore imaging

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Members of the Basidiomycota are important as decomposers and plant symbionts in most terrestrial ecosystems. This phylum contains, among others, the classes Agaricomycetes, comprising the 'true mushrooms', and the Tremellomycetes, the parasitic jelly fungi. Isolates of putative Agaricomycetes and Tremellomycetes (identified phenotypically as the genera *Sympodiella* and *Cystofilobasidium*) swabbed from cave dwelling bats in New Brunswick were sent to the Thorn lab for DNA-based identification and phylogenetic classification. DNA was extracted using a microbead beating approach. The nuclear ribosomal internal transcribed spacer (ITS) and portions of the adjacent small and large ribosomal subunits were PCR-amplified and sequenced. These sequences will be compared, using phylogenetic methods, to annotated sequences in GenBank to identify the unknown samples. For two isolates of putative Tremellomycetes, the genetic data will be complemented by morphological assessment of the septal pore structures, which will be generated using transmission electron microscopy.

Reproductive Ecology of the Pacific beetle cockroach (*Diploptera punctata*)

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Reproduction in the viviparous Pacific beetle cockroach (*Diploptera punctata*) has been studied from a physiological but not from an ecological perspective. I investigated the effect of food quality during their 80 day gestation period on female fecundity, when they were fed either high (A) or low (B) quality diets for the first 40 days following mating. The diets were reversed after 40 days to mimic possible changes between wet and dry seasons. Females continuously fed high quality served as controls. The working hypothesis was that females initially fed a low quality diet would delay the onset of pregnancy while those initially fed a high quality one would continue gestation due to significant investment in offspring at the time diet quality changed. Gestation periods between Hawaiian (H) and Thai (T) females starting on A did not differ (H = 87.7, T = 87.7; ANOVA, df = 17, P>0.05), but did for those starting on B (H = 86.92, T = 84.22; ANOVA, df = 20, P<0.05), while the control group's gestation was similar to the high quality group's (T & H = 87.3). All control group females completed their pregnancy, compared with 93% of diet A and 98% of diet B females. Interestingly, the Hawaiian females initially fed diet A produced fewer but larger nymphs (n=10.1±1.97, mass=23.6±4.8mg) than those fed diet B (n=12.3±.75, mass=22.6±4.3 mg). The inverse was seen with Thai females (n=9.8±1.2 vs. n=8.6±1.5; mass=16.7±1.4 vs mass=22.0±4.4 mg), while controls generally had high values for both parameters (H: n=12.33±1.5, mass=24.9±5.4; T: n=9.67±2.1, mass=21.3±2.6). A 2-way ANOVA suggests that diet affected this duration of gestation (P = .01), while nymph mass (P = .01) and clutch size (P <.001) were determined by geographic origin. The results of this study demonstrate that a shift in food quality may influence the gestation period, but not clutch size or nymph mass.

Epigenetic changes and depression phenotypes in a mouse model of Fetal Alcohol Spectrum Disorder.

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Fetal Alcohol Spectrum Disorder (FASD) refers to the neurological, behavioural and developmental abnormalities resulting from *in utero* alcohol (ethanol) exposure; among them an increased risk for depression and anxiety. The molecular mechanism of FASD remains unclear but involves impaired neuron development and synapse formation, possibly via alteration of the epigenome. We exposed fetuses *in utero* via continuous preference (CP) ethanol consumption by pregnant C57BL/6J mouse mothers in a two-bottle (10% ethanol vs. water) free choice paradigm. First, anxiety and depression phenotypes of CP progeny were assessed using the elevated plus maze, forced swim and tail suspension tests respectively. Female CP progeny, but not males, exhibited significantly less anxiety than controls. Also, they did not exhibit a significant depression phenotype compared to controls. Second, use of microarrays identified 36 genes that were dys-regulated in the brains of 60 day old CP progeny. Of these, four genes (*Gh*, *Prl*, *Cga* and *Gabra6*) with known roles in depression and/or anxiety were confirmed for altered expression by TaqMan based qPCR. Finally, we hypothesized that: alterations in gene-expression following ethanol exposure are accomplished and maintained by changes in DNA methylation. Thus, the methylation profile of a CpG island upstream of *Cga* is currently being characterized for ethanol-responsive changes via sodium bisulfite sequencing. In the future, whole-genome methylation changes should be considered in FASD mice and cross-referenced with gene-expression changes. This will identify strong candidate genes, but more importantly an epigenetic basis of FASD.

The Importance of Soil CO₂ Efflux during Storm Events in Estimating Forest Carbon Accounts

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Predicting carbon pools is an important focus of national and international policies for inventory reporting and the review of carbon emissions and removals. Major efforts are underway to determine the relative role of forests in the global carbon budget. Most carbon accounting estimates are based on measurements that occur at regular weekly or monthly intervals; few specifically focus on targeting hydrologic events such as melt or storms. The purpose of this project was to establish the relative importance of storms on soil CO₂ efflux from a forest in the Great Lakes-St. Lawrence forest region of Canada, which extends from southeastern Manitoba to the Gaspé Peninsula. We hypothesized that soil CO₂ efflux during storm events would be significantly different from measurements taken in-between events and this discrepancy would result in an over- or under-estimate of annual soil CO₂ efflux. We constructed an eight-year time series (2003 to 2010) of soil CO₂ efflux measurements to determine if there was a relationship between rain and CO₂ efflux. Differences in total annual soil CO₂ efflux between estimations including and not including rain events were compared to total growing season precipitation. Results showed a significant over-estimation in soil CO₂ efflux with lower growing season precipitation, and a significant under-estimation of soil CO₂ efflux with higher growing season precipitation. The implications of this project illustrate a growing need to improve our methods in describing and quantifying the heterogeneity in soil carbon pools and fluxes, so that forest carbon budgets and corresponding uncertainties are complete and accurate.

Mechanisms of conspecific and heterospecific communication in Black-capped Chickadees

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Many animals communicate subtle predator attributes, such as size or degree of threat, by varying aspects of their alarm signals, such as signal structure, signal type, or signal rate. Black-capped Chickadees produce chick-a-dee calls while mobbing perched raptors, and both the duty cycle and the number of dee notes per call vary inversely with the size of the predator. In response to chick-a-dee calls, both conspecific and heterospecific animals exhibit stronger antipredator behaviour towards calls elicited by smaller and more dangerous predators. It remains unclear, however, whether predator size is communicated through fine structural variation of individual calls (i.e., number of dee notes), through variation in the duty cycle of signaling sequences, or both. To distinguish between these potential mechanisms of communication, we independently manipulated the call structure and duty cycle of chick-a-dee calls by varying the number of dee notes per call and the calling rate, respectively. We then broadcast calls in conjunction with a perched predator model at 96 different locations within natural chickadee habitat. Both conspecific and heterospecific receivers approached the playback speaker more rapidly when duty cycle was increased and the number of dee notes per call was held constant. In contrast, receivers did not respond differentially when duty cycle was held constant and the number of dee notes per call was manipulated. We conclude that predator size is communicated by variation in the duty cycle of signaling sequences and not by variation in the fine structure of individual chick-a-dee calls.

Retinal degeneration in the *harlequin* (*hq*) mouse is characterized by early gene expression changes with associated nuclear displacement

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Retinal degeneration is increasing in prevalence but knowledge of underlying disease mechanisms is lacking. The objective of this study was to examine changes in expression of *fibronectin1* (*Fn1*), *laminin-alpha2* (*Lama2*) and *glial fibrillary acidic protein* (*Gfap*) genes in parallel with displacement and loss of nuclei in the retina of the *harlequin* (*hq*) mouse, a model of neurodegeneration. A gene expression microarray was used to determine gene expression from whole retina RNA in 5-week and 4-month-old *hq* mice compared to age-matched wild-type (WT) mice. Real-time quantitative PCR was used to examine the upregulation of *Fn1*, *Lama2* and *Gfap* genes for 5-week, 4-month and 7-month-old *hq* and age-matched WT mice. Hematoxylin and eosin staining of eye cross-sections from 5-week, 4-month and 7-month-old *hq* and WT mice was performed to analyze nuclear displacement and loss. Expression of *Fn1*, *Lama2* and *Gfap* genes was unchanged in 5-week-old *hq* compared to WT mice. *Fn1*, *Lama2* and *Gfap* were upregulated by 1.48 (p=0.09), 2.16 fold (p=0.02) and 1.33 fold (p=0.27) in 4-month-old *hq* mice and 2.78 fold (p=0.01), 3.64 fold (p=0.04) and 5.31 fold (p<0.001) in 7-month-old *hq* mice compared to age-match WT mice. Nuclear displacement was observed from inner and outer nuclear layers in *hq* mice at 4-months of age with increased nuclear loss at 7-months of age. Upregulation of *Fn1*, *Lama2* and *Gfap* gene expression increased with age and disease progression in *hq* mice. Increased gene expression of *Fn1*, *Lama2* and *Gfap* was associated with increased extracellular matrix remodelling, nuclear displacement and nuclear loss.

Functional analysis of the human E3 ligase MdmX.

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The tumor suppressor p53 is known to participate in control of cell cycle and apoptosis. Mdm2 and MdmX, E3 ligases containing RING domains, are the main regulators of p53. Mdm2 and MdmX bind the transactivation domain of p53 and inhibit its gene transcription function (Allende-Vega & Saville, 2010). Polyubiquitination of p53 by Mdm2 destines it to degradation by 26S proteasome. The levels of Mdm2 and MdmX are kept low in normal cells due to autoubiquitination activity of Mdm2 and its ability to ubiquitinate MdmX and subject them to proteasomal degradation (Lee & Gu, 2010). It was previously shown that Mdm2/MdmX heterodimer had a higher affinity for p53 than Mdm2/Mdm2 homodimer; therefore, stabilization of Mdm2 by MdmX leads to more efficient p53 ubiquitination and degradation (Wade *et al.*, 2010). However, MdmX RING domain lacks E3 ligase activity and fails to ubiquitinate p53 in absence of Mdm2. In this study, we isolated MdmX RING domain and conducted its functional analysis. Residues essential for dimerization and E2 recruitment were previously identified in Mdm2 RING domain. Due to structural homology between Mdm2 and MdmX, it was possible to assume that those residues could also be vital for dimerization and E2 recruitment by MdmX RING domain. We introduced point mutations into MdmX RING domain by site-directed mutagenesis. Mutated forms of MdmX RING were tested for the autoubiquitination activity and the ability to ubiquitinate full length Mdm2 *in vitro*. We identified a particular point mutation that allowed MdmX RING to gain ubiquitination activity. An active form of MdmX RING domain that is capable of ubiquitinating Mdm2 and directing it to 26S proteasome for degradation, thereby, stabilizing p53 within a cell becomes a drug target for cancer therapy.

A genome-wide genotyping array detects clusters of mutations in the genome of the harlequin mouse model of neurodegeneration

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Mutation research using the mouse genome has been limited to single gene targets or low-resolution genotyping arrays, restricting our knowledge of mutational mechanisms across the genome. To identify mutations across the mouse genome, we used the highest resolution, single nucleotide polymorphism (SNP) mouse genotyping array, capable of detecting 623,124 SNPs to compare DNA from two tissues of the same mouse. Genotyping differences between two tissues were compared for four mice: two *harlequin* (*hq*) mice with neurodegeneration and two age-matched wild type mice. The Mouse Diversity Genotyping Array (MDGA; The Jackson Laboratory) was used to detect the two SNP alleles on the array and loss of SNP allele calls. Mutations were scored as differences in genotype between the two tissues of the same mouse. The number of differences in SNP allele calls and occurrences of no allele calls are “on target” and “off target” mutations respectively. The estimated target size for “off target” mutations is 1.5×10^8 and 8.9×10^{12} , determined by the number of nucleotide changes predicted to generate no allele calls. Mutation load was greater in *hq* mice ($p < 0.001$) and exhibited a non-random distribution across the chromosomes in all mice ($p < 0.001$). Selective pressures on the X chromosome and instability on chromosome 17 were identified by the reduced and increased number of mutations respectively ($p < 0.001$). Mutations in *hq* mice showed overabundance on chromosomes 12, 13, and 14 ($p < 0.001$). The MDGA approach to mutation research demonstrated potential for genome-wide analysis of the load and distribution of mutations associated with complex mouse disease phenotypes.

Linker Histone Acetylation in *Saccharomyces cerevisiae*

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Histones are highly conserved eukaryotic proteins that play a critical role in modulating access to DNA during repair, replication and gene expression. The nucleosome is composed of 147 bp of DNA wrapped around a histone octamer made up of two copies of each of the core histones (H2A, H2B, H3, and H4). Nucleosomes are further condensed by association with linker histone H1. In order for enzymes to access DNA within a nucleosome, histones must undergo conformational changes to bind “less tightly” to the DNA. One way this is done is by post translational modification, such as acetylation. In this study, we are interested in determining if H1 is acetylated in the model yeast *Saccharomyces cerevisiae* (gene name *HHO1*). Histone acetyltransferases (HATs) are enzymes that acetylate specific lysine residues of histones. The Rtt109 HAT has been previously demonstrated to acetylate H3 on several sites in conjunction with another protein, Vps75. Our unpublished data shows that Rtt109-Vps75 can acetylate chicken and bovine H1 *in vitro*. My goal is to determine if Rtt109-Vps75 can also acetylate Hho1. To determine this, I expressed 6xHIS-Hho1 as a recombinant protein in bacteria and used it to conduct an *in vitro* HAT assay. We hypothesize that Hho1 acetylation occurs on a consensus sites (based on Rtt109-Vps75’s previously described activity on H3). To date we have shown that recombinant Hho1 is acetylated by Rtt109-Vps75 *in vitro*. I am currently using site directed mutagenesis to explore possible *in vivo* relevance of Hho1 acetylation.

An investigation of herbivory effects in Ontario *Festuca* grasses infected with fungal endophytes.

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Endophytes are fungal symbionts which live asymptotically in grass hosts and often produce alkaloids which act as herbivore defences. They are well studied in agronomic systems because of the economic impact they have had on livestock but research involving endophyte effects in natural systems is lacking. I conducted my research on several grasses collected from wild locations belonging to the genus *Festuca*. I tested these grasses to determine whether endophyte infection provided a herbivore defense in the grasses. Infected and uninfected plants were submitted to a choice test using garden slugs as a herbivore. The results varied depending on the grass species and location of collection. This may be an indication that the different plant and endophyte pairs produce different alkaloids causing these different effects.

Feeding Ecology of the round goby, *Neogobius melanostomus*

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The round goby, *Neogobius melanostomus*, a benthic feeder originating from the Black, Caspian, and Azov seas in southeast Europe, has been sighted in the Great Lakes and surrounding area since 1992. This invasive species has proliferated in Hamilton Harbour (Hamilton, ON), which is in part due to their ability to consume a variety of prey with Dreissenid mussels, another invasive species, being their most common dietary item. However, other species have been commonly found in round goby diets, such as crustaceans, chironomids, and molluscs. Unpublished studies have also detected fish scales and rocks in the guts of this species. Our study investigates the correlations between gut composition, reproductive status, and site contamination, as the International Joint Commission lists Hamilton Harbour as an Area of Concern. A total of 213 fish were captured at two sites in the harbour, one being relatively clean, while the other was a relatively contaminated site. Gut fullness was assessed upon dissection, using both a gut fullness index and other measures controlling for body condition. Contents were counted in both sediments and sediment samples using a stereomicroscope. Results will be discussed with respect to trends across sites, sexes, and reproductive statuses. Findings will also be related to a population census done in the harbour annually since 2004. These results will shed light on how contamination may accumulate, transfer across food webs, with new introductions into local aquatic systems, while taking organism type and abundance into account.

Characterization of a Matched Set of SOD1 P-element Excision Lines in *Drosophila melanogaster*: SOD1 Activity, Longevity, and Paraquat Sensitivity

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The superoxide dismutases (SODs) function as an enzymatic defense against the reactive oxygen molecule superoxide, a harmful by-product of aerobic respiration. The enzyme SOD1, or cytosolic SOD, protects the cell by scavenging potentially toxic superoxide radicals that have been linked to the oxidation of lipids, proteins, carbohydrates, and nucleic acids. Such damaging effects of reactive oxygen species are thought to greatly influence the overall processes of disease and aging. In this study, I characterize a matched set of SOD1 P-element excision-derived alleles in *Drosophila melanogaster* using a SOD activity assay, longevity, and sensitivity to reactive oxygen generating paraquat. The alleles examined in this study were originally thought to span from a SOD1 knockout to a SOD1 hypermorph. However, results from the longevity and paraquat sensitivity assays suggested the absence of a SOD1 knockout within this set. The SOD activity assay results confirmed the absence of a SOD1 knockout, and suggest that there was little variation in SOD1 activity among the lines. We suspect that this is likely due to a contamination of the third chromosomes in these lines. In order to confirm this, I will perform the characterization assays with a set of lines that have been previously described in the literature using these assays. Future work will focus on creating another set of SOD1 P-element excisions in a matched genetic background.

Pheromone recognition and mating success in Hawaiian and Thai populations of *Diploptera punctata*.

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The Pacific beetle cockroach *Diploptera punctata* is a viviparous cockroach found in Asia and many Pacific islands, including Hawaii. The majority of studies on this species have focused on the reproductive physiology but little is known about its mating behaviour or if, as in other cockroaches, sex pheromones are important. In my study I used two allopatric populations, one from Hawaii and the other from Thailand, as preliminary laboratory evidence suggested that interbreeding between the two populations was limited. I tested the hypothesis that differences in sex pheromones provided an effective reproductive isolating mechanism. I first examined the mating success of females, either zero or five days after their final molt when held with a male from her original population or one from the other geographic population. I then examined the response of both males and females using a Y-tube olfactometer bioassay. Females regardless of their age preferred males from their own geographic population significantly more than males from the other population. In the Y tube assays showed that Hawaiian individuals utilized volatiles to discriminate between potential mates while did not appear to be the case for those from Thailand.

Physiological effects of chronic sub-lethal waterborne Ni exposure in rainbow trout (*Oncorhynchus mykiss*).

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Increased metal concentrations in the environment from mining and smelting operations have led to impacts on aquatic biota. In the Sudbury region of Ontario, elevated levels of nickel (Ni) occur and are of concern. To examine the physiological effects of Ni, rainbow trout (*Oncorhynchus mykiss*) were exposed to varying concentrations of waterborne nickel during chronic sub-lethal exposures over three weeks. In one test, fish were exposed to 0.25, 0.75, 2.25 or 4.5 mg Ni/L for 18 d. Responses from this first test were then used to develop exposure concentrations for the subsequent exposure, 0.75 and 2.0 mg Ni/L. It was predicted that Ni tissue accumulation would be associated with adverse effects and costs that would be measured in terms of altered stress response and reduced swim performance. In other words, the cost of acclimation would affect the ability to swim and mount a stress response. Further, it was anticipated that higher concentrations of Ni would have damaging effects. Ni accumulation, plasma ion levels, swim performance and cortisol levels were assessed in Ni exposed fish with and without an acute handling stress. Kidney Ni concentrations were increased throughout the exposure and highest at 2.25 and 4.5 mg Ni/L exposures. A similar pattern was observed for accumulation in gills. Plasma Na concentrations increased initially, while Mg concentrations decreased. These results will provide further insight into how the stress response and swim performance in rainbow trout are affected by exposure to waterborne Ni.

Investigation of Germicidal UV Impacts on Viable, but Non-Culturable Marine Bacteria (VBNC).

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Ships, today, use water for ballast which they load at one dock and discharge at another. Foreign organisms are released into the water posing one of the greatest risks to oceans. New marine regulations will require ballast water to possess no viable cells greater than 0.45 μm . High proportion of marine bacteria enters into a viable but non-culturable state, in which the cells remain viable but cease producing colonies. Their detection by growth methods is ineffective. Using fluorescence dyes and examination by a flow cytometer (FCM), this experiment assessed the viability of natural and created marine bacterial assemblages after treatment with germicidal UV. Marine bacteria, together with a small population of a controlled culture, were exposed to germicidal UV light, to investigate its impacts. Total bacteria were measured using a cell permeant nucleic acid stain followed by treatment by fluorescence dye to distinguish viable bacterial numbers. A protein stain was used to follow the growth rate of natural or added cells over time by following the loss of dye per cell. The hypothesis is; flow cytometry shows the effectiveness of germicidal UV on VBNC marine bacteria to be comparable to that of viable marine bacteria cells.

The Role of the Transcription Factor Kaiso in the Development of Ovarian Cancer

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Ovarian cancer is one of the most common gynaecologic malignancies, and is currently the fifth leading cause of cancer death among women in the Western world. Among the multiple types of ovarian cancers, approximately 90% of ovarian tumours arise from the ovarian surface epithelium. Epithelial cells are characterized by strong cell-cell adhesion mediated through the adherens junction. The adherens junction is composed of the transmembrane epithelial-cadherin (E-cadherin) and its cytosolic adaptor proteins (β -, α -, and p120-catenin). E-cadherin forms homotypic interactions with E-cadherin on adjacent cells while the catenins either anchor E-cadherin (via its cytosolic domain) to the actin cytoskeleton or regulate cadherin stability and turnover. The catenins also have nuclear roles and regulate gene expression. For example, p120ctn also interacts with the transcription repressor Kaiso and relieves Kaiso-mediated repression of target genes implicated in development and tumourigenesis (e.g. matrilysin, cyclin D1). This phenomenon is usually correlated with an upregulation of N-cadherin, a characteristic of epithelial-mesenchymal transition (EMT) and an essential step in ovarian tumour invasion.

Preliminary experiments from our lab recently revealed that Kaiso was misexpressed in 50% of ovarian tumours compared to normal ovarian tissue. Additionally, Kaiso was expressed in several ovarian carcinoma cell lines. We hypothesize that Kaiso modulates cellular function in ovarian epithelial carcinomas. Due to the opposing Kaiso and cadherin expression of OVCA 420 compared to the various ovarian tumour cell lines, an inducible Kaiso-overexpressing ovarian carcinoma cell line will be generated to assess its functional role in cellular processes associated with cadherin-switching under controlled conditions. This inducible Kaiso-overexpressing OVCA 420 cell line will be used to assess the effect of Kaiso on various tumourigenic processes such as cell migration and invasion. The completion of these functional experiments should provide insight on the intracellular role of Kaiso in ovarian carcinoma progression.

Hypoxia Tolerance in Sea Lamprey *Ammocoetes*: Clues Suggesting Alcoholic Fermentation in Response to Hypoxic Exposure

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Sea Lampreys (*Petromyzon marinus*), a species of particular ecological interest in the Great Lakes, are tolerant to hypoxic environments during the larval stage of their life cycle. Interestingly, the gene coding for the alcohol dehydrogenase (ADH) enzyme is present in the muscle of these fish. Some organisms, the crucian carp (*Carassius carassius*) for example, have demonstrated the ability to avoid metabolic acidosis (due to lactate buildup) and increase ATP yield by converting pyruvate to ethanol as the end product of anaerobic glycolysis. Combined with the high glycogen stores found in the meninges of lamprey brains, the presence of ADH in the muscle tissue of these organisms suggests the possibility that they too might undergo alcoholic fermentation to avoid buildup of lactate under hypoxia. To test this hypothesis, organisms were placed in closed aquaria and dissolved oxygen was brought down to 10% by introducing nitrogen gas into the aquaria, creating a hypoxic environment. Exposure groups were anaesthetized and removed from the environment at 4, 8 and 12 hours (n=12). Muscle, brain and liver samples were removed and flash frozen in liquid nitrogen to preserve metabolites. Glycogen, lactate, ethanol will be determined using enzymatic assays and spectrophotometer analysis. Results from these assays are forthcoming.

Compost quality can affect cadmium and lead uptake in lettuce (*Lactuca sativa*)

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Bioaccumulation of metals in crop species is a major issue in the agricultural industry. Certain plants, such as lettuce (*Lactuca sativa*), grown in soils contaminated with non-essential metals have evolved complex internal tolerance mechanisms to otherwise toxic levels of elements, thus presenting a potential health risk to consumers. One approach to minimizing metal uptake in crops could be to amend the soil with organic matter (e.g. compost) so as to reduce metal bioavailability. This study aims to determine the effect that two components of compost, humic and fulvic acids (HA and FA, respectively), have on metal bioavailability to lettuce. Three soils were collected from urban gardens in Southwestern Ontario, which were mildly contaminated with cadmium and lead. Lettuce was grown in each soil type mixed with one of four composts (25% by volume) with varying HA:FA ratios. Physicochemical properties of the soil and compost mixtures, including total organic matter, pH, conductivity, and texture, were found to be relatively constant across all mixtures. Cadmium and lead content of soils and leaves were determined by ICP-AES. Despite growth in soils well above accepted contamination limits, the addition of compost lowered the amount of metal accumulated in lettuce leaves in almost all treatments. HA and FA proportions in soils were estimated as dissolved organic carbon content. Soil and compost mixtures with higher HA:FA ratio are expected to result in greater decreases in the bioavailability of metals to lettuce.

Top down control and local and regional scale environmental effects on fish and invertebrate community structure in a temperate eelgrass ecosystem

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Anthropogenic pressures on coastal marine resources have created an urgency to understand the relative influence of various food-web controls. Seagrass meadows have experienced high levels of degradation, with large scale losses being observed globally. These communities provide valuable ecosystem services including nutrient and carbon cycling, sediment stabilization, and important rearing grounds for juvenile fishes. Despite their importance, the community dynamics in temperate zone seagrass (eelgrass; *Zostera sp.*) meadows largely remain a black box. In this study, we use a unique and long-term fish and invertebrate data set collected by Parks Canada in the Gulf Islands of South-Western British Columbia to assess the relative influence of local factors such as top-down, bottom up and several abiotic factors on the community structure of fish, benthic invertebrates, and epiphyte grazers, in addition to regional spatial and temporal factors such as location and El Niño Southern Oscillation variables. Multivariate analysis reveals a top down controlled system with predator abundance explaining high levels of variation in abundance of epiphyte grazers. Local scale effects from the Fraser River plume as well as regional climate effects such as ENSO events also explaining additional levels of community variance. These results have important implications for nearshore marine spatial planning, and resource management decisions in the proposed Strait of Georgia Marine Protected Area.

The feeding responses of *Peromyscus maniculatus* and *Myodes gapperi* in their natural environment to the seeds of *Abies lasiocarpa*

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Seed producing plants often face the risk of seed loss through predation. In order to mediate this predation some plants have Plant Secondary Compounds (PSCs), which are harmful to many consumers. A tree endemic to the subalpine regions of Kananaskis Valley, Alberta, *Abies lasiocarpa*, produces PSCs and yet its seeds are believed to be consumed by the two most prominent rodents of the region: deer mice (*Peromyscus maniculatus*) and red-backed voles (*Myodes gapperi*). Recent laboratory studies showed that these seeds are not an ideal food source for either mice and voles. The current study explored the response of these rodents to the seeds of *Abies lasiocarpa* in their natural environment, relative to the seeds of a much more palatable species, *Picea glauca*. Comparisons between Giving up Densities (GUDs) and behavioral responses elucidate the true nature of the relationship between these seeds and seed predators.

Evolution of temperature adaptation in *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*

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Nothing in biology makes sense except in the light of evolution, but what exactly are the genetic mechanisms by which this occurs? Experimental evolution studies use rapidly evolving microorganisms to observe the genetics of adaptation in the laboratory, but are not necessarily a direct reflection of how evolution occurs in nature. In this experiment, the genetic mechanism of temperature evolution in the wild was explored by comparing 22 geographically and genetically diverse strains of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. The thermal phenotype of each strain was characterized by measuring their optimum growth temperature, maximum growth temperature, and energy of activation. As all strains have had their full genome re-sequenced (1-3x coverage), the sequence of their heat shock protein (HSP) genes were aligned and compared to identify genetic differences that may explain the observed differences in thermal phenotype. We hypothesized that warm-adapted yeast strains will have more stress response transcription factor (TF) binding sites in their HSP promoters, more HSP gene duplications, and fewer HSP gene deletions as compared to cold-adapted strains. Several mutations in HSP promoters have been identified, including an adjacent duplication of a TF binding site in the HSP26 promoter of *S. cerevisiae* strain S288c. This implies a greater HSP26 expression level, which may explain why S288c has a relatively high maximum growth temperature. No instances of HSP gene deletion or duplication were found, indicating that—unlike what has been reported in previous experimental evolution studies—this is not a common mechanism of adaptive evolution in natural populations.

Association between nest site distribution of the green sea turtle and vegetation on Playa Norte beach, Costa Rica.

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Playa Norte beach is located immediately north of the Tortuguero National Park on the northeast coast of the Caribbean in Costa Rica. Proximity results in similar beach habitat that is critical for nesting conditions for the largest green turtle rookery in the Atlantic basin. Nest site fidelity and high mortality rates early in life demonstrate the importance of recognizing nest site distribution in the face of human development on Playa Norte beach. Vegetation influences both hatchling orientation and nest characteristics such as duration of incubation periods, the pivotal sex determination temperature and firmness of the substratum on the beach. Despite studies indicating that hawksbills frequently nest in vegetation, little research has been done to better understand the impacts on other species of sea turtles. We identified all plant species at meter intervals along six transects of the beach, parallel to the ocean. Three transects were located where there has been high density of nests, and three where there has been low density. We found that locations with higher densities were associated with higher species richness of plants, and these data will be mapped in ArcGIS to permit further spatial analyses.

Determination of Minimal *cis*-elements Responsible for Translational Control of Lactate Dehydrogenase 1 in *Toxoplasma gondii* by Site-directed Mutagenesis

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Toxoplasma gondii is an intracellular obligate parasite responsible for toxoplasmosis. Development of novel drug targets in *T. gondii* is therefore of medical importance. During its asexual phase, *T. gondii* can differentiate from fast-growing tachyzoites into cyst-forming, slow-growing bradyzoites which are protected from the host immune system. This important transition is marked by a change in metabolic pathways, with one such change involving lactate dehydrogenase (LDH) isoforms. LDH is an enzyme necessary for glycolysis – a required pathway for anaerobic respiration in bradyzoites – which changes from LDH1 to LDH2 during this transition. Hence, LDH1 plays a key role in bradyzoite formation and is therefore a good drug target candidate. Mature mRNA contains untranslated regions (UTRs) which serve important regulatory functions. Previously, the 5'-UTR was shown to regulate the expression of LDH1 protein by repressing the translation of LDH1 mRNA. In this work, the loci within the LDH1 5'-UTR necessary for translational repression of LDH1 were determined. Through site-directed mutagenesis with oligonucleotide primers, a series of 5'-UTR deletion mutants were created for this mapping. This work can then lead to further characterization of the structural properties and interactions of such regions, which can in turn be utilized to develop novel drug targets.

Assessment of the Biodiversity of Ground and Rove Beetles in Sudbury's Industrially-Damaged Ecosystem

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Decades of logging and ore smelting in the Sudbury area has caused a decrease in biodiversity of plants and insects. Carabid (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae) were used in this study and are commonly considered indicators due to their ease of sampling, sensitivity to habitat change and occurrence in many niches. Pitfall traps were used throughout the summer of 2010 to assess the abundance and diversity of carabids and rove beetles in the unique birch transition forest adjoining the once most severely-damaged habitats. Eighteen morphospecies of carabids and ten morphospecies of rove beetles were found. Composition of the carabid assemblage changed throughout the season; however, that of the rove beetles did not. Sections of forest floor from natural habitats south of Sudbury were transplanted to the city as part of a new reclamation project and biodiversity initiative. Pitfall traps from the recipient campus sites had seven species of rove beetles not found in adjoining areas. This suggests that the transplanting experiments are successful for importing forest floor insects into the Sudbury region.

The implications of Glucocorticoids on Breast Cancer Survival and Proliferation

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Clinical evidence suggests a direct relationship between cancer and stress, as indicated by chronically elevated levels of the stress hormone cortisol, in 70% of breast cancer patients.

Cortisol is a glucocorticoid hormone that mediates its effects by binding directly to the glucocorticoid receptor to regulate numerous cellular properties including cell proliferation and survival. The glucocorticoid receptor can be activated, not only by endogenous cortisol, but also by numerous synthetic derivatives including dexamethasone. More importantly, Dexamethasone is commonly used in clinical settings as an anti-emetic in chemotherapeutic regimens treating breast cancer patients. Hence, it is of significant importance that a clear understanding of the effects of glucocorticoids on breast cancer be established. While, previous work has demonstrated dexamethasone-induced protection from apoptosis under various conditions in several human breast cancer cell lines, more work needs to be conducted to clarify the phenotype and to elucidate any molecular mechanisms at work. I hypothesize that clinically relevant levels of dexamethasone may affect the growth properties of breast cancer cells by increasing their proliferative potential and/or by protecting the cells from drug-induced apoptosis. The effects of dexamethasone, both on cell proliferation alone or in combination with a commonly used chemotherapeutic drug paclitaxel, was assessed by trypan blue exclusion. My study shows that dexamethasone does affect the growth properties of a number of breast cancer cell lines both increasing cell proliferation rates and protecting cells from drug-induced apoptosis. This work is of significance clinically as it further implicates a potential negative effect of glucocorticoid usage in chemotherapy regimens for at least subsets of breast cancer patients.

Characterization of the soybean transcription factor, GmMYB176, in *Arabidopsis thaliana*

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Isoflavonoids are secondary metabolites that are synthesized predominantly in leguminous plants via the phenylpropanoid pathway. These compounds play an important role in plant defence mechanism and nitrogen fixing symbiosis as signal molecules. An R1 MYB transcription factor, GmMYB176, regulates a critical enzyme CHS8 that is involved in isoflavonoid biosynthesis in soybean. GmMYB176 does not have a nuclear localization signal, yet it is localized in the nucleus. It has been speculated that a protein-protein interaction is involved in its nuclear localization. GmMYB176 contains three potential phosphorylated Serine/Threonine (pST) binding sites where 14-3-3 family of proteins may bind. Detailed characterization of these pST binding sites within GmMYB176 has revealed that a D2 motif is critical for its interaction with 14-3-3 proteins. The objective of this study is to investigate the effect of the D2 deletion in GmMYB176. Two overexpressing YFP-fusion constructs, pGmMYB176-YFP and pGmMYB176-D2-YFP, were transformed into *Arabidopsis thaliana*. Confocal microscopy of transgenic *A. thaliana* plants showed that GmMYB176-YFP was localized mainly in nucleus and some in cytoplasm. However, no GmMYB176-D2-YFP transgenic plants were obtained. Interestingly, all GmMYB176-YFP overexpressing plants showed dwarf phenotype compared to control. Furthermore, T-DNA knockout line of *Atlg74840* (*Atlg74840-KO*) in *Arabidopsis* was characterized. *Atlg74840* is the *Arabidopsis* homolog of *GmMYB176*. *Atlg74840-KO* plants showed delayed germination and resistance to salt stress compared to the wild type. In conclusion, overexpression of *GmMYB176* affects normal plant growth and developmental process and we speculate that *GmMYB176-D2* overexpression is lethal. In addition, *GmMYB176*-homolog in *Arabidopsis* is involved in seed germination and salt stress.

Upregulation of *Aldh2*, *Gpd2* and *Trpm6* can act as a marker of mitochondrial dysfunction in the degenerating retina of a premature ageing mouse model.

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Age-related eye disease is a growing concern in today's Canadian population. Finding biomarkers for age-related eye disease would allow early diagnosis and treatment. The *harlequin* (*hq*) mouse is an accelerated aging model with retinal and cerebellar neurodegeneration. Retinal function is compromised in *hq* as early as 2 months of age with mitochondrial dysfunction as the underlying cause. Differential expression of many genes is observed in the *hq* mouse as compared to wild-type (WT) at 4 months of age, while at 5 weeks of age expression profiles are comparable. *Aldehyde dehydrogenase 2* (*Aldh2*), *Glycerophosphate dehydrogenase 2* (*Gpd2*) and *Transit receptor potential m6* (*Trpm6*) are three genes implicated in response to mitochondrial dysfunction and are upregulated in 4 month old *hq* mouse retina. Herein, TaqMan® qRT-PCR was used to compare expression of these genes in 7-week and 7-month-old *Hq* and WT mice in both the retina and cerebellum. Intramitochondrial fluorescence of Tetramethylrhodamine methyl ester (TMRM) was used to compare mitochondrial integrity in the retina of *hq* and WT mice. Fluoro-Jade B staining of neurite extensions was used to quantify neurodegeneration in the retina and cerebellum of *hq* and WT mice. Preliminary data are consistent with overexpression of *Aldh2*, *Gpd2* and *Trpm6* in *hq* mice at 7 weeks and 7 months of age indicating existence of mitochondrial stress.

Mutagenesis, expression and purification of *atToc33G* mutants for biophysical analyses.

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Chloroplasts are plant organelles that house many different reactions which are essential to photosynthesis. Many of the proteins that chloroplasts require are encoded by the nuclear genes and translated on cytoplasmic ribosomes. These proteins must be translocated across the double-membrane surrounding the organelle. Translocation and regulation of these proteins involves the action of two protein complexes: the TOC and TIC complexes. Although the exact way in which the subunits of these complexes interact is still unknown, much is known about their individual protein components. Toc33 is a component of the TOC complex which plays a role in the recognition of pre-proteins that need to be translocated. The GTPase domain of this component (Toc33G) is the focus of my study. There are already recombinant versions of this domain which have been optimized for tryptophan fluorescence, which is used to study protein structure. The overall goal of my study is to gain structural information about conformation of Toc33G. To achieve this goal, several mutants of Toc33G were generated using overlap-extension polymerase chain reaction. Two of these recombinant proteins were expressed in *E.coli* and then purified using His-tag purification. These mutants will be analyzed in the future using tryptophan fluorescence to gain structural information. This will give further insight into the translocation of nuclear encoded photosynthetic proteins into chloroplasts.

Taking the "phyto" out of phytohormones: fungal production of plant hormones is widespread.

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Plant development is controlled by growth regulators, also known as phytohormones. There are five main groups: auxins, gibberellins, cytokinins (CK), ethylene, and abscisic acid (ABA). These are potent signalling compounds that play a fundamental role in controlling a diverse array of physiological processes like seed germination, organ development, flowering and shoot and root formation. Surprisingly, plants are not the only organisms that can produce phytohormones. For instance microorganisms such as rhizobacteria, are capable of synthesizing hormones including ABA and CK's such as kinetin, zeatin, isopentenyladenine. More recently, researchers have begun to find ABA and CK production by fungi. However, in spite of the great importance of these hormones various plant functions and the pervasiveness of fungal-plant interactions, no serious attempt has been made to isolate and study how widespread phytohormone production is in fungi. I hypothesized that CK and ABA levels would vary depending on whether the fungi interact with plants (ie. biotrophs/saprotrophs), for which I predicted that CK levels would be higher compared to non-biotrophs/saprotrophs. There is potential for both accumulation in the fungi of host plant produced CKs or it may be that fungi produce CK to control host plant response. In order to determine how widespread CK and ABA are among fungi, a large array of fungal species from southern Ontario was analyzed and the hormone levels measured by mass spectrometry (LC-MS/MS). Preliminary results show that there are medium to high levels of ABA [and very high levels of some CKs] found in all thirty-five species of fungi that were examined, regardless of whether the fungi were saprotrophs or biotrophs. This indicates that some phytohormones are pervasively present in fungi species, opening up a new avenue of research to determine their role in plant-fungus associations.

Analysis of Hox gene *lin-39* expression and function in nematode vulval development

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Studies of Hox gene *lin-39* expression and its protein function in *C. elegans* vulval development have revealed important regulatory mechanisms. To determine the extent of conservation of *lin-39* regulation and protein function, my study focuses on comparing *lin-39* expression in closely related species, *C. elegans* and *C. briggsae*. This will be done by quantifying *lin-39::GFP* reporter expression in vulval precursor cells (VPCs). *LIN-39* function will also be studied by quantifying irregular spacing of VPCs in the *lin-39* mutants of both species. Judging from the role of *lin-39* in vulval development for both species, the *lin-39::GFP* expression is predicted to be expressed in the six VPCs, P(3-8).p, during early larval stages until the time of vulval induction and increase in P6.p or its descendants during vulval induction. One exception is that GFP expression might not be present in P3.p in *C. briggsae* as it is not competent to form vulva in *C. briggsae*. As well, irregular spacing among the VPCs of *lin-39* mutant of both species is expected. Qualitative analysis of *Cel-lin-39::GFP* expression pattern in VPCs is as predicted. Regrettably, the GFP reporter construction for *Cbr-lin-39* through molecular cloning was not successful. Alternatively, a recombineering approach is being pursued. Future studies will analyze the expression of the promoter region of *Cbr-lin-39* to determine its cis-regulatory region(s). These studies show that the spacing of VPCs in *lin-39* mutants of both species is irregular. Further functional studies include performing rescue experiments for both species.

A test for genotypic influence on caste in the Eastern subterranean termite

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Termites are social insects characterized by morphological castes with distinct behaviours. While it is understood from kin theory that genes are essential for the evolution of non-reproductive helper castes, it is less clear to what degree genetic versus environmental factors regulate caste differentiation within colonies. In this study, we measure the genetic effect on caste by comparing how well microsatellite DNA genotypes co-vary with caste phenotype within colonies of the Eastern subterranean termite (*Reticulitermes flavipes*). We first sorted individual termites from four unrelated colonies into reproductive (nymph) and non-reproductive (worker and soldier) castes, then genotyped each individual at eight, highly variable microsatellite DNA loci. Initial findings suggest there is much variation between colonies with regard to caste composition and allele frequency. Initial statistical analyses indicate that the genetic effect on caste determination is weak, but two loci do show evidence of linkage to a caste determining gene. While the location of such a gene is unknown, this study hopes to provide evidence of its existence and further contribute to the overall understanding of the roles of nature and nurture in social societies.

5'-methylthioadenosine nucleosidase (MTN)-deficient *Arabidopsis thaliana* roots do the 'twist'

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The enzyme 5'-methylthioadenosine nucleosidase (MTN) is responsible for converting methylthioadenosine (MTA) into methylthioribose (MTR) and adenine in the Yang cycle. In *Arabidopsis thaliana*, there are two genes encoding MTN activity - AtMTN1 (AT4g03880) and AtMTN2 (At4g34840). To better understand the role of MTN in plant development, MTN1 and MTN2 T-DNA insertion mutants were identified and crossed to generate a double mutant, *mtn1-1 mtn2-1*, that almost completely lack MTN activity. *mtn1-1 mtn2-1* mutants have a complex phenotype that includes unfixated-handed organ twisting; this research is focused on the twisting of the roots.

Previous models of helical growth suggest that root twisting in plants is due to impaired development of the central vascular tissue. The purpose of this investigation is to visualize root twisting in specific cell types through the use of GFP reporter lines that correspond to the epidermal and vascular cells of *A. thaliana* roots. In addition, root skewing is being monitored and quantified as an indirect indicator of root twisting. Understanding the basis of the root twisting in MTN-deficient plants will provide insight into the functional significance of MTN in plants.

Change in the Inflammatory Biomarker C-Reactive Protein in Obese Children and Adolescents after a 1 Year Weight Management Program and its' Association to Total Body Fat, Trunk Fat, and Waist Circumference.

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The increasing prevalence of obesity in children and adolescents is alarming. Overweight children and adolescents are at risk for health problems during both their youth and their adulthood. The increasing obesity prevalence in Canada has translated to an increased prevalence of the metabolic syndrome. Low-grade inflammation is beginning to be viewed as a significant component of metabolic syndrome and has been associated with an increased risk of cardiovascular disease, diabetes, and the development of atherosclerotic plaques. Adipose tissue (particularly intra-abdominal adipose tissue) releases the proinflammatory cytokine, interleukin-6, which stimulates the release of C-reactive protein (CRP) from the liver. Circulating plasma CRP levels of 40 obese children and adolescents were measured with a high-sensitivity CRP ELISA assay. The objectives of this study are to a) to determine whether obese children and adolescents experience a change in plasma CRP concentration after a 1 year weight management program, and b) to determine if the aforementioned change in CRP could be explained by either a change in i) total body fat, ii) trunk fat, or iii) waist circumference. It is hypothesized that after the weight management program, obese children and adolescents will experience a decrease in plasma CRP concentration. It is further hypothesized that a decrease in total body fat and/or a decrease in trunk fat and/or a decrease in waist circumference will explain the decrease in CRP concentration.

Examining the effect of source of natural organic matter on the acute toxicity of copper to *Hyalella azteca*

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The potential for aquatic ecosystem remediation as a result of natural organic matter (NOM) providing protective effects against metal toxicity has become a significant area of research in environmental toxicology. This aim of this study is to examine the influence of NOM quantity and quality (source of NOM) on acute copper toxicity to the freshwater invertebrate *Hyalella azteca*. Cu toxicity tests were conducted in artificial media to establish an LC50 and bioaccumulation levels. Toxicity tests (96h) were done in duplicate using 200mL solutions spiked with Cu (0-2 μ M), and 10 *Hyalella* aged 2-9 days. The LC50 was determined to be 1.2 μ M Cu and accumulation sharply increased at low Cu concentrations then began to plateau at 1.0 μ M Cu. The impact of NOM quantity was tested using NOM collected from Brandy Lake, ON. Concentrations of NOM were varied from 0–11.5mg C/L and spiked with 2 or 4 μ M Cu. A three-fold increase in survival was observed from 0-2.45mg C/L NOM. This evidence suggests that Cu toxicity mitigation occurs in a concentration-dependent manner, because more NOM leads to a reduction in the amount of free Cu²⁺ available for toxic action. The influence of NOM source will be examined by testing the protective effects of three different lakes (Brandy, Daisy, and Allard) at a concentration of 5mg C/L. Chemical characterization (ISE and EEMS) of these NOM sources will be completed in an attempt to understand if protective abilities differ between NOM sources. Biotic ligand model (BLM) modelling will be conducted, aiming to predict metal toxicity based on site-specific water chemistry. Currently in the BLM, NOM is treated as one homogeneous entity. However, it is suspected that NOM sources differ in their protective abilities, thus the BLM may need to be revisited in order to account for differing sources (quality) of NOM to ensure that accurate regulatory actions can be taken.

The *Neurospora crassa* circadian clock: clock-control gene 16 expression in strains with different combinations of *frq*-white collar and *frq*-less oscillators

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Circadian rhythms are self-sustained oscillations that have a period of approximately 24 hours. The central oscillator in *Neurospora crassa* responsible for its circadian rhythm was long believed to be a single transcription/translational oscillator (TTO). The TTO mechanism has been studied thoroughly and involves the expression of the *frq* gene under the transcriptional control of WC-1 and WC-2 proteins and subsequent negative regulation of the WC proteins by the FRQ protein. The notion of a single oscillator is currently being debated after the discovery of rhythmic properties such as conidiation, nitrate reductase activity, diacylglycerol levels and gene expression in *frq*-null mutants. These properties suggest the existence of one or more *frq*-less oscillators (FLO) as opposed to a single *frq*-white collar oscillator (FWC). The purpose of this study is to replicate previously documented rhythmic expression of clock-controlled gene (*ccg-16*) RNA in a *frq*-less strain and to further study its expression in a wildtype as well as a double mutant that lacks both FWC and FLO. This study will use quantitative real-time PCR to document the *ccg-16* RNA expression in samples grown in the dark for various time lengths. Preliminary results showed that *ccg-16* RNA expression is rhythmic in the FLO strain, but arrhythmic in the wildtype and double mutant strains. The results support the existence of FLO(s) and suggest that *ccg-16* expression is regulated by the same FLO governing rhythmic conidiation. Efforts are currently being made to replicate these results.

The impact of excluding history of assembly in metacommunities.

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The processes that determine community composition are fundamental to the field of ecology. Assembly rules attempt to explain the mechanisms that allow species of a community to fit together. The sequence of species invasion can result in communities with different set of species or alternative states. Therefore, to exclude the assembly history of colonization 26 beaker communities were mixed to create a “null community”. These communities were derived from invertebrate communities inhabiting natural rock pools in Jamaica. The null community was divided into 30 beakers, which were sampled after 6 and 12 months from the start of the experiment to determine community composition. This study relies on microcosm models to analyze if determinism or stochasticity, or both shape community assembly. Hence, assembly history of colonization is thought to produce alternative states. I hypothesized that if removing the influence of assembly history affects metacommunities, then community structure will converge into one final state. In order to find final states, cluster analysis and multi-dimensional scaling (MDS) will be performed to analyze the similarity between beaker communities based on the abundances of species. Despite all the controversy about alternatives states, its research is crucial for ecosystem restoration. Understanding the pathways that leads to degraded or alternative states in ecosystems are fundamental to their recovery.

Identifying the genetic basis of interspecific female mate preference in *Drosophila simulans*

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A speciation event involves one group of interbreeding organisms diverging into two or more isolated populations. Barriers that stop gene flow between these groups can be categorized as acting either prezygotically (before zygote formation) or postzygotically (after zygote formation). Although prezygotic isolation is thought to evolve first, no genes have yet been identified for this type of isolating barrier. One prezygotic barrier is interspecific female mate preference, a form of behavioural isolation where the innate preference of females is to mate with a conspecific male. For my project I look to identify a genomic region that contains interspecific female preference gene(s) in *Drosophila*. Specifically, I use deficiency mapping to fine-map small regions of the *D. simulans* third chromosome that cause female discrimination against *D. melanogaster* males. Fine mapping these regions has allowed the systematic reduction of thousands of possible genes to just a handful of candidate genes. When confirmed, these candidate genes will be the first genes ever identified for prezygotic isolation.

The Effects of a Parasitic Infection on the Biochemical Composition and Physiological Processes of *Daphnia magna*

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Daphnia metabolism and chemical composition can be influenced by several factors such as age, food quality and environment, but one potential factor that has not been thoroughly explored is infection. There is reason to believe that parasitic infections influence *Daphnia* development and function due to the visible differences that occur under infection as well as the altered reproductive cycle and impaired immune function. We hypothesized that bacterial infection affects *Daphnia* physiology and biochemistry and predicted that there would be a significant difference between the infected and uninfected *Daphnia* across all variables. We infected *Daphnia magna* with the parasitic bacteria *Pasteuria ramosa* and observed the infection processes under three different food qualities. The experiments were taken down on days 7, 14 and 21, in order to observe the various stages of infection throughout the *Daphnia*'s life. To measure biochemical changes, the carbon, nitrogen and phosphorus concentrations were measured, as well as the DNA/RNA concentrations, alkaline-phosphatase concentration, lipid content and spore density. To quantify physiological changes, number of offspring was also measured. After preliminary analysis we found the number of offspring drastically decreased as infection progressed until the infected *Daphnia* became sterile, with the *Daphnia* in lower food quality becoming sterile sooner. The final results of this project will demonstrate the importance of understanding host-parasite relationships and the role disease may play in the evolution and development of host species.

Comparison of biodiversity and economics of various pan traps for collecting insects with emphasis on bees

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Insects are the largest group of pollinators and are vital for the maintenance, diversity and productivity of natural and agricultural ecosystems. Insect pollinators provide valuable ecosystem services and terrestrial ecosystems are incapable of functioning in their absence. Increases in the human population have resulted in alterations of habitats and as a result, many pollinators and their services have been reduced at an alarming rate. Thus, intensive surveys and effective monitoring of pollinator biodiversity across a wide range of habitats is essential. In order to conduct these surveys, a set of standardized methods and techniques are required. This will allow conservation biologists and agriculturalists to compare their findings on spatial and temporal scales. Surveys were conducted throughout Canada, in five sites, representing four Ecozones: Oak Savannah habitat in Southern Ontario (part of the Mixedwood Plain Ecozone), the Prairie Ecozone of Manitoba, the Atlantic Maritime Ecozone of Nova Scotia and the Arctic Ecozone of the northern Yukon Territory. A set of twenty-five pan traps of five different colours/paint combinations (i.e., pre-coloured white, painted yellow, painted blue, pre-coloured yellow and pre-coloured blue) were placed in a Latin square format (5 X 5), at distances of 5 – 10m from each other (distance varied based on the size of the sites). Pan traps varied in their economic cost and sample yield. I will present results of research which determined which of the different pan traps were most effective and whether this depended on the studied habitat types.

***Sinorhizobium meliloti* 1021: The Metabolic Link Between Carbon Source Utilization and Nitrogen Fixation**

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S. meliloti 1021 (Rm1021) is a commonly found soil microorganism and is a member of order of Rhizobiales. Previous research has shown that mutations in short chain dehydrogenase/reductase (SDR) genes located on the megaplasmid pSymB link carbon source utilization, nodulation, and nitrogen fixation through uncharacterized pathways. Previously studied single crossover mutants of genes SMb20492 and SMb20493 were unable to utilize leucine, lysine, succinate, monomethyl-succinate and D-fucose as carbon sources. The SMb20492 mutant was incapable of both nodule formation and nitrogen fixation. The SMb20493 mutant nodulated plants but showed an inconsistent nitrogen fixation phenotype. These two genes were chosen because they are located adjacently on pSymB. Disrupting either gene yields the same carbon metabolism deficiencies, but the effect on nodulation and nitrogen fixation is different, as previously mentioned.

My work focuses on creating unmarked knockout mutants of these genes by double homologous recombination. Two homologous upstream and downstream regions were synthesized for each gene knockout. The synthesized fragments were subsequently cloned into the suicide plasmid pK19*mobsacB* and conjugated into Rm1021 using the helper plasmid pRK600. Screening for knockout mutants is currently in progress. Future work will focus on elucidation of the relationship between carbon utilization, nodulation and nitrogen fixation pathways.

Nutritional Availability Influences Tuberin's role as a Mitotic Regulator

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Tuberous sclerosis (TS) is a genetic disorder characterized by the development of multiple tumors (hamartomas) throughout a variety of tissues. TS arises from mutations in either the TSC1 or TSC2 gene, which encode the proteins Hamartin and Tuberin respectively. Experimentation has shown that Tuberin plays a critical role in cell growth, and proliferation. Tuberin, characterized as tumour suppressor, functions as an antagonist of the mammalian target of rapamycin (mTOR) pathway, thereby acting as an inhibitor of G1/S progression. Recently, experimental data from our lab has revealed that along with Tuberin's role as an inhibitor of cell growth, it may also be a regulator of mitotic progression into the G2/M phase of the cell cycle through its interaction with cell cycle protein Cyclin B1. Our lab has shown that Tuberin can monitor the rate at which Cyclin B1 localizes to the nucleus, hence regulating cell cycle progression. Given the importance of Tuberin as a metabolic sensor, we hypothesized that the binding between Tuberin and Cyclin B1 would also be sensitive to alterations in nutrients and growth factors. We further hypothesized that this interaction may be disrupted in patients with TS. We therefore, utilized Tuberin and missense substitution mutants clinically found in TS patients in binding and localization assays with Cyclin B1 under varying nutrient levels. Our findings suggest the amount of nutrients available within the cell influences the binding between these two proteins as well as the cellular localization. Future work in this area may provide further molecular insight toward hamartoma development and the treatment of TS.

Interactions of Two Growth Promoting Plant Hormone groups: Brassinosteroids and Cytokinins

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Brassinosteroids and Cytokinins are growth promoting plant hormones that appear to have many over-lapping functions. Both hormone groups have been reported to play a role in breaking seed dormancy, cell division, apical dominance and senescence. Although the similarity of functions is evident no work has directly studied the relationship between them. Thus, we propose that perturbations in Brassinosteroids will lead to altered Cytokinin metabolite profiles. The presence and concentrations of various Cytokinins were measured from brassinosteroid -insensitive and -deficient *Arabidopsis thaliana* mutants. The analysis was performed using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Preliminary data suggests that when Brassinosteroids are underproduced or when the plant cannot detect BR's, Cytokinins concentrations are increased. These results suggest novel findings in hormone cross-talk. Specifically, the hormone groups may have overlapping function, and the lack of one may lead to overcompensation in the presence of the other. Future research can continue to build upon these novel discoveries and the implications of interaction between these two highly active growth-promoting hormones.

Factors affecting Canada Goose (*Branta canadensis interior*) nest defence behaviour on Akimiski Island, Nunavut

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Parental investment plays a major role in the behaviour of adults towards their offspring. Nest defence is one form of investment commonly seen in birds. I studied the nest defence behaviour of female Canada Geese (*Branta canadensis interior*) on Akimiski Island, Nunavut over one breeding season. My objectives are to determine how reproductive value, nest site characteristics and weather influence nest defence. Nests were approached by researchers and flushing distance was recorded. Return times were determined using motion sensor cameras. Clutch size, nest age, egg volume and lateral vegetation density at 5m and 10m were recorded. Temperature was determined based on the most appropriate nearby weather station, Kuujjuarapik, Quebec. Results using a single random nest visit from each nest suggest that vegetation density is significantly inversely related to female flushing distance. Hence, females on well concealed nests flush later than females on more exposed nests. Results suggest that nest age is inversely related to flushing distance which illustrates the older the nests, the longer the females will remain. There is also a significant inverse relationship between flushing distance and mean temperature, suggesting that at lower temperatures, females flush earlier. None of the variables examined were significantly related to return times. Based on model selection, the best model for determining flushing distance is vegetation density at 10m and nest age. When multiple nest visits, rather than a single visit, were included, there were significant differences between females in their responses with regards to both flushing distance and return time. Thus, vegetation cover, nest age, temperature and parental differences appear to influence the nest defence and parental investment in Canada Geese.

Antibiotic Resistance Patterns in *Salmonella* Isolated from a Treatment Lagoon System

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The use of antibiotics in food animals has been linked to the emergence of drug-resistant *Salmonella*, posing selective pressures on the bacteria and enhancing its adaptability to novel antibiotic challenges. As a result the persistence of environmental antibiotic resistant *Salmonella* is of increasing concern. In this study, samples were obtained from a constructed wastewater treatment lagoon receiving post-flocculation poultry waste effluent. Within the lagoon series, the presence of *Salmonella* was identified through selective isolation processes on differential media, biochemical testing, and PCR colony confirmation. The primary objective of the study was to assess antibiotic resistance patterns initially and following exposure to physiological stressors such as temperature, UV exposure, and antiseptics commonly used in hospital settings and the process of food handling. Resistance patterns of the confirmed isolates were determined using the disk-diffusion method against a panel of 12 antibiotics: chloramphenicol, streptomycin, tetracycline, doxycycline, sulfisoxazole, ampicillin, amoxicillin, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin and sulfamethoxazole-trimethoprim. 44% of confirmed isolates shared a tetracycline-sulfisoxazole-streptomycin resistant phenotype and are now undergoing PCR for the detection of genes conferring this resistance, including *sul1*, *sul2*, *strA*, *strB* and *tetA*. Monitoring response to physiological stress reflects the ability of *Salmonella* to counter environmental change. This is essential to understanding its survival in a variety of niches, both environmentally and within a host. Additionally, this research will contribute to our ability to understand and manage the environmental presence of infectious *Salmonella*.

Compensation response of the Trinidadian guppy (*Poecilia reticulata*) upon thermal acclimation

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Increased water temperatures due to global climate change may pose a particularly severe threat for ectothermic fish given that all aspects of their physiology are directly influenced by ambient water temperature. To overcome this sustained stress, fish may make partial or complete compensatory adjustments upon thermal acclimation, thereby maintaining their physiological state in different temperatures. The compensation response of Trinidadian guppies (*Poecilia reticulata*) was investigated by measuring their metabolic rate and burst speed following short-term (6 h) and long-term (70 days) exposure to different temperatures (23°C, 25°C, 28°C, and 30°C). Metabolic rate and burst speed increased with temperature for fully acclimated fish, indicating that guppies are unable to make complete compensatory adjustments. The final results of this study will be discussed in the context of global climate change, addressing the physiological and ecological significance of compensation capacities.

Isolation and Identification of Bacterial Species from an Acid Mine Drainage Site in Copper Cliff, Ontario

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Acid mine drainage (AMD) is produced by the chemical and microbial oxidation of sulphide mineral waste rock, a by-product of the mining industry. It is detrimental to the surrounding biota due to its acidity, metal toxicity, salinity and ferric iron precipitation. Despite the extreme conditions, some microbial species have adapted to this environment and play an important role in mineral (primarily pyrite, FeS₂) dissolution and acidification. The purpose of this study was to identify the species present in a water sample collected from the Copper Cliff Tailings Area, an environment affected by AMD. We are using a combination of classical microbiology and direct pyrosequencing to identify all the bacterial species present and the fraction of these that could be cultured. For culturing, water samples were inoculated onto three types of solid selective media and a fragment of the 16S rRNA gene amplified and sequenced to identify individual species matching known organisms in the NCBI database. We are also using direct DNA sequencing of a fragment of the 16S rRNA gene to identify all bacterial species present in the water samples. We have identified seventeen isolates from the solid media cultures. From the pyrosequencing, we have identified 3 dominant genera, *Acidithiobacillus*, *Legionella*, and *Acidiphilium*. The identification of the bacterial species in this AMD site is a first step towards understanding the genetic potential of this microbial community. This work will provide a reference for the use of new deep sequencing techniques for the mining industry which will help guide new bioremediation strategies.

Validating gene expression changes detected by microarray analysis in drug resistant ovarian cancer cell lines using Q-PCR

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The development of drug resistance is a major problem associated with chemotherapy of ovarian cancer. Specific changes in gene expression are thought to contribute to drug resistance. These changes in expression can be measured using microarray analysis and quantitative polymerase chain reaction (Q-PCR). Microarray analysis is used to establish genome-wide changes in gene expression, and Q-PCR is the commonly used technique for validation of microarray observations. Microarray observations and Q-PCR data may result in disagreement as each method has a fundamentally different approach for normalization and transcript specificity. In the present study, 16 genes were selected based on microarray observations of changes in gene expression in the parent and the drug resistant cell lines, for validation using Q-PCR. Primers were designed to be transcript specific and the ribosomal S28 gene was used as reference gene against which other gene expression changes were calibrated. Two sample t-test was performed to determine whether the differences in gene expression between parent and the resistant cell lines are significantly different. Of the genes being verified by Q-PCR, significant changes in expression between the parent and resistant cell line was observed for 14 genes with a p value <0.05. Q-PCR technique detected changes in gene expression in other cell lines which were not detected by microarray. To determine if any of the resistant cell lines had significant difference compared to the other cell line, one way ANOVA was performed and significance was seen for 12 genes. Overall, Q-PCR generally confirmed the changes observed in microarray.

The Identification of Non-Angiosperm Alternative Oxidases.

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The alternative oxidase (AOX) pathway is located in the respiratory electron transport chain (ETC) of the mitochondria and branches at the point of ubiquinone, bypassing two of the three proton pumping complexes of the cytochrome c oxidase (COX) pathway. Unlike the COX pathway, the AOX protein is non-proton pumping which makes it an energetically wasteful pathway as less ATP is generated during its activity. Despite being energetically wasteful, AOX has been identified in all kingdoms of life excluding Archaeobacteria. AOX is widely distributed in the plant kingdom, predominantly in angiosperms, such as *Arabidopsis thaliana*, corn, tomato, rice, and tobacco. Since the majority of AOX data has been collected only from angiosperm plants, we are investigating non-angiosperm AOX with the intent to compare and contrast the two groups. Based on the known data for angiosperms, we were able to use bioinformatics and sequence databases to identify non-angiosperm AOX sequences. Thus far, our results have shown that AOX is present a fern, moss, liverwort, lycopod, and many gymnosperm species. Next, we plan to clone several AOX genes from a variety of non-angiosperm plants and to verify their expression at the mRNA and protein levels. Lastly, we will use the primers designed for known AOX proteins in non-angiosperm plants and/or degenerate primers to discover new AOX sequences in other non-angiosperms for which no genomic resources are currently available.

Does *Lactobacillus reuteri* induce neurochemical changes in enteric sensory neurons?

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A specific strain of *Lactobacillus reuteri* (LR) can have direct effects on nerves in the gastrointestinal tract. In order to further characterize the actions of LR on gastrointestinal nerves, we tested the hypothesis that ingestion of LR can induce neurochemical changes in the sensory neurons within the gastrointestinal tract, known as the intrinsic primary afferent neurons (IPANs). According to previously established protocols, Balb/c (n=5) mice were gavaged with LR (10⁹ CFU) in Man-Rogosa Sharpe media (MRS) for 9 days. On the 10th day, the animals were sacrificed, the terminal ileum removed and processed for immunocytochemistry. Control mice (n=5) were gavaged with MRS media. Triple-labeling immunocytochemistry was used to detect sites of antibody binding to the pan-neural marker, PGP 9.5, neuropeptide calbindin, expressed predominantly by IPANs, and to the neuropeptide substance P. The immunolabeled sections were photographed and the nerve volume densities of calbindin and substance P relative to PGP 9.5 in the ileal muscle (including the enteric nerve plexi) and mucosal layers were quantified using Volocity software. The nerve volume density of calbindin-expressing nerves in the enteric plexi was found to be significantly greater in mice gavaged with LR as compared to controls (p < 0.05). No significant differences were seen in the nerve volume density of substance P-expressing nerves in the enteric plexi or nerve volume density of either neuropeptide in the mucosa. These findings suggest that LR can induce neurochemical changes in the cell bodies of calbindin-expressing IPANs in the enteric plexi, but not in their mucosal processes.

Functional and Expression Analysis of a Chromatin Remodeling Protein in *Tetrahymena thermophila*

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Chromodomain helicase DNA-Binding Domain (CHD) proteins are ATP-dependent proteins involved in chromatin remodeling. We are addressing questions about the identity of RNA associations with two specific CHD proteins that are involved in processes of heterochromatin/RNAi-dependent irreversible gene silencing and developmentally programmed genome rearrangement in *Tetrahymena thermophila*. Our studies take place in this animal model organism during the sexual cycle (conjugation), as this is the period during which reorganization and silencing occur. It is proposed that these RNA molecules, in conjunction with CHD proteins, allow for targeted formation of heterochromatin during development. Tagged constructs for each of the two CHD proteins were made by engineering a vector with a selectable antibiotic (paromomycin) resistance marker. These tags will be used in pull down experiments to analyze, using deep sequencing RNA seq technology, RNA associated with these proteins during conjugation. We will report the construction and analysis of the tagged vectors. We are presently transforming these vectors into mating-compatible strains in prelude to performing the pull down assays and analyzing the RNA molecules associated with these proteins. We will describe progress made towards this analysis.

Population Structure of Sexual Isolates of *Candida ipomoeae*

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Recent discovery of populations of sexually reproducing *Candida ipomoeae* contests the idea that this yeast species propagates strictly by asexual reproduction. The purpose of this study is to examine the effects of sexual reproduction on the population structure of sexual *C. ipomoeae*. I hypothesized that genetic recombination is occurring in the sexual populations of *C. ipomoeae*. The presence of recombination was determined by comparing haplotype diversity of six sexual isolates to that of previously identified asexual isolates. Specifically, allele sequences of 12 DNA markers were studied in the sexual isolates and compared to the same markers in the asexual isolates. A new allele was found in each of two DNA markers. Additionally, three new haplotypes were discovered among the six sexual isolates studied. In spite of the small sample size, it is possible to conclude that recombination is occurring in the sexual *C. ipomoeae* populations. I also hypothesized that previously identified asexual isolates had not lost the ability to reproduce sexually, and will undergo sexual reproduction when crossed with the opposite mating type of sexual isolates. Crosses between asexual and sexual isolates were studied for the presence of fertile asci. Two asexual isolates formed asci containing two ascospores when mixed with h^+ sexual isolates, indicating sexual reproduction had taken place. Other asexual isolates did not form asci when mated with either sexual mating type. These findings indicate that some isolates have retained the ability to reproduce sexually and other isolates have lost this ability.

Effect of FMRFamide on *Drosophila* larval whole body muscle contraction.

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Biology, York University.

This project will commence with the replication of the DPKQDFMRFamide effect on the *Drosophila* larval whole body muscle contraction. This will serve as a standard for comparison. The individual effect of proctolin and PDNFMRFamide on *Drosophila* larval whole body muscle contraction will be examined. If time permits SDN FMRFamide will also be investigated. The nervous system of the animal is completely removed to observe muscle contraction solely depending on the peptide present. Basic anatomy and physiology of the animal will also be reviews during the presentation. An overview of the peptides will also be given.

The role of integrin-linked kinase in TGF- β receptor type II receptor stability during wound healing

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Pettigrew, Heather; University Western Ontario, Biology

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Wound healing is a complex process including the differentiation of fibroblasts into myofibroblasts which mediates wound contraction. This differentiation is induced by several stimuli including the transforming growth factor-beta (TGF- β) through activation of SMAD. However TGF- β responses in dermal fibroblasts are abnormal in the absence of ILK. Specifically TGF- β signalling does not occur properly, and SMAD phosphorylation and activation are impaired, through poorly understood mechanisms. One possibility is that ILK affects TGF- β signalling at the receptor level. The purpose of this study is then to determine the effect of ILK deficiency on TGF- β type II receptor (TGF- β RII) in primary mouse dermal fibroblasts. It is hypothesized that ILK is required for the stabilization of the receptor. This can be confirmed by looking at the apparent half-life of TGF- β RII following cell treatment with cycloheximide in ILK deficient cells using cre-loxP technology. Receptor levels will be analysed through immunoblotting and densitometry. The apparent half-life of the receptor is expected to decrease when ILK is not present, therefore indicating decreased stabilization.

Identification of causal mutations in rare patients by automated DNA sequencing and whole exome sequencing.

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The identification of causal mutations by DNA sequencing presents a comprehensive and definitive approach to characterizing the genetic basis of diseases. This study hypothesized that automated DNA sequencing and whole-exome sequencing techniques may be employed to diagnostically explain the genetic cause of phenotypes seen in rare patients, as shown through models of severe childhood obesity and an unidentified progeroid syndrome. Automated DNA sequencing was conducted on eight patients for the exons of the *LEP*, *LEPR*, *POMC*, *MC3R*, *MC4R*, *PCSK1* and *FTO* genes, which contain mutations that have been previously shown to cause severe early-onset obesity. This method was also conducted on the *WRN* gene for twenty patients that presented with unknown progeroid syndromes or suspected Werner's syndrome. For one isolated progeroid syndrome case, whole-exome sequencing was performed using the Illumina Genome Analyzer II_x. To determine the functional consequences of the mutations found by these methods, mutations are analyzed through a series of online databases and prediction tools: Single Nucleotide Polymorphism database (dbSNP), Online Mendelian Inheritance in Man (OMIM), GeneCards, Human Genome Mutation Database (HGMD), Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping Program v.2 (PolyPhen-2), and Automated Splice Site Analyses. Future findings from this investigation will elucidate the important applications of novel sequencing technologies to the study of rare genetic conditions.

The role of radiocarpal morphology in prey handling behaviour among Phyllostomid species

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Through the qualitative and quantitative analyses of digital images of the radiocarpal skeletons of 40 species of bats (4 families), the relationship between morphology and function was investigated. Bat species consisted of mainly Phyllostomids that traverse a range of feeding habits. A few Mormoopid, Noctilionid, and Rhinolophid species were included to broaden the sample. The significance of varying degrees of prey manipulation (species were graded on a scale ranging from extensive to none) on associated radiocarpal morphology was focused on. As the current status of Chiropteran phylogeny is unclear with evidence supporting old and new arrangements, the phylogenetic implications of wrist morphology was also considered. The bats examined showed some significant differences in wrist associated features. Across the entire sample, frugivores and omnivores exhibited the most robust radiocarpal morphology. Specific features that were pronounced in these species include scaphoid surface area/perimeter as well as trapezium and trapezoid surface areas. For example, despite their large size in relation to a majority of bat species *Vampyrum spectrum* and *Trachops cirrhosis* (both feed on small vertebrates) exhibited drastically larger scaphoid bones than nectarivorous species *Glossophaga soricina*. Differences in most wrist associated features between species signify the possibility of selective pressures. Further study of Chiropteran radiocarpal morphology can aim to elucidate the origin of these differences and their evolutionary implications.

A test for trade offs and dispersal limitation in a zooplankton metacommunity

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Metacommunities explicitly incorporate the forces that structure communities: dispersal limitation and environment conditions. However, environmental variables need to be explicitly measured or manipulated to determine the importance of environmental conditions. This is a problem given the wide variety of potential candidates or in fairly homogeneous environments. Chase and Leibold (2004) suggested an omnibus test to detect environmental processes without measuring environmental variables. They hypothesized that reducing community density and determining whether the communities after this manipulation were similar to the community structure before the reduction would indicate the importance of environmental conditions through trade-offs, without actually identifying the environmental variables responsible for these trade-offs. We used this methodology with rock pool zooplankton communities from the freshwater portion of the salinity gradient in subarctic Churchill, Manitoba. The rock pools were manipulated by experimentally reducing zooplankton community density and tracking the response for 11 days. Communities after the reduction were dissimilar from communities before the reduction, indicating the importance of local, random factors determining community composition in freshwater pools, or neutral dynamics. Together with previous work in this rock pool system, our results suggest that metacommunities are structured by a combination of different groups of habitat specialists that respond to the different salinity concentrations. Within these groups of specialists, the species behave neutrally. This is probably a very general pattern in communities, different groups of neutrally-behaving specialists, or neutrality embedded within species sorting.

Systematic Reviews of Knowledge Translation Interventions: Next Steps to Optimize Implementation

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Two systematic reviews regarding the effectiveness of knowledge translation interventions in cancer patients were analyzed. This study was undertaken to determine the relationship between study characteristics and the magnitude of effectiveness of the outcomes in individual studies, and also the relationship between the quality of studies and the magnitude of effectiveness of the outcomes in individual studies. In addition, the systematic reviews will be tested for similar patterns or trends. Patient characteristics, outcomes and methodological and intervention study characteristics were extracted from primary studies of the two systematic reviews. The underpinning primary journal articles were critically appraised to measure quality. Co-relational analysis of the data will include Pearson correlations to determine if there is a relationship between quality and magnitude of effectiveness of outcome scores. Moreover, descriptive statistics will be calculated for key variables and exploratory analysis will be conducted to determine any associations between outcome measures and key variables. The conclusion of this study will provide insight into how best to effectively implement and operationalize knowledge translation interventions.

The temporal and spatial expression of TSC2 throughout neural development.

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Tuberous sclerosis (TS) is a multisystem disorder that is characterized by the development of primarily benign tumours called hamartomas. TS is linked to a loss of heterozygosity in two genes, TSC1 and TSC2 encoding the proteins Hamartin and Tuberin respectively. In vivo Hamartin and Tuberin form a heterodimer that functions as a tumour suppressor to inhibit the mammalian target of rapamycin (mTOR). mTOR inhibits protein synthesis through the phosphorylation and subsequent activation of the S6K. Furthermore, Tuberin is known to prevent the Cdk inhibitor p27 from localizing to the nucleus, thereby inhibiting cell growth. Tuberin may be involved in many key processes that lead to proper brain development, such as the formation of the notochord and one of the primary sites for hamartoma formation in TS is the brain. We hypothesize that regulation of Tuberin levels, localization and activity is an essential component of neural development. This study utilized murine brain samples collected from embryonic stages on through to early adulthood and immunohistochemical analysis to observe the differences in protein levels of endogenous Tuberin, as well as phospho-S6K and cellular localization of p27 as indicators of Tuberin function. Understanding when Tuberin levels are elevated and when the protein is activated during neural development will implicate critical events that may be most susceptible to altered Tuberin signalling in TS patients.

Insights on Histone H3 Lysine 9 Acetylation by Rtt109 in yeast.

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Acetylation of K56 by Rtt109 on newly synthesized histone H3 has been shown to be important in its deposition onto nucleosomes during chromatin assembly in yeast. Another prominent posttranslational modification on H3 prior to its deposition onto nucleosomes is K9ac. In vivo, K9ac is performed by both histone acetyltransferases (HATs) Rtt109 and Gcn5. Asf1 and Vps75 are two histone chaperones that aid Rtt109 in the acetylation of H3. In vivo, both chaperones are essential for K9ac whereas for K56ac only Asf1 is required. Asf1 binds and chaperones H3-H4 dimers to present them for acetylation, whereas Vps75 binds Rtt109 to enhance its K9ac activity. Even though there have been a lot of structural studies on these three proteins, the mechanism by which they carry out H3 acetylation is still poorly understood. In this study we have used protein sequence alignments to identify motifs in Rtt109 that have been under selective pressure to be conserved through evolution; the motifs can potentially represent important sites required for catalysis. Deletion of one of the identified motifs in *S.cerevisiae* eliminated K9ac while maintaining K56ac in vivo. Co-purification studies showed that deletion of this motif does not affect the Rtt109-Vps75 interaction. From these findings we suggest that the identified motif might play a role in the interaction between Rtt109 and Asf1 during K9ac.

Emergence timing in Chinook salmon (*Oncorhynchus tshawytscha*): effects of nest density

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Chinook salmon (*Oncorhynchus tshawytscha*) make their redds (nests) in gravel at the bottom of rivers. After spawning, females cover their fertilized eggs with a layer of gravel to protect the embryos during development. Eventually, fry emerge from the gravel and establish feeding territories within the river. Fry that emerge earlier are able to claim a territory and avoid being displaced downstream to less suitable habitat. We tested the hypothesis that emergence timing is sensitive to cues of competitor density, and predicted that early emergence will occur in nests of higher densities compared to nests of lower densities. Gametes were collected from the Sydenham River, Owen Sound, Ontario, fertilized, and then monitored until hatching. At hatching, 28 high and 13 low density 'nests' (groups of alevins incubated in cups) of 20 and 10 alevins, respectively. Alevins within a cup had similar hatch dates and were allowed to continue their development in gravel substrate. At emergence from the substrate, fry were euthanized, measured, and weighed. In contrast to expectation, density did not influence emergence timing or size. However, emergence was significantly earlier among fry from later hatched cups. This pattern of catch-up is consistent with there being benefits associated with early emergence.

Meiosis Specific Function and Regulation of the Anaphase Promoting Complex.

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The Anaphase Promoting Complex (APC) is an E3 ubiquitin ligase that is responsible for mediating the successful transition from metaphase to anaphase in mitosis and meiosis I and II. The APC is activated by Cdc20 (Fizzy in *Drosophila*) for mitotic progression by binding to Cdc27 and APC2. However for proper meiotic progression Fzy, as well as another activator, Cort, are both necessary. It is not understood why meiosis requires two separate APC activators while one is sufficient for mitosis. I hypothesize that the two activators convey substrate specificity or activate APCs composed of varying subunits. Inhibition of the 26S proteasome *in vivo* followed by immunoprecipitation and mass spectroscopy will be used to identify APC^{Cort} protein interactions, and specifically identify those that are substrates. I have shown that the 26S proteasome can be inhibited in the female germ line by the presence of increased cyclin levels and embryos arrested in meiosis. When the Cort-Cdc27 interaction is abolished, meiosis is still completed and resulting flies are viable. I am working on obtaining mass spectrometry results to identify specific proteins interacting with the APC^{Cort}. From this, we know that the Cdc27 interaction is not necessary for Cort mediated activation of the APC, and it is therefore possible that Cort is part of a varying APC from APC^{Fzy}.

Role of ROS in the activation of the p20k lipocalin gene by contact-inhibition and hypoxia.

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Quiescent cells observed in the G₀ phase in chicken embryo fibroblasts (CEF) undergo a programmed expression of growth arrest-specific (*GAS*) genes. A key model *GAS* gene, p20K, a member of the lipocalin family of lipid-binding proteins, also known as chicken p21, or extracellular fatty acid-binding protein (EX-FABP) has high levels of expression upon conditions of contact-inhibition. Previous studies have identified the role of CAAT/enhancer binding proteins beta (C/EBP β) in regulation of the p20K gene by a 48-bp region known as the quiescence-responsive unit (QRU). Reactive oxygen species (ROS) have shown to influence gene expression in intracellular concentrations, as observed in regulation for transcription factors NF- κ B and AP-1. However, it remains unknown whether C/EBP β is regulated or sensitive to ROS levels directly altering the induction of p20K. In this study, CEF under conditions of contact-inhibition and hypoxia will be treated with antioxidant *N*-acteyl-L-cysteine (NAC) to decrease ROS levels. The effect of altering the levels of ROS on expression of p20K will be examined. Further analysis includes examining the p20K promoter through a transient reporter assay, based on chloramphenicol acetyltransferase (CAT). From previous studies, it is likely that the activation of p20K by hypoxia and contact-inhibition in CEF is sensitive to the inhibition of ROS production, resulting in decreased p20K expression. Preliminary results do suggest antagonizing the effects of ROS production is affecting p20K expression.

Thermal plasticity of chill coma: the relationship between chill-coma onset and recovery in *Drosophila melanogaster*

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Chill-coma onset and recovery are widely-used metrics of thermal tolerance in insects. The physiological mechanisms underlying chill-coma are poorly understood, and are generally regarded to be the same for onset and recovery. To investigate whether chill-coma onset and recovery have the same underlying physiological mechanisms I generated variation in thermal tolerance in adult *Drosophila melanogaster* by a combination of rearing (14 °C, 21.5 °C and 27 °C), acclimation (5 days at 6 °C or 29 °C) and hardening (1 h at 0 °C or 36 °C) conditions. Acclimation had a significantly greater effect on chill-coma onset temperature and recovery time than hardening treatments across all three rearing temperatures. Chill-coma onset and recovery were positively correlated; however, females recovered from chill-coma significantly faster than males across most treatment conditions. Thus, these results support that the physiological mechanisms of chill-coma onset and recovery are linked, but may, in part, differ subtly between males and females.

Congenital cataracts: getting a clearer picture of the role of Pitx3 and the filamins.

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The *Pitx* family of genes comprises a group of homeobox transcription factors involved in the formation of many structures during development. *Pitx3* is involved in the formation of the lens, pituitary and somites, and asymmetrical organs. *Pitx3* null mutant mice and humans can develop congenital cataracts. Preliminary work suggests that this gene could be a transcriptional regulator of the three filamin genes A, B and C, a group of actin binding proteins which are thought to organize actin in the developing lens. One reported mutant of *filamin* displays a pattern of congenital cataracts. Two objectives have been formulated that will provide evidence to support the hypothesis that *Pitx3* is a transcriptional regulator of the filamin genes. The objectives include determination of the wild type spatial-temporal expression of the filamins during lens development, as well as changes in filamin expression patterns pursuant to *Pitx3* knockdown. Using *Xenopus laevis*, these objectives were studied by means of in situ hybridizations and morpholino-mediated translational knockdown of *Pitx3*. Results indicate the filamins have a differential wild type expression. Filamin A and B are expressed in the somites, branchial arches and head region with Filamin B also being expressed in the lens. Filamin C is expressed in the somites, presumptive heart and lens. Moving forward, experiments upon the relationship between the *filamin* promoters and the *Pitx3* protein product will give more insight into the relationship between the *filamins* and *Pitx3*.

Circadian Cycling of the Ecdysteroid Hormone 20-hydroxyecdysone in the Ovaries of the Adult Insect *Rhodnius prolixus*.

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Ecdysteroids are the molting hormones in the larval stages of the insect *Rhodnius prolixus*. Ecdysteroids are present in high concentrations during moulting and have been shown to cycle with a circadian rhythm. In adult insects ecdysteroids are known to function in reproduction. The ovaries of some species have been shown to produce ecdysteroids during vitellogenesis, with ecdysteroids being involved in ovary maturation and oviposition. This study focuses on the rhythmic cycling of ecdysteroids in the ovaries and hemolymph of adult mated females. Insects were given a blood meal to initiate a reproductive cycle; 6 days after feeding, ovaries and hemolymph were collected every 6 hours for 4 days. The insects began in an entrained environment (12h Light/12h Dark) and were transferred to aperiodic conditions (continuous darkness) on the 2nd day. Concentrations of ecdysteroids were determined using a radioimmunoassay (RIA). The results indicate rhythmic fluctuations in ecdysteroid concentration in the ovaries and hemolymph, including in the absence of environmental cues indicating a possible circadian rhythm of ecdysteroids in adult female *Rhodnius prolixus*.

The effects of salicylate on GABA_B receptor expression in auditory structures

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Type B γ -Aminobutyric acid (GABA_B) is a major inhibitory neurotransmitter receptor in the mammalian central nervous system. Presynaptic GABA_B receptors regulate the release of the neurotransmitters including glutamate and GABA, while postsynaptic GABA_B receptors are involved in the mediation of inhibitory postsynaptic events. Therefore GABA_B receptors are important for the balance between excitation and inhibition in the auditory system. Previous studies suggest that exposure to salicylate may cause a reduction in synaptic inhibition in auditory neurons, which may in turn have implications for hearing problems such as tinnitus. The present study examines how salicylate affects the distribution and expression of GABA_BR2 receptors in auditory structures. Pairs of Wistar rats were respectively injected intraperitoneally with sodium salicylate dissolved in physiological saline and an equivalent volume of saline (as a control). Immunohistochemical experiments were conducted to examine the regional and cellular distribution of GABA_B receptors in the auditory system. Our preliminary results indicate that salicylate down-regulates the expression of GABA_BR2 receptors in the auditory system. This confirms that salicylate toxicity decreases GABA-mediated inhibition and consequently leads to a relative increase in excitation. This change in the balance between excitation and inhibition likely results in enhanced neural activity in the auditory system, ultimately leading to tinnitus. These findings contribute to our understanding of the neural mechanisms responsible for tinnitus.

The inhibition and reversal of semaphorin 3A-induced growth cone collapse in adult sensory neurons by enteric glia

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It has been shown using a clip-compression spinal cord injury model in the rat that transplantation of enteric glia (EG) from the lining of the small intestine into the injured spinal cord promotes the regeneration of neurofilament-positive dorsal root axons into the injury site and that these axons are intimately associated with the EG (Jiang *et al.*, 2003; Jiang *et al.*, 2009). *In vitro*, it has been shown that bath-applied enteric glial-conditioned medium promotes neurite outgrowth in adult dorsal root ganglia (DRG) derived sensory neurons and that these effects are at least in part mediated by the release of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT-3) by enteric glia (Hansebout, 2009). An important feature of spinal cord injury is the formation of a glial scar, which expresses chemical signals that are inhibitory to axonal growth, among other pathological effects; whether or not the application of EG-conditioned medium can allow the growth cones of DRG-derived sensory neurons to overcome the collapsing effects of inhibitory guidance cues that are released from the glial scar is now of interest. This study uses one such molecular cue, semaphorin 3A, to elucidate the answer to this question. Semaphorin 3A is involved in the guidance of neurite tips in the developing nervous system, but it has also been shown that semaphorin 3A is released by scar-associated cells in the injured spinal cord (Pasterkamp *et al.*, 1998; Pasterkamp *et al.*, 1999; De Winter *et al.*, 2002) and that its release causes the collapse of growth cones located on sensory axons, preventing them from penetrating the injured area (Kaneko *et al.*, 2006; Niclou *et al.*, 2006; Pasterkamp *et al.*, 1998). NGF, GDNF, and NT-3 have been shown to inhibit the collapsing activity of semaphorin 3A in adult sensory neurons (Wanigasekara & Keast 2006); therefore we hypothesize that EG-conditioned medium will inhibit and perhaps even reverse semaphorin 3A-induced collapse, since EG release all of these neurotrophic factors (Hansebout, 2009). Finding an effective method of inhibiting or reversing semaphorin-induced growth cone collapse may be important to future treatments of spinal cord injury as such an inhibition would allow the penetration of sensory axons back into the site of injury and would potentially revert responses such as neuropathic pain and autonomic dysreflexia that arise after spinal cord injury (Krentz *et al.*, 2000; Marsh *et al.*, 2002). Initial data suggests that enteric glial-conditioned medium completely inhibits growth cone collapse in adult dorsal root ganglia-derived sensory neurons when administered 1 hour before semaphorin 3A and when co-administered with semaphorin 3A; the effects of EG-conditioned medium administered after the initial induction of semaphorin 3A-mediated collapse are currently being elucidated. Judging from previous studies, it would seem that the observed neuroprotective effects are due to the release of growth factors by EG.

Endophytes in Mongolian Grasses

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The relationship between grasses and fungal endophytes has tremendous applied significance and is both detrimental and beneficial. On one side of the spectrum, there are the economic benefits of increased plant growth and resistance to insect pests. On the other side, however, is the poisonings of domestic animals from endophyte-produced alkaloids. Therefore, it is important to quantify the level of infection present in a plant. Currently, testing laboratories use histological staining followed by microscopic analysis to determine infection levels. This is a very tedious methodology and requires considerable expertise on part of the technician. The purpose of this research is to determine the quantity of endophytes in *Festuca* plants from Mongolia. Commercial immunoblot test kits from Agrinostics Ltd. Co. have been widely used for detection of the presence of *Neotyphodium* endophyte in grass tillers. This study shows that the use of these immunoblot kits can be extended into quantitative analysis for *Neotyphodium* as well. Tiller imprints on the card were dissected into Red, Green and Blue channels using computer software, and a statistically significant correlation between the red and green intensities on the immunoblot card and *Neotyphodium* hyphal density in plants was. Further confirmation and implementation of this rapid method of determining the quantity of *Neotyphodium* would be a giant step forward in research on fungal endophytes.

Rearing effects of hypoxia on the male reproductive capacity of *Pseudocrenilabrus multicolor victoriae*.

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Environmental hypoxia can affect the general biology of fish. Over the last decade, it has been demonstrated that hypoxia is an endocrine disruptor and can impair fish reproductive capacity. Most of the known effect of hypoxia exposure in male fish include a generalized reduction in testicular mass, androgen levels and sperm number. Despite the great importance of sperm morphometry in determining reproductive success in fish, and the detrimental effects of hypoxia as well, little efforts have been made to determine whether hypoxia exposure will cause changes in sperm morphometry, and whether the reduction in reproductive success may be hindered by changes in sperm morphometry. In this study, I used males of the African cichlid, *Pseudocrenilabrus multicolor victoriae*, in order to answer to the first question, does hypoxia have any effects on sperm morphometry? F₁'s from two different populations with divergent oxygen regime, a swamp (hypoxic) and lake (normoxic) were raised in lab controlled conditions under hypoxia and normoxia. After one year under these divergent dissolved oxygen conditions, the sperm traits (head height, head width, midpiece length and tail length) were measured. Since fish raised under hypoxia conditions are under stress, their gonad mass should be smaller than those raised under normoxia. It was speculated that sperm from fish under hypoxic conditions would have longer tails to allow for faster swimming speeds.

Defining Interaction Networks Involving SKP2 and SPY1 Proteins Involved in Human Cell Cycle Regulation and Oncogenesis

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E3 SCF ubiquitin-ligase complexes are responsible for signaling the proteolytic degradation of certain proteins via ubiquitination of the target protein. The SCF core-complex's F-box protein contributes to the target specificity of the complex. Human SKP2 (S-phase kinase-associated protein 2), an F-box protein, has been shown to interact with a cell cycle regulatory protein, speedy 1 (SPY1), however the functional significance of such interactions have yet to be investigated. SPY1 is required for normal entry into the S-phase and promotes survival of mammalian cells following DNA damage.

BiFC (bimolecular fluorescence complementation) will be employed to characterize specific protein-protein interactions involving SKP2 and SPY1, which will provide information on the sub-cellular localization of the interaction and the importance of the fusion-orientation of the interacting autofluorescent protein constructs. This has been approached by splitting the single-peptide yellow fluorescent protein (YFP), and linking each fragment to each of the proteins of interest. When two proteins of interest interact, the YFP fragments re-associate to reconstitute an autofluorescence signal. The vectors developed as part of this study incorporate Gateway[®] technology for expedited and efficient cloning of the gene of interest into the BiFC vector.

Various BiFC vectors have been constructed and will be utilized to investigate the interaction between SPY1 and SKP2 *in vivo*, namely the cellular location and cell cycle phase of such interaction. The interaction's significance with respect to the SCF complex's potential role in targeting SPY1 for degradation will also be studied.

Effect of Metabolic Rate on EE2 Uptake in *Fundulus heteroclitus*.

Lisa Robertson^{*1}, Tamzin Blewett¹, Deborah MacLatchy² & Chris Wood¹

¹*McMaster University*; ²*Wilfrid Laurier University*

This study focuses on the link between EE2 and metabolic rate in *Fundulus heteroclitus*. Ethynylestradiol is the major component of birth control and is unable to be fully broken down by sewage treatment, resulting in varying background water levels.

The aim of this study was to determine whether or not metabolic rate (increased by swimming) influences ethynylestradiol uptake, potentially via a similar mechanism as oxygen uptake in *F. heteroclitus*. Our hypothesis is that since EE2 and oxygen share several molecular characteristics, oxygen uptake will be a predictor of EE2 uptake in *F. heteroclitus*. Thus, if EE2 enters at the gills in a manner similar to oxygen, increased exercise (increased metabolism) will increase EE2 uptake.

This hypothesis was tested using swim respirometry (swimming the fish at different controlled speeds in a closed chamber) and radiolabeled EE2, later analyzed in a β counter to determine EE2 sites of accumulation. It was found that patterns of EE2 uptake *did* support the hypothesis – more was taken up at higher swim speeds (higher metabolic rates). Thus oxygen consumption and EE2 accumulation showed a positive correlation. EE2 also tended to accumulate in the liver (where toxicants are metabolized), the gall bladder (where metabolized toxins are incorporated into bile), and the gut (where bile is dumped and eventually excreted).

EFFECTS OF STEROIDAL HORMONES ON THE INFECTIVITY OF MICROSPORIDIA TO FISH CELLS

Rebecca Rumney & Lucy Lee,

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Aquaculture is an important source of fish for humans world-wide. With the decline of fish harvest yields, significant efforts are ongoing to raise the populations of food-fish to meet consumer demand. High stocking densities and other environmental conditions increases the prevalence of disease among these fish. Microsporidia are a group of obligate intracellular parasites known to cause tissue degradation and disease in fish. The microsporidian *Loma morhua*, is commonly found in Atlantic Cod (*Gadus morhua*) and other food fish as an emerging parasite of concern. As a result, the development of effective measures for treatment and prevention of disease is necessary in aquaculture. Certain environmental conditions, steroidal hormones for example, affect the infectivity of such pathogens. To examine this phenomenon, rainbow trout, zebrafish and haddock cell lines were examined to determine the effects of steroidal hormones on the infectivity of the model microsporidian, *Anncaliia algera*, in fish. Using fish cell lines to monitor pathogens *in vitro* will help investigate disease, treatment and toxicity on hosts as a model for the live specimen and control microsporidia infections in aquaculture. The purpose of my thesis research is to use fish cell culture to study the effect of steroidal hormones on the infectivity of microsporidia on their hosts. This is economically important as microsporidiosis has substantial consequences to the profitability of aquaculture and fisheries and results obtained from this study could be significant in our understanding of effective treatment for diseased fish.

The temporal and spatial expression of TSC2 throughout neural development.

Hugh Quinn*, Elizabeth Fidalgo da Silva and Lisa A. Porter

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Tuberous sclerosis (TS) is a multisystem disorder that is characterized by the development of primarily benign tumours called hamartomas. TS is linked to a loss of heterozygosity in two genes, TSC1 and TSC2 encoding the proteins Hamartin and Tuberin respectively. In vivo Hamartin and Tuberin form a heterodimer that functions as a tumour suppressor to inhibit the mammalian target of rapamycin (mTOR). mTOR inhibits protein synthesis through the phosphorylation and subsequent activation of the S6K. Furthermore, Tuberin is known to prevent the Cdk inhibitor p27 from localizing to the nucleus, thereby inhibiting cell growth. Tuberin may be involved in many key processes that lead to proper brain development, such as the formation of the notochord and one of the primary sites for hamartoma formation in TS is the brain. We hypothesize that regulation of Tuberin levels, localization and activity is an essential component of neural development. This study utilized murine brain samples collected from embryonic stages on through to early adulthood and immunohistochemical analysis to observe the differences in protein levels of endogenous Tuberin, as well as phospho-S6K and cellular localization of p27 as indicators of Tuberin function. Understanding when Tuberin levels are elevated and when the protein is activated during neural development will implicate critical events that may be most susceptible to altered Tuberin signalling in TS patients.

Insights into the function of Toc159 as the chloroplast preprotein import receptor

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Toc159 is an essential protein for the import of nucleus-encoded transit peptides into the chloroplast, and it is located on the outer membrane of chloroplasts. It has been identified as one of the main receptors for preproteins targeted for the chloroplast, and recent research in Dr. Smith's lab has identified some novel preprotein interactors that have yet to be characterized. These unknown proteins were identified by screening the *Arabidopsis thaliana* cDNA protein library for protein-protein interactions with the Toc159 G domain receptor. Four of the unknown proteins were identified, sequenced and then analyzed with a program, TargetP, which predicted them as chloroplastic proteins based on their N-terminal transit sequence. The purpose of this study is to determine if these putative chloroplast resident proteins are targeted to the chloroplast. First, I will use *in vitro* transcription and translation to produce the protein products from the cloned vector/insert constructs. Then a chloroplast import assay will be carried out in the presence of the proteins to determine if they are indeed chloroplastic. I expect that these proteins will be found either imported, or bound to the outside of the chloroplasts. Resulting data from this study will help increase our knowledge of the Toc159 receptor family, as well as begin to characterize previously unknown chloroplastic proteins.

The effect of doxorubicin on cellular senescence and p66^{Shc} expression in human dermal fibroblasts

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Cellular senescence is a permanent cell cycle arrest that occurs in most differentiated somatic cells after a restricted number of cell divisions or prematurely after some forms of cellular stress. Senescence is influenced by the levels of p66^{Shc}, a stress response protein, whose concentration varies in response to intracellular oxidative stress. This study investigated the effects of oxidative stress-induced premature senescence of human dermal fibroblasts (HDFs) through doxorubicin treatment by evaluating the expression of p66^{Shc} under these conditions. Western blot analysis revealed that cells treated with 0.1 μ M doxorubicin collected immediately after treatment showed an increase of total Shc protein (p46^{Shc}, p52^{Shc} and p66^{Shc} protein) levels and p66^{Shc} protein levels. Doxorubicin treatment in HDFs appears to reduce the amount of Shc protein when compared to untreated cells when cells were collected at day 1 through to day 5 time points. These results show that doxorubicin treatment of HDF cells leads to an initial increase of p66^{Shc} expression, which is not sustained. These results provide better insight into the role of p66^{Shc} in cellular senescence. In addition it suggests that the use of chemotherapeutic agents such as doxorubicin may have practical applications in studying cellular senescence. An hourly time course experiment would provide insight on the effect of doxorubicin on HDF cells at initial treatment. A grant awarded to DHB from the Canadian Institutes of Health Research (CIHR) funded this project.

Are fat bodies of the adult *Rhodnius proxilus* a rhythmic target for the steroid hormone ecdysone?

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Ecdysteroids are the primary molting hormones in *Rhodnius proxilus*, guiding the insect's development from L1 to the adult stage. As during development, ecdysone is expressed rhythmically, but in adults the target organs and/or tissues for this hormone are currently unknown. One possible candidate would be the fat bodies (FB) of the animal, where ecdysone would modulate the synthesis of vitellogenin, which is required for oocyte maturation in female adults. Moreover, in males, ecdysone might play a role in spermatogenesis. Ecdysone is a steroid hormone, and as such it acts through an intracellular receptor expressed in target tissues, the ecdysone receptor (EcR). In order to elucidate if fat bodies are indeed a target for ecdysone in adults, immunohistochemistry (IHC) and laser confocal microscopy will be used. FB tissues samples will be submerged in primary antibody solution containing anti-EcR 9B9, and afterwards in secondary antibody anti-mouse. Furthermore, for determining if FB is a rhythmic target for ecdysone during oogenesis and spermatogenesis, samples will be analyzed at 4 separate time points each day at 6 hour intervals, for the days of: 3, 5, 7 and 9 after a blood meal (which triggers the start of the reproduction cycle).

Identification of Interactomes for chromatin related proteins in *Tetrahymena Thermophila*

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In all eukaryotic cells, DNA is packaged into a protein complex called the nucleosome which in turn forms chromatin. The physical structure and physiological activity of chromatin are regulated through a diverse set of posttranslational modifications, histone exchange, and structural remodelling. The aim of this study is to discover the “interactomes (a set of interacting proteins)” for several chromatin related proteins using *Tetrahymena thermophila* as a model organism.

HIR1, HAT1 and GCN5 histone acetyl transferases function in chromatin assembly. BRCA2 is a tumour suppressor in mammals that is essential for homologous DNA recombination (HDR), a process for the error free repair of DNA double-stranded breaks (DSBs). The activity and substrate recognition of many HATs, chromatin-mediated transcriptional silencing proteins and tumour suppressor proteins is typically regulated by their association with other factors in the context of multi-protein complexes. The goal of this study was to find out the interacting partners for HAT1, GCN5, HIR1 and BRCA2 proteins in *T. Thermophila* by affinity purification combined with mass spectrometry. I will present my work in constructing the vectors necessary for targeting the FZZ tandem affinity epitope tag to the four genes in the *Tetrahymena* macronucleus. Plasmid DNA preparation, restriction digestion, PCR, DNA sequencing, cloning and biolistic transformation are some of the most prominent research techniques that would be employed in this research project.

Through the use of *Tetrahymena Thermophila*'s unicellular model system, we hope to understand by analogy how defects in chromatin assembly can contribute to disease progression in humans.

Hormonal cues in seeds of *Lupinus perennis* populations with contrasting germination rates

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The Wild Blue Lupine, *Lupinus perennis*, is a rare plant which is the obligate host to larvae of three threatened butterfly species, specifically the Karner Blue. It has been reintroduced to the Alderville Black Oak Savanna restoration site where it has only been successful in four microhabitats to varying degrees. Because Wild Blue Lupines have low germination rates, which may affect the success of the populations, this paper looks into the hormonal cues involved in germination. It was expected that the dormancy inducing hormone Abscisic acid (ABA) would be present in high levels in seeds that germinate poorly while those which germinate well would contain higher levels of Cytokinins (CKs) which are growth promoting hormones. Seeds of plants from 20 plots within the four microhabitats were harvested and planted in standard greenhouse conditions. % germination was recorded. Seeds of these plots were also analysed for levels of ABA and CKs. % germination and hormone levels were analysed for correlation. Hormone-germination relationships are more complex than anticipated. Findings will be discussed.

New droplet assay for analyzing thermal preferences in the nematodes *Caenorhabditis elegans* and *C. briggsae*

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The nematode worm *Caenorhabditis elegans* exhibits behavioral adaptation to the cultivation temperature. The worms acclimate to developmental temperature and migrate towards the preferred temperature when placed on a temperature gradient. In classical studies of thermotactic behavior in *C. elegans*, the movements of worms were mostly investigated through population accumulation pattern on a linear gradient. However, these population-based assays provide inadequate understanding about temperature-dependent behaviors in nematodes at an individual level. In this study, I measure the thermal response of *C. elegans* N2 strain using a new droplet assay which allows fast data acquisition and single-worm analysis. I also compared thermotaxis in *C. briggsae* (strains AF16 and HK104), a closely related species to *C. elegans* to identify natural variation within and between species. My results show that the N2 strain migrates either up or down the thermal gradient depending on its cultivation temperature, whereas AF16 and HK104 strains exhibit migration up gradients towards the warmer growth temperature but no evidence of downwards migration in response to lower cultivation temperature. These results suggest that thermotactic behaviors have evolved independently in *C. briggsae* and *C. elegans*. Our new droplet-based assay will allow us to research new aspects of thermal behavior of the *Caenorhabditis* genus and to further investigate underlying mechanisms of thermosensation.

Analysis of *lkt* mRNA from non-haemolytic mutants of *Mannheimia haemolytica* A1.

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The bacterial pathogen *Mannhaemia haemolytica* A1 has been implicated as the primary cause of bovine pneumonic pasteurellosis or “Shipping fever”. This disease is a major cause of economic loss in the cattle industry. *M. haemolytica* A1 produces a collection of virulence factors to infect the host: one of which is the leukotoxin (Lkt). Lkt is a pore-forming cytolysin which specifically targets bovine leukocytes but displays non-specific binding to erythrocytes, the cause of the haemolytic phenotype of the bacterium. The genetic locus responsible for the production of Lkt has been cloned and characterized. The Lkt operon encodes four genes in the following order: *lktC* encodes a trans-activator protein which activates the Lkt protein through acylation; *lktA* encodes Lkt; *lktB* and *lktD* encode the peptides which form the Lkt transporter. Very little is known about the regulation of Lkt operon expression. In a previous study, a collection of non-haemolytic mutants were isolated after EMS mutagenesis. Western immunoblot analysis using an anti-Lkt antibody showed that presence of Lkt in the mutants varied. Reverse transcription PCR analysis is being used to examine the presence or absence of transcripts from the *lktCA* region or the *lktBD* region of the operon. This will allow for the characterization of the Lkt operon transcripts in the mutants and could lead to identification of mutants with mutations outside of the Lkt operon.

Effects of Inbreeding Depression on Sperm Morphology in Lake Trout *Salvelinus*

Namaycush

Jennifer Smith

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Small, isolated populations frequently suffer the effects of inbreeding depression due to the higher instances of mating between related individuals. Previous studies have focused on the negative implications of inbreeding on offspring survival and reproduction. More recent studies have shown similar negative implications of inbreeding in more cryptic traits underlying reproductive success, such as sperm quality. The purpose of the current study was to examine the effects of inbreeding on sperm morphology in four experimental lines of lake trout (*Salvelinus namaycush*) each differing in levels of inbreeding (unrelated matings, maternal half-sibs, paternal half-sibs, and full sibs). Digitized images of sperm heads and tails were taken for each male and Image-J software was used to analyze a variety of size and shape metrics. A one-way analysis of the data will be used to measure the effects of inbreeding on sperm morphology.

Live-cell Imaging of the Pumilio Proteins in the Fission Yeast *Schizosaccharomyces pombe*.

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The PUF, or Pumilio protein family is a highly conserved group of proteins present in many different eukaryotes. PUF proteins share a defining C-terminal RNA-binding domain, which contacts target mRNAs to affect various aspects of mRNA metabolism, including mRNA stability and translational efficiency. The genome of the fission yeast *Schizosaccharomyces pombe* encodes nine such PUF proteins, but little is known with regards to their function and regulation. As part of a broader, collaborative genome-wide study aimed at better understanding the role of these Pumilio proteins, the localization and intracellular dynamics of each EGFP-tagged *S. pombe* PUF protein were examined in real time over the course of the cell cycle using spinning disk confocal microscopy. Localizations studied have revealed that the proteins Puf6p and Nop9p share a nucleolar distribution, proteins SPAC4G8.03, Mpf1p and Mcp2p are found in granular structures in the cytoplasm, and SPBP35G2.14, SPBC56F2.08, SPAC6G9.14 and Puf3p are distributed through out the cytoplasm. Furthermore, the cytoplasmic proteins undergo a change to their localization, as they become distributed in a granular fashion in response to heat shock.

The effect of salinity on the toxicity of *Heterosigma akashiwo*

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Heterosigma akashiwo is a raphidophyte that forms harmful algal blooms that can kill fish. The toxicity of *H. akashiwo* can vary from one bloom to another, within a single bloom, across time, and among locations. As an euryhaline genus the level of toxicity may correspond to salinity differences. The possibility that salinity affects the level of toxicity in a bloom was explored in this experiment. It was hypothesized salinity would affect the level of toxicity and buoyancy of the cells, such that sinking rates would increase as salinity decreased and that sinking cells would be more toxic than floating cells. Cultures of six strains of *H. akashiwo* were grown at a 15:1 molar ratio of nitrate to phosphate, 18°C, and different salinities. The salinity treatments were 30, 20, 15, and 10 psu. Culture growth was monitored and then processed in early stationary growth phase. Floating and sinking cells were separated and rates of floating and sinking were determined. Toxicity was measured using standard brine shrimp bioassays. The culture growth rates, floating and sinking rates, and toxicity of floating and sinking cells were compared within and among treatments.

Immune gene expression in the Eastern subterranean termite, *Reticulitermes flavipes*

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Combating disease and infection is a major problem that has shaped the life history of all living things. The innate immune response is an ancient answer to this problem, and it is characterised by pathways that regulate the expression of antimicrobial genes. Studies from *Drosophila* and other immune models reveal that specific pathways are activated by different types of pathogens. Here we infect the Eastern subterranean termite with a fungus and a gram-negative bacteria, and measure differences in expression at loci chosen from the TOLL and IMD immune pathways. A qPCR analysis suggests that termites do up-regulate pathway specific transcripts in a manner predicted from *Drosophila*. The immunity factor *Relish* is differentially responsive to bacterial challenge, while a *Gram-Negative Binding Protein* is most responsive to fungal infection. Unlike flies, however, termites are highly social and we are currently testing whether immune gene expression varies as a function of social context.

Potential Antimicrobial Properties of Select Natural Products Using a Resazurin Microtitre Plate Assay

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The growing number of infections caused by antibiotic resistant microorganisms has increased the need for novel antimicrobials. The diverse phytochemicals produced by plants prove to be an excellent source of such antimicrobial compounds. This study investigated the potential antimicrobial activity of 26 natural products against the following microorganisms; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. A resazurin microtiter plate assay was used to determine antimicrobial activity and the minimum inhibitory concentration (MIC) of the natural products against the target microorganisms. Only one natural product showed antimicrobial properties; the olive leaf against *C. albicans* at an MIC of 1000 µg/mL. The viable plate count curves, however, showed that olive leaf extracts do not have any antimicrobial properties against *C. albicans*.

Increased para-inflammatory response is associated with neurodegeneration in the cerebellum of *harlequin* mice

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Neurodegenerative disorders such as Alzheimer's, Huntington's and Parkinson's diseases are becoming prevalent in our increasingly aged population. The *harlequin* (*hq*) mouse model exhibiting neurodegeneration due to knock down of *Apoptosis-inducing factor* (*Aifm1*) gene expression was used to demonstrate the involvement of para-inflammatory pathways in neurodegenerative disease. Affymetrix[®] gene expression microarrays (Santa Clara, CA) were used to determine transcriptome differences (1.5-fold change, $p < 0.05$) in the cerebellum of *hq* compared to wild-type (WT) mice at 4 months of age. TaqMan[®] real-time qPCR was used to confirm changes in the expression of genes relevant to para-inflammation. Seventy-one genes involved in para-inflammation had altered expression in *hq* cerebellum. In particular, *Cathepsin S* (*Ctss*) was upregulated 2.06 fold ($p < 0.001$), *Cluster of differentiation 68* (*Cd68*) was upregulated 2.10 fold ($p = 0.002$), but *Inducible nitric oxide synthase* (*Nos2*) was not upregulated in the cerebellum. TaqMan[®] assays for *Nos2* and *Ctss* revealed 2.24 and 1.66 fold upregulation, respectively. The retina transcriptome showed 215 para-inflammatory genes with altered expression associated with *hq* retinal degeneration. In the retina, *Nos2*, *Ctss*, and *Cd68* were all upregulated: 2.59 fold ($p = 0.025$), 1.98 fold ($p = 0.04$), and 2.13 fold ($p = 0.02$), respectively. TaqMan[®] assays confirmed these *Nos2* and *Ctss* changes in gene expression with fold changes of 2.59 ($p = 0.025$), and 1.93 ($p = 0.04$), respectively. Gene-expression changes in para-inflammatory pathways implicate the immune system early in the process of *hq* neurodegeneration. Also, the gene expression of such markers of inflammation have potential as molecular targets for drug design and/or monitoring treatment efficacy.

The effects of isotocin on conspecific group preferences in *Neolamprologus pulcher*

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Differences in social affiliation, grouping behaviour, and conspecific tolerance both between and within species can in part be explained by the effects of neuropeptides on social behaviour. Isotocin, a neuropeptide homolog of oxytocin found in teleost fishes, has been found to induce pro-social behaviours in different species. These pro-social behaviours, including group-choice decisions for social organisms, have profound fitness implications. In the study species *Neolamprologus pulcher*, a dominance hierarchy exists for each sex, and there is a tradeoff between increased safety in larger groups but a shorter queue to breed in smaller groups. In the current study, group-preferences for a large versus a small group of conspecifics were conducted in a controlled laboratory isotocin dose-response experiment. Results showed that males prefer to associate with a larger group while females prefer to associate with a smaller group regardless of dose. Also, both sexes showed lower aggression rates and increased exploratory behaviour when administered isotocin compared to a control group. Finally, dose-response curves were determined for each sex showing inverted-U shape curves with females more sensitive to the neuropeptide than males. These results provide the first account of isotocin's effects in a cichlid fish and support an underlying framework for further isotocin studies on group-preferences.

The impact of extreme environmental conditions on embryonic development and hatching of the mangrove rivulus (*Kryptolebias marmoratus*)

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The mangrove rivulus, *Kryptolebias marmoratus*, inhabits small pools of water in highly variable mangrove swamps, where conditions are often hypoxic and both tidal and seasonal cycles cause fluctuations in water levels. During periods of poor aquatic conditions, adults may leave the water and are able to survive terrestrially. Captive fish are known to release embryos both aquatically and terrestrially, suggesting that developing embryos may be exposed to a range of environmental conditions. This makes the mangrove rivulus an ideal candidate for the study of the effects of hypoxia and air exposure on early development and hatching. The first objective was to determine the embryonic stages of the mangrove rivulus under controlled conditions using laboratory reared rivulus embryos. The second objective was to measure the effects of air exposure on the developmental. Growth rates of air exposed embryos were morphometrically compared to controls. Air exposure had no significant effect on development. The third objective was to investigate how acute hypoxia influences hatching. Developed embryos were exposed to one of four acute treatments: water control, water hypoxia, air control, and air hypoxia. Control treatments never elicited hatching, while hypoxia in either medium triggered hatching in >90% of embryos. Furthermore, water hypoxia caused significantly faster hatching than the air hypoxia. These results suggest that aerial embryonic development in wild mangrove rivulus is possible, potentially allowing embryos to avoid poor aquatic conditions. Furthermore, it was found that acute hypoxia is a hatching cue for developed embryos, which may allow escape from sudden environmental perturbations.

Changing substrate specificity of *Arabidopsis thaliana* AROGENATE DEHYDRATASES (ADTs)

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Phenylalanine biosynthesis depends on six *AROGENATE DEHYDRATASES* (ADTs) encoded within the *Arabidopsis thaliana* genome. *ADT1* and *ADT2* encode enzymes with ADT and PREPHENATE DEHYDRATASE (PDT) activity and therefore recognize both arogenate and prephenate as substrates. Unlike these two enzymes, *ADT3*, *ADT4* and *ADT5* are only capable of utilizing arogenate. *ADT6* displays both ADT and PDT activity in biochemical *in vitro* assays however lacks PDT activity *in vivo*. The specific amino acids conferring substrate specificity to ADTs and PDTs are currently unknown, hence we cannot use sequence comparison to determine if an enzyme can accept arogenate or prephenate. Arogenate and prephenate are structurally very similar and differ only by one amino group. However presence or absence of this amino group causes a charge difference, and we predict that recognition of this charge defines substrate specificity of ADTs and PDTs. To determine which amino acids need to be changed to convert an ADT into a PDT, error-prone PCR was used to randomly mutagenize the arogenate-specific *ADT4* sequence. We are planning to generate *ADT4* mutant libraries that can be used for complementation assays. For such an assay *pha2* knockout yeast will be transformed with mutagenized *ADT4* cDNA libraries to select for sequence changes that have converted an ADT into a PDT. As the substrates are very similar we predict that only one or two amino acid changes are sufficient to convert one enzyme into the other.

Complementation of non-haemolytic mutants of *Mannheimia haemolytica* A1.

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Mannheimia haemolytica A1 is a bovine pathogen that causes pneumonic pasteurellosis resulting in significant economic loss for the cattle industry. The most important virulence factor produced by the bacterium is a leukotoxin (Lkt) which is a pore-forming cytolysin specific for bovine leukocytes but non-specific for erythrocytes resulting in cell lysis. The non-specific binding of Lkt to erythrocytes produces a zone of clearance on blood agar plates. Lkt is produced by a four gene operon which has been cloned and sequenced. *lktA* codes for the structural protein of Lkt, *lktC* codes for an activator enzyme that is responsible for acylating Lkt to its active form. Together, *lktB* and *lktD* code for a secretion apparatus that secretes the toxin extracellularly. A collection of non-haemolytic *M. haemolytica* mutants were previously generated by EMS mutagenesis. Total proteins from these mutants were examined by Western immunoblot analysis using a monoclonal antibody specific for Lkt. Lkt was not detected in some of the mutants as expected, however, Lkt was detectable in some mutants in spite of a non-haemolytic phenotype. These mutants could have a defect in any one of the four genes, or possibly elsewhere in the genome suggesting other factors may be required for fully functional leukotoxin production. In this project, recombinant plasmids containing either the 4 kbp *lktCA* genes or the entire 8 kbp *lktCABD* operon will be constructed for complementation experiments with the mutants to aid in determining the location of the defect.

S-adenosylhomocysteine hydrolase: a cytokinin binding protein in developing *Arabidopsis thaliana* seeds?

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S-adenosylhomocysteine hydrolase (SAHH) is an enzyme that, in the presence of adenosine kinase (ADK), permits methionine regeneration in the activated methyl cycle of *Arabidopsis thaliana*. SAHH and ADK are co-expressed in all tissues, except in roots and maturing seeds. The role of SAHH in the methylation cycle requires ADK, so the absence of ADK in these areas suggests that SAHH has an alternate function. Godge *et al.* (2008) have shown that native SAHH binds the plant hormone cytokinin (CK) *in vitro*, so this project investigates the possibility that SAHH is acting as a CK-binding protein in developing *Arabidopsis* seeds and a contributor to intracellular homeostasis. By expressing an artificial miRNA (amiRNA) targeted for SAHH (amiSAHH) under the influence of seed-specific promoters, the effects of decreased SAHH levels in seed can be studied. DNAs containing such an amiSAHH construct were transformed into wild-type *Arabidopsis* plants using *Agrobacterium*. These plants will be crossed with a CK-reporter line of *Arabidopsis* (ARR5::GUS) to observe changes in CK levels. We hypothesize that the expression of amiSAHH will increase free CK levels in the seed, and affect seed development. Expression of amiRNA targeted for ADK (amiADK) is used as a negative control. Further investigation into the possibility that SAHH is acting as a CK-binding protein involves growing ARR5::GUS and SAHH1::GUS plants on varying concentrations of CK. Analysis of the results may further elucidate the function of SAHH in *Arabidopsis*.

Characterization of the CDK1-1N mutant.

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Progression of eukaryotic cell cycle is controlled by the activity of serine/Threonine kinases known as cyclin-dependent kinases (CDKs). During G1 and G0 phase, cdk is kept in check by cdk inhibitors (CDKi), which prevents premature entry into the S phase. CDKi is degraded in late G1 phase by the E3 ubiquitin ligase SCF (skp2), the cell cycle regulatory protein cks1 and subsequent degradation by the 26S-proteasome. Recognition of CDKi by SCF (skp2), and its subsequent ubiquitination depends upon cyclin/CDKs mediated phosphorylation. In mammals, Skp2 and Cks1 are shown to associate with the SCFSkp2 complex and are required for growth and diploidy maintenance

Previous studies have shown that Cks85A, the Cks1 homolog in *Drosophila*, associates with CDK1. In this experiment we study the significance of this interaction by using cdk1-1N mutant that are not able to interact with cks85A and mutant ck85A (cks30AP90A) that are unable to interact with endogenous cdk1 using coimmunoprecipitation. Furthermore, we study the phenotype of cdk1-1N in larva, by examining the phenotype of larvae in cdk1 null background rescued by the CDK1-wt versus mutant rescued by CDK1-1N. It has previously been identified that CDK1 plays a non-catalytical role in the SCFskp2 complex for maintaining diploidy. We will investigate the significance kinase activity of CDK1 in this complex by studying a dead version of kinase cdk1 (cdk1-KD) and testing its ability to rescue the phenotype of cdk1-1N mutant.

AP-2 α plays role in lens epithelial cell maintenance subsequent to lens vesicle separation

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Objective: The Activating Protein-2 (AP-2) transcription factors are a family of genes playing essential roles in the development of multiple tissues, including the eye. Previous work in our lab has illustrated a cell autonomous role for AP-2 α in early lens development. In the current study, we investigated the requirement of AP-2 α in later stages of lens development, subsequent to lens vesicle separation in order to understand the role of AP-2a in maintenance of the lens epithelium.

Methods: The MLR10-Cre mouse line was utilized to create a conditional deletion of AP-2 α from the lens following its separation from the overlying ectoderm. Eyes and lenses were examined at embryonic and postnatal stages using histological and immunofluorescent techniques.

Results: Immunostaining for AP-2 α indicated its deletion from the lens epithelium at E11.5 and later. A few cells in the epithelium of the mutants at all stages expressed AP-2 α positive cells due to the mosaic behavior of the cre activity. Hematoxylin and Eosin staining revealed morphological abnormalities by E18.5, in which vacuoles were evident throughout the fiber cell region of the lens. This defect was greatly attenuated by P4 and multilayering of the epithelium was also observed. Lens epithelial and fiber cell markers Pax6, E-cadherin and β -Crystallin were expressed normally throughout development.

The required role of AP-2 α in the lens during later stages, subsequent to lens vesicle separation has been revealed. These mutants offer a model for the continued examination of the role of AP-2 α in maintenance of the lens epithelial phenotype.

Consumer and resource driven impacts on root dynamics and carbon sequestration in a degraded grassland ecosystem

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Root dynamics represent a significant gap in the present understanding of how terrestrial carbon (C) cycles will respond to environmental change – especially in grasslands, which cover ~40% of the earth's surface and store ~25% of the global C pool. Anthropogenic changes in resource availability and consumer pressures could alter grassland C storage through impacts on root dynamics (e.g. tissue quality/quantity, decomposition), influencing atmospheric CO₂ levels. However, how these changes affect belowground processes are unknown, despite the fact that 50-90% of annual net primary productivity (NPP) in grasslands occurs in the roots. Total NPP, tissue quality, decomposition, and root production/mortality are known to regulate grassland C dynamics, ultimately determining whether grasslands function as C sinks (deposits>withdrawals) or sources (deposits<withdrawals). The effects of consumers and resources may determine the direction of this balance. I tested two interacting hypotheses regarding two representative changes to degraded grasslands – reduced consumer effects (little/no grazing) and increased fertility (eutrophication) – using minirhizotron technology and field sampling in a degraded *Alopecurus pratensis* grassland at the Cowichan Garry Oak Preserve on Vancouver Island, British Columbia. Under the **eutrophication hypothesis**, grazing suppression and eutrophication should lead to net soil C loss because decomposition rates of higher quality litter [lower C:N] exceed sequestration. Under the **grazing hypothesis**, this feedback is broken due to the positive association between soil fertility, grass palatability, and the necessity for grazed individuals to invest in structurally complex C compounds that reduce grazing but also decomposition. The presence of grazing is therefore predicted to determine whether nitrogen-enriched grasslands act as C sources or sinks.

The relationship between body size and sensitivity to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), in larval sea lampreys

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For over fifty years, pesticide, 3-trifluoromethyl-4-nitrophenol (TFM), has been used extensively to control the invasive sea lamprey (*Petromyzon marinus*) populations in the Great Lakes; however, much remains unknown concerning the factors that influence its efficiency. Since larval lamprey exposed to TFM are forced to rely on anaerobic glycolysis to meet their metabolic demand, such possible factors include the relationship of the body size of the animal and the amount of metabolic stores. In our first experiment, larval lamprey ($N=50$, weight= 0.4 – 2.9 g) were exposed to LC50 concentration of TFM at 5.0 mg/L for 16 hours. The weight, length, and condition factor ($CF = \text{weight}/\text{length}^3 \times 10^6$) were compared to the survival time using regression analysis. In the second experiment, larval lamprey ($N=50$, weight= 0.4 – 2.9 g) underwent a control experiment for 16 hours, were euthanized upon its completion, and the brain, liver, kidney, and carcass were removed and investigated for metabolic store levels of glycogen, glucose, ATP, PCr, lactate, lipids, and fats. The strongest relationship found for TFM sensitivity and body size was the weight of the animal ($r^2=0.3850$, $p<0.05$), followed by the body length of the animal ($r^2=0.3385$, $p<0.05$), and the condition factor ($r^2=0.1766$, $p<0.05$). Research efforts are still investigating the relationship between body size and metabolic stores. Current evidence, however, suggests that the weight of the animal may be indicative of toxic concentration of TFM necessary to control larval lamprey population.