Ontario Biology Day 2008
Abstracts

Abdellah, Melissa and Stephanie Doucet

Biology, University of Windsor

The evolution of female ornamentation in birds.

Male ornamentation and courtship displays receive a great deal of attention in the literature; however, elaborate traits have also evolved in females of many species, including birds. One theory proposes that female ornamentation is simply a by-product of genetic correlation with male traits. However, other authors suggest that natural and sexual selection may directly influence female ornamentation. Nonetheless, there is little empirical evidence to support this idea. The goal of our study is to determine the most influential factors driving the evolution of ornamentation patterns across various bird species. We collected plumage reflectance data from 745 museum specimens, representing 127 New World species, by measuring ten body regions for three males and three females of each species. We calculated Euclidean distances between plumage reflectance and the reflectance of background vegetation for both males and females, and compared these scores to various behavioural and ecological variables collected from published papers, books, and species accounts. Our results indicate that females are less conspicuous than males against background vegetation. Although both sexes showed positive relationships between contrast against the background and sexual dichromatism, this relationship was stronger in males. Selection for crypsis seems to be influential in female ornamentation, predominantly when females are the sole providers for their offspring. Accordingly, female plumage was more cryptic in species using open-cup nests rather than closed nests, and for species nesting at heights that are known to have higher predation risks. Additionally, social selection seems to play a role in systems with biparental care. When males invest more in offspring by incubating, feeding, and nest building, females tend to me more colourful. These findings emphasize the importance of male parental care in the development of elaborate ornaments among females, supporting the notion that elaborate traits in females result from an interaction between natural and sexual selection.

Presenting Author: Melissa Abdellah

Abu Saleh, Ola

Molecular and Cellular Biology, University of Guelph

Changes in GAPDH Localization and its Association with PABPN1 in Oculopharyngeal Muscular Dystrophy.

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant, adult-onset genetic disease characterized by progressive eyelid drooping, swallowing difficulties and proximal limb weakness. OPMD is a result of a polyalanine expansion in the poly (A) binding protein nuclear 1 protein (PABPN1). Some of PABPN1's functions include pre-mRNA polyadenylation and transcription regulation. The mutant PABPN1 forms aggregates in the nucleus of skeletal muscle cells. In a previous study it was found that the enzyme Glyceraldehyde-3- phosphate dehydrogenase (GAPDH), which is a multifunctional enzyme, involved in glycolysis and possibly programmed cell death, co-localizes with the mutant PABPN1-A17 in the nucleus. In this research project, the possible relationship between GAPDH and the mutant PABPN1 (PABPN1-A17) is studied. First the nature of this association is determined using Co-IP. The level of GAPDH is then looked at using Reverse Transcriptase PCR. All of the experiments were done with mammalian cells transfected with the wildtype and mutant PABPN1 genes. If the study shows a chemical interaction between GAPDH and PABN1-A17 and an increase in GAPDH transcription this may suggest that the mechanism of cell death caused by PABPN1 polyalanine expansion in OPMD is through Programmed Cell Death signaled by GAPDH.

Presenting Author: Ola Abu Saleh

Akelaitis, Mark

Terrestrial and Aquatic Ecology, Laurentian University

Comparison of component communities associated with galls of diplolepis spinosa (hymenoptera: cynipidae) in distant parts of its range.

The cynipid wasp, Diplolepis spinosa (Ashmead) induces spherical galls on the stems of wild roses. This wasp and its galls are widely distributed across much of Canada from the Ontario–Quebec border to British Columbia. Galls are found only on Rosa woodsii Lindl. In southern Alberta and on Rosa blanda Aiton in southern and central Ontario. Mature galls were collected in the spring of 2007 from Waterton Lakes National Park in southwestern Alberta and from Timmins and Manitoulin Island in 2002 and returned to the laboratory for analysis and emerging of inhabitants. Gall size and the inhabitants per gall were compared for the two sites, the two central Ontario sites being considered as one site. Galls at the Waterton site are larger and less spinier than galls in Ontario and contain more inhabitants per gall. At both sites, larger galls contain more parasitoids than do smaller galls. Waterton galls are inhabited by an inquiline of the genus Periclsitus and contain more parasitoids per gall. Inquilines are not present in central Ontario galls and contain less parasitoids on average per gall. Other inhabitants associated with galls include a cynipid inquiline of the genus Periclistus and chalcid parasitoids of the genera Eurytoma, Pteromalus, Aprostocetus, Torymus, Eupelmus, and Ormyrus, along with an ichneumonid of the genus Orthopelma. The abundance of inquilines and parasitoids is correlated with gall size.

Presenting Author: Mark Akelaitis

Alam, Farhana

Biology, McMaster University

Standardization of vitellogenin biomarker assay for estrogen exposed to male, round goby fish.

The round goby (Neogobius melanostomus) is an invasive species that has expanded throughout the Great Lakes and has taken over native fish habitat. The expansion of gobies has the potential to affect trophic interactions and transfer contaminants throughout the food chain. A source of contaminant to many fish species in the Great Lakes are Endocrine Disrupting Chemicals (EDCs), specifically xenoestrogens. Estrogen exposure causes multiple abnormalities, which have been visible in many fish species. In order to assess estrogen exposure, vitellogenin has been used as a biomarker and actin has been used as an internal standard for normalization. Vitellogenin is an egg precursor protein produced normally in female fish liver during egg production, and facilitates in energy storage for larval development. Vitellogenin is significantly induced while interacting with estrogen or estrogenic EDCs. Therefore, when male species start expressing vitellogenin, it is an indication of estrogen exposure. Cloned and sequenced vitellogenin, actin, 18SrRNA and rpl8 genes are used for primer design to develop a biomarker assay for estrogen exposure in round gobies. Estrogen exposure studies done in the past on other male fish species had shown induction of vitellogenin. Consequently, it is speculated that the male round goby fish will also induce vitellogenin when exposed to estrogen compounds. For the purpose of measuring accurate vitellogenin induction, round goby liver samples are used in quantitative PCR to evaluate vitellogenin at various estrogen concentrations. QPCR requires standardizing reactions for each gene with efficiency results ranging from 90 – 105%. Reactions are run in two different dilutions, 1:4 and 1:5, in duplicates. The standardization efficiencies and curves have not yet been optimal with fluctuating efficiency values. After the biomarker assay is standardized, an estrogen exposure study can be done on male round gobies and determine their Lowest Observed Effect Concentration.

Presenting Author: Farhana Alam

Alexiu, Michelle, Irena Creed and Jane Bowles

Biology, University of Western Ontario

Biodiversity and production of a tallgrass prairie along a 1000 year chronosequence.

The Quest for Understanding Ecosystem Services of the Tallgrass Prairies (QUEST) initiative is a collaborative research project of the University of Western Ontario, Tallgrass Ontario, and The Heritage Center of Walpole Island First Nation. The QUEST project is assessing the ability of tallgrass prairies to sequester carbon by investigating a series of prairies following a 1000 year chronosequence and a moisture gradient. The chronosequence contains an agricultural field, old field, regenerating prairie and mature prairie, while the moisture gradient consists of mature prairie in dry, mesic, and wet environments. This thesis examines the trends in production and biodiversity along the same gradients. Production was measured as above-ground and below-ground biomass, while biodiversity was examined through plant species richness and composition. Total production did not change along the chronosequence, but the proportion of below-ground biomass was highest in older sites. Plant species richness was highest in the intermediate aged site, but floristic quality measured by the number of prairie indicators and highly conservative species was highest in the mature prairie.

Presenting Author: Michelle Alexiu

Allen, Andrea

Integrative Biology, University of Guelph

How do zooplankton species perceive the dispersal limitations of a landscape?

Metacommunity analyses are valuable undertakings when trying to understand the complex underpinnings of community structure for they can allow researchers to examine different scales of spatial organization and to include environmental variables. Natural microcosms, such as those found in the rock bluffs of Churchill Manitoba, provide an ideal natural system in which to measure the spatial dispersal patterns of zooplankton metacommunities. Samples were taken from 81 rock bluff pools with environmental variables measured and zooplankton community composition analyzed. Past research had emphasized that both local variables and spatial organization affect the structure of zooplankton communities . However, one of the limitations of these past studies is the crude method used to incorporate space as a proxy for dispersal limitation in the multivariate analyses. The goal of this study was to search for the optimal method of modelling spatial relationships between zooplankton communities. We compared three classes of spatial models, each incorporate an increasing amount of biological information: polynomial response surfaces, principal coordinates of neighbouring matrices which decompose space into broad, medium and fine scale spatial patterns, and isolation methods that incorporate network theory and species presences. We found strong evidence for the effectiveness of both fine scale spatial patterns and some very specific isolation methods. These results suggest that dispersal in zooplankton is not limiting at small spatial scales (<300 m), and will provide the basis for future research on the importance of metacommunity dynamics, not only in zooplankton communities, but also for other systems with passive dispersers.

Presenting Author: Andrea Allen

Armstrong, Zachary, Norm Huner and Susanne Kohalmi

Biology, University of Western Ontario

Subcellular localization of Arabidopsis thaliana ADT-GFP fusion proteins.

Arogenate dehydratases (ADTs) catalyze the final step in phenylalanine biosynthesis through dehydration/decarboxylation of arogenate to phenylalanine. Six ADTs have been identified in Arabidopsis thaliana. In silico analyses predict that the gene sequences code for proteins which include a putative N-terminal transit peptide to direct the ADTs to specific organelles. At least three of the peptides are predicted to direct localization of the protein to the chloroplast. Previous work has shown that the presence of the transit peptide is required for localization to specific subcellular organelles. In addition, a unique localization pattern in tobacco suggests that the Arabidopsis transit peptide is sufficient to target ADT1 to the chloroplast but lacks species specific components required for the successful transport through the chloroplast membrane. To investigate ADT targeting in more detail, ADT1, ADT3, and ADT5 were cloned 5' to a green fluorescent protein (GFP) tag to either allow for the expression of the full length (FL) fusion protein or a mature (M) version lacking the N-terminal putative transit peptide. The fusion constructs were sub-cloned into an Agrobacterium vector to be expressed transiently in Arabidopsis thaliana and Nicotiana tabacum. Expression patterns of GFP can be visualized to localize the fusion proteins in vivo.

Presenting Author: Zachary Armstrong

Ashick-Stinson, Chloe

Biology, Laurentian University

The effect of chronic exposure to particulate air pollution on the leukocyte community of the NOD mouse lung

In today's modern society, air pollution, and increasingly, particulate air pollution has become a mounting concern for many sectors of society, and is no longer simply a problem of those who work in high-pollution environments. Increasingly, research has supported that particulate air pollution (specifically the fine and ultra-fine) is an important factor to consider in many diseases, and is no longer limited to that of respiratory disorders. Concern has been raised about the effects of particulates on those members of society with long-term chronic disorders, including diabetes, and environments of high air pollution have been linked to the development and acceleration of this disease in children. This research has investigated the leukocyte community of a model diabetic organism, the NOD mouse, as an indicator of the effects of inhaled particulate on the immune system of an organism with the long-term chronic disease of diabetes. It has been found that particulate air pollution tended to decrease the levels of macrophages and neutrophils, two important cell populations for inflammation, in the NOD mouse when compared to the control mouse, but the interaction of diabetes and air pollution seemed to increase slightly the levels of lymphocytes and eosinophils in the lung community,. Extensive macrophage activation was also seen in the pollution-exposed NOD mice. In summary, this work contributes to a greater body of evidence that diabetic populations are particularly susceptible to the effects of chronic air pollution and may have reduced capabilities of combating subsequent infections due to their altered leukocyte environment.

Presenting Author: Chloe Ashick-Stinson

Baldwin, Sarah

Integrative Biology, University of Guelph

The effect of genome duplication on reproductively isolating characteristics in Chamerion angustifolium.

In the northern Boreal Forest in the Rocky Mountains, tetraploid and diploid Chamerion angustifolium cytotypes are found at different latitudes, but a hybrid zone is located where the distributions overlap. Many notable observations have been made with regards to differences in morphological and physiological differences that may decrease the flux of gene flow between these groups. Most notably, tetraploid pollen sires a disproportionate number of offspring in controlled mixed pollinations with diploids pollen on both cytotypes. In addition, flowering time asynchronies and differences in flower size, plant size, and pollen size have been suggested as prezygotically isolating barriers. Genome duplication may result in traits such as plant and flower size that prevent gene flow from diploids to the original tetraploid population. However, many researchers are arguing that many genetic changes occur after genome duplication making this process slower and more complicated than commonly though. I hypothesize that characteristics involved in reproductive isolation are not present immediately after genome duplication. Pollen number, size, and aperture number; flowering time and size; and total plant growth rate have been compared between diploids and tetraploids from established wild populations to neotetraploids which have just undergone genome duplication. If there is no significant difference between these traits in neotetraploids and the extant tetraploids, the hypothesis can be rejected.

Presenting Author: Sarah Baldwin

Balogun, Ibironke (Elizabeth), Karla Williams and Marc Coppolino

Molecular and Cellular Biology, University of Guelph

Investigating the role of syntaxin 4 in MMP trafficking.

Matrix metalloprotinases (MMPs) are zinc dependent proteolytic enzymes which are known to function to degrade several components of the extra cellular matrix (ECM) such as collagen. These enzymes are involved in homeostatic and developmental processes such as embryogenesis, tissue remodeling and wound. MMPs are known to enhance the neovascularization of tumor cells during invasion and metastasis due to their ability to remodel the ECM. Utilizing green fluorescent protein (GFP) tagged MMP-2 and MMP-9 constructs will allow for the observation of intracellular localization during trafficking and secretory events. Both MMPS of interest are known to be trafficked to the site of ECM degradation. SNAREs (soluble N-ethylmaleimide-sensitive factor attachment receptor) are family of proteins shown to play a major role in the trafficking of MMPs. SNAREs are involved in almost all intracellular trafficking. Syntaxin 4, a t-SNARE (target SNARE) is known to play a role in the trafficking of membrane type 1- MMP (MT1-MMP), which in turn aids in the activation of MMP2. The inhibition of either of the SNAREs appears to impair the MMP trafficking. There is evidence that supports the trafficking of MMPs is an important factor in the regulation of ECM remodeling by tumor cells, as they are able to degrade the ECM. Thus, the study of the specific pathways by which the MMPs can be inhibited can be of great importance for future research.

Presenting Author: Ibironke (Elizabeth) Balogun

Barnard, Jacqueline

Molecular and Cellular Biology, University of Guelph

HSE Promoter Silencing as a Mechanism to Sensitize Tumor Cells to Hyperthermia.

Heat shock proteins function as molecular chaperones, and to protect the cell from apoptosis during times of cellular stress. These proteins are over expressed in cancer cells, which are able to survive after cellular stress such as hyperthermia. Heat shock factor 1, a transcription factor, initiates the transcription of these proteins by binding to a heat shock element, HSE, on DNA. HeLa-PC-rtTA, PErtTA, and MCF-7 cell lines were transiently transfected with plasmids containing modified HSF1 domains that silence HSE promoter function, and analyzed for HSF1-KRAB and Hsp70 protein levels following heat shock. RT-PCR and Western blot analysis were performed to measure these levels.

Presenting Author: Jacqueline Barnard

Baynton, Scott and Andrew Bendall

Molecular and Cellular Biology, University of Guelph

Transcriptional analysis of bone-specific promoters.

The transcription factor promyelocytic leukemia zinc finger was investigated in regards to its effect on the transcriptional activity of the homeodomain proteins Dlx5 and Dlx6 on the promoter regions for the bone-specific proteins osteocalcin and bone sialoprotein using luciferase reporter constructs within HEK 293 and HEK 293T cell lines.

Presenting Author: Scott Baynton

Berkers, Tanya and Marc-André Lachance

Biology, University of Western Ontario

Sequence-based yeast identification.

Identification of yeast species is best accomplished by sequencing a ribosomal RNA gene and comparing the sequence to those in a database. Sequences for the D1/D2 domain of the large subunit rDNA of all known yeast species are available on the online NCBI database and have the potential to fulfil that requirement. Unfortunately, the database contains many mislabeled, redundant, or poor quality sequences that render identification problematic, especially for inexperienced users. The aim of this project was to solve these problems. Ideally, an identification database should contain only accurate sequences that represent authentic strains of each known species. Several potential approaches were investigated for ease of setup, quality of results, and usability. The simplest and most elegant solution was to create an inventory of authentic sequences to be used as a filter for the existing online BLAST program. Regrettably, that approach was not acceptable to NCBI staff. Other options involved the use of commercial or publicly available search engines such as those provided by NCBI or by J. Mullins at the University of Washington (Viroblast). A standalone BLAST search engine was successfully tested with a local database of authentic sequences for the type strains of all known Candida species. The next steps will be expansion of the database to all other yeast genera and implementation on a publicly accessible server.

Presenting Author: Tanya Berkers

Bertrand, Claudia

Integrative Biology, University of Guelph

DNA barcoding for land plants: Testing COI, Matk and trnH/psbA for a universal multi-marker.

DNA barcoding utilizes short species-specific DNA fragments for biodiversity analysis. A 650bp fragment of mitochondrial gene Cytochrome c oxidase 1 (COI) has been successful tested as a DNA barcode for animal species. As for plants, COI has been dismissed as a suitable barcode due to slower rates of evolution in plants but convincing data has yet to be collected. The disagreement on a universal marker has prevented the use of barcoding on plants. Many candidate genes have been proposed with recent reports showing matK, a chloroplast gene, as an appropriate barcode. However, biases in sampling efforts have focused primer design to flowering plants and, as a result, gymnosperms and lower plants have been dismissed in these analyses. This study utilizes a wide range of Costa Rican land plant specimens, including specimens outside of flowering plants, to evaluate the effectiveness of three potential markers: COI, trnH/psbA and matK. We decided to test COI as a potential marker particularly for its universality with animal barcoding. Our analysis using neighbor joining trees showed matK and COI to both discriminate morphological species of flowering plants but the resolution in several groups is limited to genus level. The non-coding trnH/psbA region has shown to be universally amplifiable but its analysis is difficult in large taxonomic groups (i.e. above genus-level) due to difficulties in alignments. However, we propose its use to complement COI or matK, to increase species-level resolution. Our preliminary results suggest that COI can be reallocated as a candidate barcode but further studies on a multi-marker barcode system using matK or COI combined with trnH/psbA be applied to barcoding plants. Our current research is focused on developing wide range PCR primers for both matK and COI to facilitate their amplification in all land plant lineages.

Presenting Author: Claudia Bertrand

Bourgaize, Shannon, Tom Cieslak and Brian Timmons

Biology, McMaster University: Kinesiology, University of Toronto: Pediatrics, McMaster University

An in vitro model of skeletal muscle responses to exercise during human growth

Presenting Author: Shannon Bourgaize

Breivik, Leah

Biology , McMaster University

Insular Malay civet (Viverra tangalunga) population dynamics: the effects of reduced interspecific competition.

The Malay civet (Viverra tangalunga) inhabits old world rainforests located throughout the Malesian subkingdom of the Paleotropics. Due to their nocturnality and the difficult terrain of their environments, little research has been conducted on these carnivores. Their range encompasses many of the islands that make up the Malay archipelago, including Buton Island in Indonesia. This area is unique due to deep ocean trenches (Wallace's line) that separate Buton Island and neighbouring Borneo 'mainland' into two different biogeographical zones, Wallacea and Sundaland, respectively. This region is rich in biodiversity and threatened due to human activity and increasing population pressure. Wallacea is a recognized biodiversity hot spot and Operation Wallacea has been dedicated to research in this area since 1996. With the aid of their research stations, this study was conducted to examine Buton Island's Malay civet population and investigate the 'island syndrome', which predicts that the island population will have higher density, reduced home-range sizes, and increased home-range overlap, in comparison to their mainland counterparts on Borneo. On Buton Island, Malay civet population density (minimum density = 2.21; ETA density = 7.01; Mbh density = 3.15 animals/km^2) was found to be higher than that on the mainland counterpart, Borneo (minimum density = 2.17, ETA density = 6.25; Mbh = N/A animals/km^2). Home-range size was found to be significantly smaller on Buton Island (43.3±11.8ha) than on Borneo (88.5±8.7ha) (t=3.09, P=0.006). With respect to overlap, Malay civets demonstrated extensive overlap in both locations and cleared corridors (i.e. trails and roads) were largely the sites of this overlap. Therefore, home-range overlap was not significantly different between the sites; however inter-gender overlap was significantly higher on Buton (t=3.42, P=0.006).

Presenting Author: Leah Breivik

Brown, Graham

Biology, McMaster University

Embryonic expression of unique and homologous transcription factors in c. elegans suggests a conserved evolutionary history.

Transcription factors play an integral role in the regulation of metazoan development and specification of cells and tissues. The genome of Caenorhabditis elegans encodes 215 transcription factors (TF), which lack orthologs in two closely related Caenorhabditis species C. briggsae and C. remanei (Gupta, 2007). Since TFs are highly conserved and are integral in development, the cause for the absence of these TFs in C. elegans' sister species should be investigated.Using the resources of a BLAST analysis and the Wormbase, InParanoid and Treefam databases, we have classified these C. elegans TFs into three groups; those with orthologs in C. briggsae, those with no found orthologs in C. briggsae, and those which may or may not have orthologs in C. briggsae based on conflicting information from the various databases. 20% of the TFs had strong evidence supporting a C. briggsae ortholog, 40% had conflicting evidence supporting a C. briggsae ortholog, and 40% showed no known C. briggsae ortholog. The TF group with C. briggsae orthologs possessed three times as many RNA mutant phenotypes as the TF group with no C. briggsae orthologs, suggesting that the conserved TFs played a more significant role in C. elegans development. All three TF groups showed a trend towards early expression patterns, suggesting a conserved evolutionary history for TFs in C. elegans.

Presenting Author: Graham Brown

Bufalino, Mary Rose and Lucy Lee

Biology, Wilfrid Laurier University

In vitro growth comparisons between neural cell lines derived from goldfish and crayfish.

Neural cell lines have been extremely useful for elucidating mechanisms of neural cell function, regeneration and disease for mammalian species. Despite the widely known regenerative capability of neural tissues in many aquatic organisms, few cell lines have been derived from neural tissues of aquatic species. In this study, we report on a newly established neural cell line from goldfish cerebellum (GFB3C) and compare growth characteristics in response to various parameters (temperature, osmolality, nutritional supplements, growth/differentiation factors and response to common aquatic contaminants such as metals) with an aquatic invertebrate cell line (OLGA-PH-J/92) derived from the cerebral ganglia of crayfish. Both goldfish and crayfish have been used as model organisms to study neuronal function and survival in polluted waters. The OLGA cell line grew best at 27*C whereas GFB3C grew well at 22-30*C. For both cell lines, best growth was observed in Leibovitz's L-15 medium supplemented with 10% Fetal Bovine Serum, compared to other common mammalian or insect culture medias. OLGA grew best at lower osmolalities than GFB3C cells, which was consistent with their invertebrate origins. Addition of glucose (L-15 contains galactose), or growth factors did not affect growth of either cell line. Formation of neurospheres, a characteristic of neural stem cells was also investigated. OLGA cells did not form neurospheres, whereas GFB3C cells formed spherical aggregates quite readily. These cells were tested for differentiation capabilities using retinoic acid or adhesion to poly-L-lysine as differentiation agents. Neuronal like cellular processes could be induced when GFB3C neurospheres were plated onto poly-L-lysine coated plates. Finally, in terms of toxicity, both cell lines were highly tolerant to metal exposure, which correlates with their in vivo behavior. Both could be useful for understanding mechanisms of neural growth and differentiation in vertebrate and invertebrate species as well as in toxicity response and resistance in aquatic animals.

Presenting Author: Mary Rose Bufalino

Bundalovic-Torma, Cedoljub

Biochemistry Origins Institute, McMaster University

A model of horizontal gene transfer during the early evolution of cells.

The origin of modern cells is a question that remains at large. Rooting the tree of life does not reveal anything more about the last common ancestor(LCA) other than it had already possessed the basic biochemical pathways common to extant life and thus was anything but a "primitive" state. Furthermore, deviations of the phylogenies of many proteins with that of the rRNA tree have led many researchers to suggest that the transfer of many genes between species, or Horizontal Gene Transfer(HGT), was the main mechanism which accelerated the evolution of early cells prior to the LCA. Furthermore HGT is believed to have been so rampant during this early stage of cellular evolution that the wholeconcept of darwinian inheritance(vertical transmission of genes from parent to daughter progeny) did not exist and the LCA was therefore an entire population of entities which were rapidly and readily exchanging their collective gene pool. There are some important questions to ask. Wouldn't the transfer of non functioning genes be more detrimental to cellular fitness given high HGT rates and what then causes HGT rates to diminish and cellular life to take on increasingly vertical genetic transmission? Using a computer model of an early cell progenote we hope to shed some light on these questions and more on the process of cellular complexity.

Presenting Author: Cedoljub Bundalovic-Torma

Bustos, Claudia, Lynda Corkum, Kathy Slater and Daniel Mennill

Biological Science, University of Windsor and Operation Wallacea, UK

Acoustic characteristics of the vocalizations of mantled howler monkeys (Alouatta palliata) in the fragmented low-land forests of Honduras.

A central question in the study of primate behaviour concerns the extent to which primate vocalizations are acoustically adapted for specific communication functions. No current studies have analyzed the acoustic characteristics of Mantled Howler Monkey vocalizations living in fragmented forest populations. The goal of this study is to provide an in-depth description of the fine structure of the vocalizations of Mantled Howler Monkeys (Alouatta palliata). Close-range (<20 m) recordings were collected from seven different mantled howler monkey troops in the low-land forests of Cofradia, Honduras. We analyzed the 10 best representations for each vocalization for each group. Barking and howling were the most frequently recorded vocalizations, although we identified 10 types of vocalizations in total. The loud, namesake howl vocalization had the highest frequency characteristics (average frequency of maximum amplitude 423 Hz) which may reflect an adaptation to maximize transmission of this territorial signal. Soft barks, in contrast, showed the lowest frequency characteristics (average frequency of maximum amplitude 286 Hz) which suggests that they may play an intra-group communication function. Examination of form and function in vocal communication will reveal links between structure of vocalizations and receiver responses. Such links will add novel insight on the adaptive significance of the various calls identified in mantled howler monkey vocal repertoire.

Presenting Author: Claudia Bustos

Carnevale, Jasmyne, Dorothy Myers, Azadeh Golipour and Lisa Porter

Biological Sciences, University of Windsor and Medical Genetics, University of Toronto

The novel cell cycle regulator, Spy1 regulates lactogenesis.

Epidemiological studies investigating the affect of parity on the risk of breast cancer development in women suggest that full term pregnancy and lactation are associated with a decreased occurrence of mammary carcinogenesis (5,6). Pregnancy and lactation are stages of post-embryonic development by which the mammary gland undergoes tightly regulated and specific temporal patterns of cell proliferation and differentiation respectively. It is accepted that disruption in these highly regulated processes is intimately linked to carcinogenesis (2,3,4). This study aims to determine the role of Spy1, a unique cell cycle activator and atypical cyclin, in the development and morphogenesis of the mammary gland undergoing lactogenesis. Spy1 is expressed at high levels in the proliferating gland and is downregulated naturally during lactogenesis, importantly Spy1 levels are upregulated in invasive ductal carcinoma of the breast (1). To directly address the role of Spy1 in mammary gland morphogenesis in vivo a deoxycycline-inducible syngenic mouse model was initiated to overexpress Spy1 during the postnatal development of the mammary gland throughout puberty, pregnancy and lactation. This is the first in vivo study to demonstrate that aberrant Spy1 expression in the mammary gland during development stimulates premature lactogenesis leading to abnormal morphogenesis. Importantly, this work demonstrates that Spy1 may represent a novel target in the diagnosis and/or treatment of breast cancer.1. Zucchi, I.et. al (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 18147–18152; 2. Evan, G. I., and Vousden, K. H. (2001) Nature 411, 342–348; 3. Lowe, S. W., Cepero, E., and Evan, G. (2004) Nature 432, 307–315; 4. Fridman, J. S., and Lowe, S. W. (2003) Oncogene 22, 9030–9040; 5. Largent J.A., Ziogas A., and Anton-Culver H. (2005) Breast Cancer Res 7, 541-54; 6. Furberg H et. al. (1999) Int. J. Epidemiol 28, 396-402

Presenting Author: Jasmyne Carnevale

Castillo, Sarrah

Biology, Laurentian University

Emerging infectious diseases (EIDs) in leopard frogs (Rana pipiens) in relation to a watershed system.

For many, watersheds are very unfamiliar but they are important areas for aquatic organisms. Watersheds support not only many terrestrial mammals but also many aquatic and semi-aquatic animals such as adult and tadpole frogs. Unfortunately there have been only a few studies that have focused on how changes in watersheds affect anuran populations. In the last decade, global amphibian populations have declined drastically and have become a major issue of the late 20th century. Many reasons have been proposed for these declines in numbers, but emerging infectious diseases (EIDs) have been thought to be among the major causes. EIDs are disease that have increased in geographical range, moved into a new host or have been recently discovered or newly evolved. Two EIDs that affect amphibians are chytrid fungus and Ranavirus. Throughout the summer of 2007 toe clips were taken from a total of 166 frogs found in 7 sites located from the top to the bottom of a watershed in Sudbury, ON. Molecular analysis was performed on the toe clips in order detect the presence or absence of Ranavirus. Results of this study showed that Ranavirus was present in 5 individuals found at the lowest site of the watershed, supporting my hypothesis that infection rates increase from the top to the bottom of a watershed.

Presenting Author: Sarrah Castillo

Chan, Olivia

Pathology and Molecular Medicine, McMaster University

Alterations in tight junction proteins of genital and intestinal epithelial cells by HIV-1 and HIV-1 proteins.

At the end of 2006, UNAIDS estimated that there were approximately 38.5 million people worldwide living with a human immunodeficiency virus (HIV) infection. Studies from patients suffering from HIV-1 related encephalitis indicate that HIV-1 can alter permeability of endothelial lining, while intestinal mucosa studies indicate a reduction of integrity of epithelial lining, both suggesting viral entry by disruption of tight junction (TJ) proteins. This study examined whether such findings can explain HIV-1 entry into the genital and intestinal tract by similar TJ disruption. Individual viral proteins gp120 and Tat were also investigated for their roles in mediating TJ disturbances. Monolayers of primary genital epithelial cells and T84 cell line (human intestinal epithelial) were infected with HIV-1, and transepithelial electrical resistance (TER) were measured prior and post HIV-1 infection. It was demonstrated that an HIV-1 infection induced a 43.2% and 23.1% TER decrease in genital and intestinal cells, respectively. Immunofluorescent staining for TJ proteins claudin-1, -2, -4, ZO-1 and occludin was also performed. ZO-1 staining following infection in endometrial and T84 cells resulted in the greatest reduction in expression among other TJ proteins investigated. This was demonstrated by prominent ZO-1 fragmentation and the lack of clear cell-cell border outlining. This is indicative of increased cellular permeability and TJ degradation following HIV-1 infection. Gp120 and Tat were individually added to primary cells, and TER readings indicate a minor reduction (18.3%) following treatment. Confocal images of ZO-1 expression following protein exposure resulted in slight reductions of TJ staining compared to whole-viral infection. Taken together, current findings provide valuable insight into HIV-1 pathogenesis by illustrating the pathway underlying HIV-1 entry and penetration by compromising epithelial TJ protein barrier function. The findings reported here have important implications for other co-infections that may take advantage of this HIV-1 induced permeability.

Presenting Author: Olivia Chan

Charlton, Meaghan

Molecular and Cellular Biology , University of Guelph

Gene expression in ram spermatogonial stem cells during postnatal development.

N-Cadherin and E-cadherin are examples of junctions involved in the orientation and development of germ cells and sertoli cells in the testis. Due to the necessity of forming a proper interaction between these cells in order to be successful during spermatogenesis, it is the objective to determine the expression levels of N-cadherin and E-cadherin in ram sheep testis. Primers were designed first in order to run PCR and later quantify the mRNA. Sheep sequences of these two molecules are unknown therefore by examining the conservation across three close and well studied species, mouse, human, and cow, it would be possible to infer an appropriate sequence and area to pick primers from. This was accomplished using ClustalW , an online multi-sequence alignment tool, for both the nucleotide sequences and amino acid sequences. Perl Primer helped in designing the primers and allowed for certain areas to be chosen for primers. After cross-referencing with exon-intron borders and areas of conservation, primers for N-cadherin and E-cadherin were selected. Many attempts of PCR have been done and results thus far have been inconclusive. Primers made have been either too general or too specific. New primers were recently designed and currently undergoing testing to determine proficiency during the PCR process. Overall results to determine the expression of E cadherin and N cadherin are inconclusive. . Another step to accomplish in later work is to stain sections by following immunohistochemistry protocols which allows for specific antibody staining of these two molecules in order to accomplish the objective in determining the levels of expressing of the N-Cadherin and E-cadherin.

Presenting Author: Meaghan Charlton

Clemow, Scott

Biology, Wilfrid Laurier University

The establishment of two critical techniques to study pea nodulation.

Nodule formation is the result of a mutualistic relationship between the roots of certain plants and appropriate genera of bacteria. Model plants have greatly advanced our knowledge on nodulation over the last decade. This progression has pushed scientists to revisit pea as past nodulation studies did not account for pre-infection events in that species. The long-term objective of this research is to characterize further the role of the plant hormone cytokinin in nodulation. My short-term objectives are twofold: 1) to produce transformed composite plants, i.e. plants with transformed roots but wild type shoot, and make them nodulate. 2) to develop a successful spot-inoculation technique to visualize the first anatomical changes of the roots in response to the bacteria. For the first objective, plants were cut through the stem and inserted into fibrgro© cubes, inoculated with Agrobacterium rhizogenes in open petri plates, placed in trays and covered with plastic domes. The plants were later inoculated with Rhizobium leguminosarum. Because the A. rhizogenes used in this study carries a GUS reporter gene, a GUS assay was performed to determine which roots were transformed and the nodules on those roots were counted. For the second objective, plants were pre-germinated on petri plates for 48 hours, transferred to germination pouches containing water and inoculated with R. Leguminosarum carrying a lacZ reporter. These plants were harvested 5 days after inoculation, exposed to lacZ staining solution and embedded in agar for sectioning. The sections were visualized under a light microscope for signs of infection. Composite plants with nodulated transformed roots were obtained but the transformation efficiencies were low. Spot-inoculation was, however, successful with early infection events caught at the site of infection. These two techniques will be useful in our lab as a transformed pea system would allow the alteration of cytokinin levels via insertion of specific genes while spot-inoculation would allow the visualization of the effects these changes would have on nodulation.

Presenting Author: Scott Clemow

Craig, Ryan, Mohammad Al Sorkhy and Lisa Porter

Biological Sciences, University of Windsor

The Nature of the Spy1/Nedd4 Interaction.

Spy1 is a cyclin-like protein required for progression through the G1/S phase of the cell cycle (Porter et al., 2002). Elevated Spy1 protein levels are attributed to overriding the DNA damage response and enhancing cell proliferation (Zucchi et al., 2004); accordingly aberrant levels of this protein have been implicated in tumorigenesis (Barnes et al., 2003; Gastwirt et al., 2006). Given Spy1's contributions to normal and abnormal growth, elucidating its production and degradation mechanisms is imperative. Recently our lab has demonstrated that Spy1 is degraded in a cell-cycle-dependent manner via the ubiquitin-proteasome system, and identified Nedd4 as the E3 ubiquitin ligase that promotes its degradation (Al Sorkhy et al., submitted). The objective of this study was to identify the important sites within Spy1 which initiates this process as well as to elucidate sites which mediate the binding to Nedd4. Utilizing a panel of truncation mutants of Spy1 we were able to narrow down the region which was necessary for degradation. Analysis of this region identified several putative phosphorylation sites which may target Spy1 for ubiquitination as well as a conserved PPxxxxY motif which may mediate binding to Nedd4. These regions were mutated using site-directed mutagenesis and their functional importance assessed using a series of binding and ubiquitination assays. To date, we have identified three key phosphorylation sites; S22, T33 and T15 are essential for targeting the Spy1 protein for ubiquitination. We have further synthesized non-degradable forms of Spy1 utilizing these sites and we have demonstrated that these do not initiate a cell-cycle arrest but instead result in uncontrolled cell growth. This demonstrates that in opposition to other key cyclin families, misregulation of the Spy1 family does not stimulate cell cycle checkpoints and is instead capable of promoting aberrant cell growth. Given these significant findings, further investigation into the regulation of Spy1 may reveal novel strategies for understanding the etiology and progression of specific growth disorders.

Presenting Author: Ryan Craig

Cruceanu, Cristiana

Molecular and Cellular Biology, University of Guelph

The role of dlx genes in regulating cell proliferation.

The members of the Dlx family of homeobox genes play important roles in developmental pathways in a variety of tissue types such as bone and cartilage. They have highly conserved sequences across vertebrate species and are homologous to the Drosophila gene Distal-less. The genes encode transcription factors that have been implicated in various cell differentiation pathways. Particularly, Dlx5 and Dlx6 seem to be expressed at different levels of the complex process of bone formation. They have been shown to play a central role in regulating the proliferation and differentiation of intermediary cell types such as chondrocytes and osteoblasts. However, it is not yet clear whether these transcription factors inhibit proliferation of differentiating cells or rather activate the actual differentiation processes. Our results show that Dlx5 and Dlx6 inhibit proliferation of chondrocytes, as was seen in ATDC5 cells, a murine model cell line designed for study of chondrocyte differentiation.

Presenting Author: Cristiana Cruceanu

Cudmore, Caitlin

Molecular and Cellular Biology, University of Guelph

Expression and purification of type I antifreeze protein.

Antifreeze proteins (AFP) are found in many cold-climate species. They bind to ice (by an unknown mechanism) modifying and inhibiting its growth, as well as separating the freezing and melting temperature of water (thermal hysteresis activity), thus preventing damage to cells and organisms. Winter flounder Type I AFP is a widely studied 37 amino acid, alanine-rich, a-helical protein. Studies to determine how this protein binds ice are centred on observing the function of mutant proteins to determine the effect of amino acids with different side-chain properties than the wildtype. In this work wildtype AFPI and two mutants with two of four threonines replaced by either two serines or two valines were expressed. However growth, expression and purification were highly variable and did not always occur and therefore a new expression system using pET22b and BL-21 Escherichia coli cells was made. This system will be used to express and purify AFPI and its mutants, and growth in minimal media will be optimized for future experiments using NMR.

Presenting Author: Caitlin Cudmore

Daniell, Shane

Molecular and Cellular Biology, University of Guelph

Molecular characterization of the B-band LPS biosynthesis cluster of Pseudomonas aeruginosa O1.

Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen and is divided into serotypes based upon the long polysaccharide chain, known as the O antigen, which is a constituent of lipopolysaccharide (LPS). LPS is a cell-surface glycolipid virulence factor found in Gram-negative bacteria that includes lipid A, core oligosaccharide and the O antigen. Although a relevant clinical isolate, making up approximately 21% of isolates in a recent study, the molecular genetics of O-antigen biosynthesis remain a mystery in P. aeruginosa O1. The O antigen of this organism contains sugar residues that are found in well-characterized serotypes such as P. aeruginosa O5 and O6; therefore, using what is known about these bacteria, O-antigen biosynthesis can be studied in P. aeruginosa serotype O1. Open reading frames (orfs) in O1 that displayed sequence similarity to genes of known function in O5 and O6 were tested via cross-complementation. Of particular interest are orf14 and orf16 which are putative epimerase/reductase enzymes based on sequence homology to O5 and O6 respectively; in addition, the potential bifunctionality of these enzymes will also be investigated. Preliminary results indicate that these orfs from O1 could not complement knockout strains, perhaps suggesting that the genetically engineered histidine tags were interfering with protein function in vivo. Current work is focused on re-cloning the orfs minus the histidine tags and re-testing their cross-complementation ability.

Presenting Author: Shane Daniell

De Avila, Miguel

Molecular and Cellular Biology, University of Guelph

Effects of deimination of myelin basic protein on the exposure of immunodominant epitopes in multiple sclerosis.

Myelin basic protein (MBP) is an abundant protein component of myelin in the nervous system, and is considered a candidate autoantigen for initiation or preservation of the disease multiple sclerosis (MS). Individuals with MS have a high amount of an MBP variant that has reduced charge due to deimination, namely the charge isoform C8. The most positively charged component, C1, is the most abundant in healthy adults, with a charge of +19. Component C8, with a charge of +13, is not as effective at forming tight lipid complexes around axons, leading to instability of myelin. By adding a spin label to cysteine mutants of both the C8 and the C1 component, topological differences of the protein were studied via electron paramagnetic resonance (EPR). These studies focused on the interaction of a secondary immunodominant epitope that spans residues 141 to 154, under membrane mimetic conditions. Additionally, further biochemical analyses were employed to determine binding affinity of MBP to calcium-bound calmodulin (Ca2+-CaM). Previous studies have shown that another possible function of MBP is to interact with the cytoskeleton of oligodendrocytes by interacting with calmodulin, thus losing its binding affinity for actin (Boggs and Rangaraj, 2000). These binding events can cause dramatic changes in cell shape and morphology in oligodendrocytes. Experiments using gel-shift assays were designed to test for the binding affinity of C1 MBP with Ca2+-CaM.

Presenting Author: Miguel De Avila

De Catanzaro, Rachel and Patricia Chow-Fraser

Biology, McMaster University

The impact of anthropogenic disturbance on aquatic turtle assemblages of Great Lakes coastal marshes.

An emerging issue in conservation biology is the striking decline in many freshwater turtle species in areas with high anthropogenic activity. Agricultural, urban, and recreational development that alter terrestrial and wetland habitat are believed to have caused many regional extirpations, particularly in the Lakes Erie and Ontario. Here, we use data collected from wetlands of Lakes Erie, Ontario, and Huron from 2001 to 2007 (83 wetland-years) to evaluate the impact of watershed alteration and wetland degradation on the occurrence and abundance of aquatic turtles. Data collected from Georgian Bay, a relatively undisturbed portion of Lake Huron, were examined in greater detail to evaluate the impact of low levels of human activity (cottage development) and other wetland habitat features on the abundance of the common musk turtle. Painted turtles, common musk turtles, and common snapping turtles were the most abundant species in our study, while Blanding's turtles and common map turtles were relatively rare and no spotted turtles were found. Painted turtles were encountered disproportionately in degraded wetlands and their abundance was negatively correlated with wetland quality ($P<0.001$), while abundance of common snapping turtles was highest in sites of intermediate quality. Common musk turtles were absent from degraded wetlands and increased in abundance as wetland quality improved. Within Georgian Bay, higher common musk turtle abundances were associated with wetlands with a high dock density, as well as those with a high sediment organic content. Wetlands with a high dock density had elevated levels of nitrogen and phosphorus. While the common musk turtle is intolerant of extensive alteration of habitat, low levels of human activity in a relatively pristine area may benefit this species, possibly by increasing nutrient concentrations that can enhance wetland productivity. However, the impact of cottage development on the occurrence of the spotted turtle and Blanding's turtle remains unclear.

Presenting Author: Rachel De Catanzaro

De Souza, Rebecca

Biology, McMaster University

The identification of IR33, a novel regulator of sister chromatid cohesion and separation in Drosophila melongaster.

The division of genetic material in diploids is of utmost importance during the process of mitosis. In order to safeguard this process, cell cycle regulators have evolved to ensure the appropriate separation of sister chromatids. IR33 is a Drosophila melongaster cell cycle mutant in which nuclei arrest after the 13th nuclear division of embryogenesis due to sister chromatid bridging. Previous research mapped this mutation to the region of the genome encompassed by the larger deficiency Df(2L)TW137 as well as to the smaller deletion within the larger, Df(2L)Exel8038. The research preformed in this study, however, contradicts these results as well as uncovers the presence of escapers within the Df(2L)TW137 and IR33 cross . To date the exact gene affected by this mutation remains unknown, P-element transposition was attempted to potentially create new alleles of the affaced gene with no results.

Presenting Author: Rebecca De Souza

Del Gobbo, Jenna, Jurek Kolasa and Shubha Pandit

Biology, McMaster University

Analysis of habitat heterogeneity as a factor of synchrony in Jamaican rock pool microcosms.

Populations exhibit synchrony when their numbers rise and fall together at several sites over their distribution, that is, correlated population fluctuations over localized or wide-scale geographical areas. Ecologists, especially those concerned with population conservation, have sought to understand the mechanisms driving population synchrony, as populations that fluctuate in synchrony may face a greater risk of extinction. Three of the fundamental mechanisms of synchrony include: dispersal among populations, correlated environmental perturbations (i.e., the Moran effect), and predation. Additionally, it has been suggested that habitat heterogeneity may be a factor of synchrony. This study addresses the effects of habitat heterogeneity and distance on synchrony within a model system of 49 Jamaican rock pools inhabited by 70 invertebrate species. A saline gradient was used as quantitative means of reflecting landscape heterogeneity as experienced by individual species. Specialist and generalist invertebrate species are distributed across the salinity gradient narrowly and broadly, respectively. It is likely that they exhibit different dynamics. We hypothesized that specialist (low heterogeneity) species will exhibit high synchrony whereas generalist (high heterogeneity species) will exhibit low synchrony. Furthermore, we hypothesized that with increasing distance, synchrony should decrease. The Pearson cross-correlation function was used as a measure for synchrony. Species tolerance was determined by means of niche breadth values from HyperNiche software, maximum/minimum salinity tolerance ranges, and standard deviation of salinity tolerance. PC-ORD software was used to determine the effect of distance between pools on synchrony. We found that increasing heterogeneity was associated with a decrease in synchrony. Though no trend emerged for all species collectively between distance and synchrony, individual species often/occasionally responded to increasing distance with synchrony decreased. Further data analysis where the effect of distance is eliminated through ANCOVA will follow.

Presenting Author: Jenna Del Gobbo

Delaney, Kristen

Biology, University of Western Ontario

Potential for changes in CO2 efflux from organic deposits in a temperate swamp under changing climatic conditions.

Understanding the quality of organic carbon stored in peat lands is critically important in determining the implications of future climatic changes on the efflux of carbon dioxide (CO2). Of particular interest is whether or not the quality of carbon is uniform with depth. Peat lands are known to store large amounts of carbon and act as carbon sinks. However predicted climatic changes could convert peat lands from sinks to sources by exposing the normally waterlogged peat. This study explores the implications that an increase in severity of summer drought may have on the CO2 efflux from a temperate peat land in the Turkey Lakes Watershed. It was hypothesized that the quality of carbon is not uniform, and that the potential for CO2 production is greatest in the upper layers of peat. Peat cores were taken in December 2007 from the Turkey Lakes Watershed located in the Algoma Highlands of Central Ontario. Cores were divided up into 10 cm increments from 0-80 cm below the surface. The peat samples were incubated at a constant temperature for 26 days and CO2 production was monitored daily. The 0-5 and 6-10 cm samples produced the most CO2 over the course of the incubation followed by the 11-20 and 21-30 cm samples. As depth increased the total CO2 production decreased, in other words the deeper the peat the less CO2 was produced. This study supports the hypothesis that the quality of carbon is not uniform. Production of CO2 was greatest in the upper layers of peat, indicating that the quality of carbon is best at the surface. The results also lend support to the idea that an increase in summer drought will not have a profound impact on CO2 efflux from temperate peat lands.

Presenting Author: Kristen Delaney

Deroo, Scott and Vojislava Grbic

Biology, University of Western Ontario

Characterization of the HUA2-RPR domain.

HUA2, a plant specific gene has been shown to regulate FLOWERING LOCUS C (FLC), a floral repressor, and AGAMOUS (AG), a regulator of carpel and stamen development. Specifically, our lab has shown that HUA2 affects the splicing and 3' pre-mRNA processing of AG. Yeast two-hybrid screens using HUA2 as bait identified UBP1, RBP45 and AtPrp40 as interactors. These proteins are RNA splicing factors that function in eukaryotic pre-mRNA processing, suggesting that HUA2 is involved in the splicing of primary transcripts. HUA2 consists of four evolutionarily conserved protein domains previously characterized in other eukaryotic systems. The HUA2 protein is comprised of a PWWP domain found in proteins associated with chromatin modification and hypothesized to bind DNA non-specifically. In addition, HUA2 has a putative nuclear localization domain that directs proteins to the nucleus, and a protein-protein interaction through the proline rich domain. Finally, HUA2 contains an RPR domain, proposed to interact with the carboxy terminus domain (CTD) of RNA polymerase II. The CTD of RNA Polymerase II plays a crucial role in gene transcription. It recruits various protein complexes required for transcription and pre-mRNA processing. Since HUA2 contains a putative nuclear localization domain, and an RPR domain, we hypothesize that HUA2 interacts with the CTD of RNA polymerase II through the conserved RPR domain. This interaction may allow coupling of synthesis and splicing of the primary transcript. Using a yeast-two hybrid system, we analyzed the possible interaction between the RPR domain of HUA2 and the CTD of RNA polymerase II.

Presenting Author: Scott Deroo

Dhiyebi, Hadi and Michael Wilkie

Biology, Wilfrid Laurier University

Why does ethanol protect against ammonia toxicity in rainbow trout but not the goldfish?

Ammonia toxicity is thought to involve overactivation of the N-Methyl-D-Aspartate (NMDA) receptor, which plays a role in learning, memory and development in the nervous system. Our goal was to determine the NMDA receptor's role in ammonia toxicity and tolerance in the ammonia-tolerant goldfish and the ammonia-sensitive trout. We therefore attempted to block the NMDA receptor with ethanol, which is known inhibit the NMDA receptor in mammals and protects against ammonia toxicity. Following intraperitoneal injection of ethanol (0, 1, 2, 4, 8 and 16 mmolkg-1 body weight) fish were subjected to high external ammonia (HEA) (goldfish= 9.5mmolL-1; rainbow trout=2.0mmolL-1) for 2 days. In trout, an ethanol dose of 4 mmolkg-1 body weight increased survival by 78%, but had no effect in goldfish. Thus, the overactivation of the NMDA receptor may explain ammonia toxicity in trout, but not in the goldfish. We conclude that a lower sensitivity to ammonia-induced NMDA receptor overactivation explains the unusually high ammonia tolerance of the goldfish.

Presenting Author: Hadi Dhiyebi

Dibal, Comfort, Janet Yee and Paul Frost

Biology and Chemistry, Trent University and Biology, Trent University

The influence of food quality on the susceptibility of the crustacean Daphnia magna to infection by the bacterium Pasteuria ramosa.

Host nutrition can strongly affect the success of disease pathogens either by affecting the resources available to the pathogen or by altering host defensive abilities. Poor host nutrition could either increase or decrease the growth and proliferation of a pathogen. As such, the nutritional modification of host-parasite interactions could allow for more successful control measures of pathogen growth and reproduction. Although many studies have characterized host-pathogen interactions, few have characterized the influence of elemental nutrition on the susceptibility of a host to pathogenic infections. In this experiment, I studied the influence of elemental food quality on the susceptibility of the crustacean, Daphnia magna, to infection by the bacterium, Pasteuria ramosa. Specifically, I varied the carbon to phosphorus (C:P) ratio in the algal diet of Daphnia, who were challenged with a fixed dose of Pasteuria ramosa. Quantitative polymerase chain reaction (qPCR) was then used to determine the amount of bacterial DNA in Daphnia magna after seven days. DNA extraction results from Daphnia showed a significant decrease in DNA content in poorly fed Daphnia (C:P 600 and 900) than better fed Daphnia (C:P 100 and 300), which reflects the smaller mass of these animals. Furthermore, qPCR results showed differences in the infection rates of Daphnia that were eating different phosphorus food, which supports my hypothesis that nutrition affects the susceptibility of Daphnia to infection by Pasteuria ramosa. This study has implications in understanding the role that nutrition plays in infection and the mechanisms by which infectious bacteria colonize Daphnia, and potentially other host organisms.

Presenting Author: Comfort Dibal

Dodds, Holly , Claire Jardine and Tom Nudds

Integrative Biology/Pathobiology, University of Guelph

Antimicrobial resistance in small mammals on farms and conservation sites.

Antimicrobial resistance is a growing concern in human and veterinary medicine. Bacterial infections that were once easily controlled with antibiotics are now becoming serious health problems as a result of the development of AMR bacterial strains. Understanding how AMR develops and is maintained in bacteria is essential in regaining control of these infections. AMR fecal bacteria have been detected in a variety of wildlife species and it is possible that wild animals may act as reservoirs of AMR infections. There is evidence in humans and domestic animals that AMR can also develop through contact with antimicrobial drugs. The role of antibiotic use on the development of AMR in wild species remains unclear. The purpose of this project is to determine if antimicrobial resistance develops in wildlife as a consequence of exposure to antimicrobials in the environment. Small mammals were trapped in Guelph, ON and surrounding area on five sites in conservation areas where animals will have no known exposure to antibiotics and on five livestock farms where antibiotics are routinely used. Small mammal fecal samples were collected and cultured for Escherichia coli. E. coli isolates were tested for resistance to 14 antimicrobial agents and the prevalence of AMR on conservation sites and livestock farms was compared. Of 45 animals successfully cultured for E. coli, nine tested positive for AMR. Seven of those nine were trapped on farm sites, and two were trapped on conservation areas. Preliminary results indicate a higher prevalence of AMR in farm-trapped animals. This study will add to our understanding of how AMR develops in wildlife and the potential impact of antibiotic use on the environment.

Presenting Author: Holly Dodds

Douglas, Stuart

Molecular and Cellular Biology, University of Guelph

The functional analysis of Luman Recruiting/Repression Factor (LRF) and Luman/LZIP using a knockout C57BL mouse model.

Luman, or LZIP in mice, is a transmembrane protein found within the membrane of the endoplasmic reticulum (ER). The cellular stress response activates an intramembrane proteolysis event that results in the release and nuclear translocation of the cytosolic amino terminal. This permits association with specific gene enhancers and the transcription activation of several gene products functionally involved in the Unfolded Protein Response (UPR). A Yeast Two-Hybrid Binding Assay identified Luman Recruiting/Repression Factor (LRF) as a Luman binding protein. LRF consists of 639 amino acids and contains two leucine zipper domains, amino acids 488-509 and amino acids 521-576, which function to bind Luman/LZIP and target the complex to specific nuclear foci. This interaction resulted in a repression of Luman-directed gene activation; LRF is thus believed to be an important regulator of the cellular stress response. This report first examines the interaction between LZIP and LRF in mice. It is hypothesized that tissues will co-express the proteins because of their functional relationship. Western Blotting provided an expression profile of the specific proteins in a collection of 17 tissue samples from wildtype C57BL mice. A correlation between expression of LZIP and LRF existed. Furthermore, LRF was only detected in tissues expressing a cleaved LZIP, supporting a functional role of LRF in the cellular stress response. Secondly, LRF knockout C57BL mice were assayed as it was hypothesized that the loss of LRF would result in a measurable phenotypic abnormality. Urinalysis was used to identify a specific organ or physiological function affected in null mice. A haematological complete blood count (CBC) was performed as the null mice were hypothesized to produce less lymphocytes. Birth weight and subsequent growth were assayed because it was hypothesized that null mice would be born smaller. Results from the phenotypic screens are pending and will be presented.

Presenting Author: Stuart Douglas

Eastwood, Michael and Jim Karagiannis

Biology, University of Western Ontario

Molecular and genetic analysis of Schizosaccharomyces pombe smh1.

Nonsense-mediated mRNA decay (NMD) is a conserved eukaryotic pathway responsible for the targeted degradation of mRNA transcripts harboring premature translation termination (nonsense) codons. The core NMD machinery is composed of Upf1, Upf2 and Upf3, proteins which have been identified as essential NMD effectors in all eukaryotes examined to date. In higher organisms such as mammals four additional factors in Smg1, Smg5, Smg6 and Smg7 are required for a fully functional pathway. NMD is believed to play a role in wild-type gene regulation, an idea that is consistent with observations made in several organisms that specific physiological defects are associated with an impaired NMD pathway. One particularly interesting example of this occurs in the fission yeast Schizosaccharomyces pombe, where NMD-deficient cells are hypersensitive to oxidative stresses resulting from exposure to reactive oxygen species or other oxidizing agents. Despite being an excellent model for studying cellular growth and stress response pathways, relatively little work has gone into understanding NMD in S. pombe. To expand this knowledge, a previously uncharacterized open reading frame displaying significant sequence homology and similar domain structure to metazoan Smg5 and Smg7 was denoted as smh1 (Smg-homolog 1) and chosen for further investigation as a potential player in the fission yeast NMD pathway. Analysis of a strain expressing Smh1p as a C-terminal GFP fusion has revealed that the smh1 gene product localizes to distinct cytoplasmic speckles potentially representing mRNA processing bodies. This pattern is consistent with human Smg7 localization and suggests that Smh1p may have a role in mRNA decay. A strain has also been developed in which the entire smh1 open reading frame has been deleted and is undergoing further analysis for stress-related phenotypes.

Presenting Author: Michael Eastwood

Edmunds, Karen and Tom Nudds

Integrative Biology, University of Guelph

A case study of consistency in COSEWIC species designations - mammals.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada) generates status reports for Canadian species of animals and plants that are considered to be at risk of either extinction or extirpation. Quantitative criteria within the reports is evaluated to determine the status of the organism (EN-Endangered/TH-Threatened/SC-Special Concern/NAR-Not at Risk or DD-Data Deficient). Status reports are submitted to the Species at Risk Act (SARA) Office that is mandated to protect those species at risk by implementing species recovery strategies and management plans. The integrity and consistency of this initial evaluation and designation process is essential in order to realistically reflect the status of the organism in its natural environment. Inconsistencies or biases could result in an inaccurate designation and potential inefficient program implementation and misdirected expenditure of effort and money. Inconsistencies in species designation status at the COSEWIC level have been detected by a graduate student at the University of Guelph during an examination of 54 species of freshwater fish. Predicted results obtained by rigorous adherence to COSEWIC guidelines by the researcher, were significantly different that those observed in the status reports. This raises questions about the assessment criteria and the possibility of uncertainty and bias within the framework. I have continued to investigate this potential by examining mammals that have been assessed since 2002 and generating predictions of their risk status. I have followed the methodology already used and adhered to a strict protocol to ensure the highest level of consistency and have compared results with observed species designations already in place by COSEWIC. Discrepancies and/or lack of discrepancies will be presented and will either support or not support my hypothesis that the COSEWIC criteria are being applied properly. Significant discrepancies will reflect the potential that the criteria are not being applied properly. The implications are that species at risk in Canada could be in danger of extinction or extirpation if the appropriate protection and conservation programs are not properly in place, or that money and effort is being expended inefficiently and inconsistently.

Presenting Author: Karen Edmunds

Elliott, Amy

Biology, University of Western Ontario

The Effects of freeze-thaw cycle amplitude on release of soluble of soil nitrogen.

Freeze-thaw fluctuations in soil temperature are common in temperate regions (35-65◦) and are of particular ecological interest due to the control they are thought to exert over nutrient cycling and thus overall ecosystem functioning. Current and ongoing climatic changes have the potential to disrupt natural soil freezing dynamics by altering overwinter temperature and snow-cover regimes, potentially changing the frequency, duration, or amplitude of freeze-thaw events. Previous studies of the effects of freeze-thaw cycles have produced mixed results and so the specific implications of changes to overwinter dynamics remain unclear. It is conjectured here that this may be partially explained by the lack of a standardized approach, especially in the often extreme amplitude of temperature cycles and freezing rates imposed. Given the disparity in the literature is hypnotized here that the relationship between temperature, cycle amplitude, and release of nitrogen is nonlinear, such that beyond a threshold amplitude there are consistently large releases of soil nitrogen. Moreover, that across the same temperature range, increasing freezing rate will result in larger release of soluble nitrogen, likely due to increased microbial death, disruption of soil aggregates and fine root cell lysis.

Presenting Author: Amy Elliott

Elliott, Tyler and T. Ryan Gregory

Integrative Biology, University of Guelph

Devising a test of the protective hypothesis of non-coding DNA.

The protective hypothesis of non-coding DNA has existed for over 30 years as a possible effect of some non-protein coding DNA. According to this hypothesis, the majority of an organisms' genome is composed of non-coding DNA because it buffers the effects of mutagens and titrates them away from protein coding introns. In this study I set out to design and evaluate a possible assay to test whether non-coding DNA does in fact provide a protective role in the genome of an organism. I used Drosophila melanogaster and Drosophila simulans as my model organisms and the alkylating agent ethyl methanesulfonate (EMS) to induce mutations. A screen was designed to look at phenotypic and sex ratios changes in the F2 offspring produced from matings between mutated males and virgin females. Difficulties were experienced in adjusting for interspecific differences among the different Drosophilids, and it became apparent that using multiple species was not the best route to test this hypothesis. The screen in itself is an excellent means to test the protective hypothesis as long as comparisons are made between different strains of the same species.

Presenting Author: Tyler Elliott

Ershadi, Mahsa, Erin Bassett , Judith West-Mays and Trevor Williams

Department of Biology , McMaster University: Pathology and Molecular Medicine, McMaster University: Craniofacial Biology and Cellular and Developmental Biology, University of Colorado

Non-cell autonomous roles of AP-2a genes in the RPE phenotype: the characterization of mammalian eye development in AP-2a germ-line knockout.

Although its accessibility and role as an ideal model for studying embryonic tissue induction and differentiation makes the vertebrate eye one of the most extensively studied organs, the specifications of ocular morphogenesis, particularly the molecular mechanisms responsible for retinal determination, remain largely unexplained. Therefore, an improved understanding of ocular development is essential for prospective treatments and prevention of visual impairment. The mammalian eye develops via a complex morphogenetic process that is highly dependent on the strict regulation of proximate tissue interactions for normal domain patterning. Activating protein-2a (AP-2a) is a critical regulator of wild-type vertebrate embryogenesis and has been shown to regulate genes expressed in multiple ocular tissues. Although previous studies have shown that this transcription factor is not expressed in the retinal pigmented epithelium (RPE), AP-2a germ-line knockout (KO) mice, exhibit a mutant phenotype in which the dorsal RPE is replaced by an inverted, duplicated neural retina (NR)-like tissue. Characterization of this abnormality using histological and immunofluorescent techniques will help to determine whether this defect arises from a disruption of early optic vesicle (OV) patterning, or rather if the loss of RPE characteristics occurs after optic cup (OC) formation. In order to further analyze the temporal and spatial alterations in the developing OV/OC that lead to this RPE defect, we have examined AP-2a KOs in detail at multiple embryonic stages (E9.5-E14.5), using RPE- and NR-specific markers, Mitf and Otx2, and Chx10 and Pax6, respectively. Our studies have shown that in the complete absence of AP-2a, the RPE defect begins as early as embryonic day 9.5 (E9.5), at the OV stage. These data suggest that AP-2a has non-cell autonomous effects on OV/OC development, by influencing the expression of genes responsible for specification and/or maintenance of the wild-type RPE phenotype. This investigation will provide insight into some important candidates of genetic ocular diseases.

Presenting Author: Mahsa Ershadi

Fell, Victoria and Andrew Bendall

Molecular and Cellular Biology, University of Guelph

The effect of Dlx proteins on the transcription of the Collagen Type II promoter and enhancer.

Dlx genes are important regulatory transcription factors in development of vertebrates. Dlx5 and Dlx6 specifically are positive regulators of chondrocyte differentiation in endochondral ossification. These genes are known to be coexpressed with the gene Collagen type II, an important marker of a differentiated chondrocyte. A reporter plasmid containing either the promoter or enhancer sequence of the Col2a1gene was co-transfected along with increasing levels of expression plasmids containing the coding regions of Dlx5 or Dlx6 into HEK 293 and ATDC5 cells.

Presenting Author: Victoria Fell

Filion, Helene

Biology, Laurentian University

Habitat selection of re-introduced elk (Cervus elaphus) in the Burwash/French River area.

Ontario has attempted to restore elk (Cervus elaphus) to the Province a number of times during the last century. The latest effort occurred in 1997 and is on-going in four regions: Lake of the Woods, Lake Huron's North Shore, Burwash/French River and Bancroft/Haliburton Highlands. Better understanding how elk select habitat in these areas is critical to managing and re-introducing this species in Ontario. This study statistically analyzed critical elk habitat based on tree-type selection and slope variation using radio-telemetry fixes obtained from 1998 to 2000 for the re-introduced Burwash\French River elk population. Correlation between habitat type and actual fixes were compared for statistical significance against a similar comparison between habitat type and randomly generated fixes. Both winter and summer elk locations showed highest selection for White Pine ($F=89.448$, $P = 0.000$ for summer and $F=84.112$, $P=0.000$ for winter). The presence of ridges appears to be another important component of elk habitat with a majority of elk-selected winter samples located on the southern facing slopes of ridge complexes. Knowing the required components of critical elk habitat will help wildlife managers properly evaluate areas suggested for elk re-introduction and will shed light on their potential carrying capacity.

Presenting Author: Helene Filion

Gagnon, Sara

Molecular and Cellular Biology, University of Guelph

The influence of the V-ATPase 16kDa subunit on regulating cell growth and structure in breast cancer cells grown in three-dimensional cultures.

The V-ATPase 16kDa (16K) subunit is a key component of the V-ATPase transmembrane proton pump. Protons pumps have been found to influence cancer formation through the effects of a hypoxic environment. However, 16K has also been found to interact with B1-integrin, a cellular regulator responsible for cell growth, differentiation, and migration. We investigated 16K expression across three different cell line derivatives, namely, benign, tumor forming and invasive cell lines. B1-intergrin and 16K co-localization was investigated due to recent research that implicates overexpression of B1-intergrin in cancer formation in breast cancer cells. Blocking B1-integrin with antibodies has been found to revert cancerous cell phenotypes to those more closely resembling normal cell phenotypes. Since B1-intergrin and 16K interact, we would expect changes in one to influence the other. Finally, a three dimensional culturing method was utilized to provide an environment for these cells that more closely replicates that found in vivo, in order to better understand the processes that 16K regulates.

Presenting Author: Sara Gagnon

Gardham, Bryan

Molecular and Cellular Biology, University of Guelph

Redox modulation alters activity of starch metabolism enzymes in Glycine max.

Chemical redox modulators were used to treat samples of leaf tissue and photoheterotrophic seed tissue of Glycine max (soybean) to determine if starch synthases (SSs), starch branching enzymes (SBEs), and starch degrading enzymes (SDEs) are regulated in a similar manner to the redox modulation of ADP-glucose pyrophosphorylase (AGPase) [Tiessen et al. 2002]. When subjected to chemical treatments starch synthases were found to be activated when reduced, and inhibited when oxidized in both leaves and seeds. However, a diurnal experiment suggests potential peak hours of artificially reduced starch synthase activity occurring independently of light. This hints at a possible flux-like regulation of metabolites towards starch and back to other metabolism pathways, and/or a secondary control mechanism on the activity of starch synthases. Although redox treatments appear to alter the activity of SBEs, a clear cut pattern of regulation could not be deciphered. A diurnal series of native SDE polyacrylamide gel electrophoresis (PAGE) gels of crude leaf extracts, suggest that amylolytic activity is enhanced during the day when proteins are in a reduced state, and are inhibited when oxidizing conditions prevail. Band positioning of the native gels under different redox treatments suggest a change in protein configuration of SDEs.

Presenting Author: Bryan Gardham

Garrido, Denise

Biology, Laurentian University

Identification of cell surface biomarker associated with the acquisition of drug resistance in MCF-7 breast tumour cells.

CD44+/CD24low/- cell surface biomarker expression within side populations of heterogeneous tumor cell populations has been correlated with “stem cell-like” characteristics that allow them to circumvent traditional forms of cancer therapies, while also re-establishing a heterogeneous tumor following treatments. Using these two antigens as markers correlated to cell resistance, several MCF-7 drug-resistant cell lines (doxorubicin-, epirubicin-, taxol-, and taxotere-resistant cell lines), which were created in our laboratory, were treated with CD44 and CD24 monoclonal fluorescent antibodies. If the cell lines were truly being selected for “stemness” according to the CD44+/CD24low/- studies, an increased level of CD44 antigen (and thus a higher fluorescence level) associated with a decreased expression of CD24 would be expected as resistance to drug concentrations also increases. Following analysis of the fluorescence levels by flow cytometry, a linear contrast and ANOVA showed a significant linear decrease in CD24 expression present in the doxorubicin- and taxol-resistant cell lines but not in the epirubicin- and taxotere-resistant cell lines. Additionally, there was no statistically significant difference in the CD44 expression on any drug-resistant cell lines implying that selection for a richer CD44+ population did not occur with selection for cell lines resistant to higher drug concentrations.

Presenting Author: Denise Garrido

Gauthier, Nicole

Biomedical Biology, Laurentian University

High resolution 2D gel analysis to determine actin isoform specificity of the B1 antibody.

A novel protein was discovered during an initial study to determine if cancer specific isoforms of the DNA replication protein PCNA (Proliferating Cell Nuclear Antigen) occurred in breast and ovarian cancer cells. The protein (called B1) was found as a cross-reacting signal when using a novel antibody (B1 antibody) which was generated against a binding domain in PCNA. The B1 protein was found to be mainly expressed in non-malignant breast and ovarian cells and reduced or absent in malignant cells. Previous studies using immunoprecipitation and analysis by MALDI-TOF indicated B1 to be a cytoplasmic actin. While six isoforms of actin are known, only two are cytoplasmic, ß- and .-actin, which occur in all tissues including breast and ovarian cells. The two cytoplasmic actins differ by 4 residues. As it was not clear if the B1 protein corresponded to ß-or .-actin, Western blots of 2-D gels were performed using, B1, ß-actin, and PAN-actin antibodies. Due to the original expression pattern of B1 in malignant cells, it was hypothesized that the B1 protein was an isoform(s) of ß-actin and would be specific to non-malignant cell lines. By performing high-resolution 2-D gel analysis a number of isoforms of ß-actin were found in breast and ovarian cells. It was observed that the B1 expression appears to be similar in one of the malignant as compared to the non-malignant breast cell lines, while a significant difference was observed in ovarian cell lines, with expression being stronger in non-malignant versus malignant cell lines. The B1 isoforms from both ovarian and breast cell lines were observed to be a subset of ß-actin isoforms. Future prospects for this work are the eventual development of a biomarker for the detection of breast and ovarian cancers.

Presenting Author: Nicole Gauthier

Gerges, Noha, Peter Krell, Jeff Hodgson and

Reprogramming the chitinase and cathepsin expression profile of Autographa californica multiple nucleopolyhedrovirus (AcMNPV).

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) was modified with the hopes of creating a reprogrammed virus. CHIA and V-CATH are key enzymes in the process of liquefaction of the insect, and it is proposed that if their native promoters are replaced with stronger ones, then the overall virulence of AcMNPV should increase. Four recombinants of the AcMNPV virus were created via homologous recombination: a GFP selectable virus, a GUS selectable virus, a rescued virus (RES) and the reprogrammed virus (REP). 2 GUS plaques, from previously plaque purified plates, were compared to the GFP virus and characterized through the use of a GUS enzyme assay, restriction digest and Southern blot, all of which confirmed that the GUS marker was present in those viruses. Viral DNA was purified, isolated and used to infect SF9 insect cells for plaque purification in regards to the GFP, RES, and REP viruses. Plaques, from all four virus isolates, with a resulting phenotype of chiA+/GFP- were searched for to be used in further experiments. As of current, GUS, RES, and REP viruses with the desired phenotype have been isolated and are currently used for further experiments. Characterization of these viruses via Southern blots has confirmed that the desired genotypes have been successfully created.

Presenting Author: Noha Gerges

Gooderham, Krista, Julie Marentette and Sigal Balshine

Psychology, McMaster University and Psychology,

Invasive changes to an invasive species (morphological abnormalities in the brain, fin, and external genitalia of the round goby in hamilton harbour).

Aquatic pollution and exposure to contaminants have long been major environmental and health concerns. Toxins, such as polychlorinated biphenyls (PCBs), can transfer through the food web resulting in the biomagnifications of chemicals, with higher trophic level carnivores acuminating the highest concentrations. A new possible vector for such toxin transfer is the round goby (Neogobius melanostomus), a recent invader of the Great Lakes. N. melanostomus is known to be consumed by sport fish and aquatic birds and due to its diet and benthic lifestyle it may be highly contaminated in particular areas. How contamination may impact brain morphology and external morphology has never before been investigated. We compared contaminated and non-contaminated round goby populations in Hamilton Harbour by measuring external morphological characters as well as brain lobe size. Gobies inhabiting highly contaminated areas had much shorter pelvic fins, a trademark of fin erosion, than relatively non-contaminated fish. Contaminated males also displayed evidence of feminized urogenital papillae or MIPS (Morphologically Intermediate Papilla Syndrome). Results of the brain measurements will also be discussed. The results suggest that contamination exposure leads to anatomical abnormalities that are likely to influence reproductive behaviour and cognitive abilities.

Presenting Author: Krista Gooderham

Grant, Chelsea

Molecular and Cellular Biology, University of Guelph

Iron response in Mycobacterium avium subspecies avium 104.

Iron is an essential nutrient required by almost all bacteria, including pathogenic Mycobacteria. Iron regulated proteins can be identified by an analysis of their differential expression under varying degrees of iron limitation. The iron response of Mycobacterium avium subspecies avium strain 104 (MAA) was examined. First, the effect of an iron chelator on the growth of this species was investigated and found to have an inhibitory effect. This inhibitory effect was determined to be a result of the lack of iron available for these bacteria, and not a result of toxicity. Therefore, dipyridyl (the chelator) was used to provide iron-limited media for further experiments. In fact, growing Mycobacteria in conditions containing varying levels of iron caused a change in their morphology. Furthermore, cultures were grown in iron-limited as well as iron-sufficient conditions, lysed by bead beating, and run by SDS-PAGE. The gels containing soluble and insoluble proteins were stained with either Coomassie Brilliant Blue, or Silver stain. The protein profiles differed slightly, with proteins of 114, 106, 78, 51, and 30 kDa appearing in the iron-limited conditions but not in the control. The identity and function of these proteins remain unknown at this time.

Presenting Author: Chelsea Grant

Green, Ellen, Lynda Corkum and Jan Ciborowski

Biological Sciences, University of Windsor

Spatial and temporal variation in burrowing mayfly nymphs (Ephemeroptera: Hexagenia limbata and H. rigida) recolonizing the western basin of Lake Erie.

Beginning in the early 1990's, burrowing mayfly species recolonized the western basin of Lake Erie after 30 years of hypoxia at the sediment-water interface. Long-term monitoring of adult mayflies at shoreline areas showed that Hexagenia rigida was much more abundant than H. limbata during the initial recolonization period, but has been replaced gradually by H. limbata. We hypothesized that the temporal shift in dominance from H. rigida to H. limbata would be confirmed by the distribution and abundance of nymphs of each species, which can be distinguished by unique pigmentation patterns (confirmed by genetic analysis). We also hypothesized that H. rigida, the original colonizer, would occupy shoreline areas and H. limbata (the historically dominant species) would eventually colonize all areas. We identified nymphs collected in Ponar grabs each spring from a grid of 41 sites distributed throughout western Lake Erie from 1997 to 2004. The relative abundances of H. rigida and H. limbata of nymphs exhibited the same temporal sequence as adults. Mean body size did not differ between species within years, but mean body sizes differed significantly among years (a reflection of sampling date). In 1997 and 1998, H. rigida nymphs were most abundant. Both species were equally numerous in 1999. Since then, H. limbata has become increasingly dominant. In 2004, H. limbata represented 90% of all nymphs collected. Hexagenia rigida occurred alone at the mouth of the Detroit River and at the eastern edge of the western basin (facilitating distribution among islands). Both locations represent sources of recolonization. Hexagenia limbata has come to dominate the central region of the basin, possibly because it may be able to withstand hypoxia better than H. rigida.

Presenting Author: Ellen Green

Gross, Jeffrey and Teresa Crease

Molecular and Cellular Biology, University of Guelph and Integrative Biology, University of Guelph

Does Pokey have an effect on the transcription rDNA in Daphnia obtusa?

Since their discovery in the 1940s mobile elements or transposons have been heavily studied. Of particular interest are mobile elements which are able to exist in the highly repetitive tandem arrays of the ribosomal RNA multi-gene family or rDNA. It is expected that the mechanisms of concerted evolution would have eliminated mobile elements from rDNA over time. Yet several mobile elements persist in rDNA. Pokey is one such element, existing in the rDNA of several species in the subgenus Daphnia. This study uses a PCR approach to investigate the effect of Pokey on rDNA transcription in Daphnia obtusa. Since Pokey specifically targets the 28S rRNA gene for insertion, oligonucleotide primers were designed to amplify the region of the 28S rRNA gene containing the Pokey insertion site and used in various combinations with Pokey-specific primers to yield amplicons that indicate the presence of the 5' 28S-Pokey junction of inserted 28S rRNA genes, the 3' 28S-Pokey junction of inserted 28S rRNA genes and uninserted 28S rRNA genes. These regions were then amplified out of DNA and total RNA extractions from Daphnia obtusa. Regions that were present in DNA extractions but absent in RNA extractions were inferred to be transcriptionally inactive. While this study is still in progress, preliminary results suggest that transcription of 28S Rrna genes is interrupted by Pokey elements some time after the transcriptional machinery has crossed the first 28S-Pokey junction into the Pokey element itself.

Presenting Author: Jeffrey Gross

Han, Katherine

Plant Agriculture, University of Guelph

Inducible and Constitutive Expression of ß-glucuronidase (GUS) in Transgenic Nicotiana benthamiana Cell Cultures and Whole Plant Tissues.

The production of therapeutic proteins in plants has been demonstrated using various expression systems. Plants are attractive organisms due to their ability to yield large amounts of protein at a cost effective rate. Transgenic Nicotiana benthamiana plants were propagated by co-cultivation with Agrobacterium tumefaciens using Cauliflower Mosaic Virus 35S and harvest-inducible (HI7) ß-glucuronidase (GUS) fusion constructs conferring kanamycin resistance. Second generation (T2) HI7-GUS and 35S-GUS lines were screened for the transgene using PCR and histochemical GUS assays. Methylumbelliferyl- ß-glucuronide (MUG) assays were performed to flurometrically detect the levels of protein expressed in each line. The lines that produced the highest levels of protein were selected for further propagation (T3 generation) in both soil and on callus-inducing media. Inducing treatments such as wounding, heat, abscisic acid (ABA), and a combination of heat and ABA were applied to whole leaf tissues and dedifferentiated calli to compare its effect on gene expression. It was hypothesized that the GUS gene could only be induced in whole tissues and not in dedifferentiated calli with the treatments having an additive effect on expression. Through histochemical staining of 35S-GUS leaves, it was established that GUS expression was evenly distributed throughout the leaf and petiole sections while there were variations in specificity seen in HI7-GUS lines. Expression of the HI7-GUS promoter is commonly localized within the veins and vascular tissues of leaves and stems, respectively. However, line 7 with the HI7-GUS promoter exhibited similar expression to that seen with the 35S-GUS promoter. GUS staining of the calli indicated that the HI7-GUS promoter could be induced in dedifferentiated cells. Preliminary results indicated that there were no significant differences in the levels of expression in regards to the various treatments applied to both leaves and calli. However, GUS gene expression was higher in calli than in the leaves.

Presenting Author: Katherine Han

Hanna, Jillian, Micheal Wilkie and Thomas Woodcock

Biology, Wilfrid Laurier University

Evaluating the potential use of PIT tag technology to study brown trout, Salmo trutta L., movements & overwinter survival in the Grand River.

The Grand River, running through Southwestern Ontario, contains an economically important brown trout, Salmo trutta L, fishery in its upper reaches. This tailwater, below the Shand Dam in Elora, Ontario, cannot currently support a self-sustaining brown trout population and over 20 000 trout are stocked into the tailwater annually. The purpose of my study was to determine the feasibility of using PIT tags with a portable PIT system to monitor brown trout in the Grand River and therefore gain insight on how to support the trout populations. Tag loss and mortality were determined to be low for tag sizes, 12-mm and 23-mm, and it was determined that the implantation procedure could easily be learnt by volunteers and hundreds of fish could be tagged with a couple days. As well heamatological (hemoglobin, heamatocrit, and mean cell hemoglobin concentration as well as osmolarity, [sodium], [chloride]) and stress (glucose and lactate) parameters were measured at various times after the fish were tagged to ensure the implantation and tags were not harming the fish. From these measurements it was determined that the process was not negatively affecting the brown trout. Lastly field studies were performed to determine microhabitat use by the brown trout in the Grand River. Due to the small detection ranges of the equipment, and the escape response displayed by the fish significant data was unable to be collected. Therefore it was concluded that larger antennas and more powerful transceivers are required to track fish in such a large river as the Grand.

Presenting Author: Thomas Woodcock

Hanner, Robert, Roy Danzmann and Amber King

Integrative Biology, University of Guelph

Conserved non-coding elements as a nuclear candidate for DNA barcoding and phylogeny estimation in fishes.

Even though DNA barcoding has had success in identifying and discovering organisms on a species level using the mtDNA 5'-CO1 gene, particular evolutionary events that are biparentally inherited are not imprinted in the maternally inherited mtDNA. Therefore there is a need for a nuclear marker to firstly confirm the identification of organisms and secondly corroborate the discovery of new species. Conserved non coding regions (CNEs) are segments of DNA that are conserved across a diversity of species that are immersed in the highly mutating intronic genomic DNA and can therefore be used to serve as universal primer site. In this study, I investigated the variability of the intervening regions of CNE956-CNE957 and CNE 957-CNE 959 to determine their potential to serve as a nuclear marker to complement DNA barcoding. Firstly, these regions were optimized and then tested them across a wide diversity of fish species. The region spanning CNE 957-CNE 959 was discarded as it exhibited a polymorphic locus. The region CNE 956-CNE957 was tested more extensively across 4 classes, 27 orders and 50 families of fishes, already tested with the mtDNA gene. Of the 92 species tested, only 34 species amplified. Sizes of the forward and reverse sequences were 507.52 ± 24.98 and 512.65 ± 20.98 respectively inferring the presence of INDELS in intervening regions or primer regions. Furthermore, when comparing phylogenetic trees, this particular region exhibited a lower rate of evolution then the mtDNA region. Overall, region CNE 956- CNE957 and CNE 957-CNE956 are not good candidates for phylogeny estimation and DNA barcoding. However, studies should continue to investigate other CNEs to act as a nuclear candidate for DNA barcoding or phylogeny estimation.

Presenting Author: Amber King

Hathaway, Amy

Molecular and Cellular Biology, University of Guelph

Hyperthermia induced apoptosis.

Heat shock induces a variety of stress responses in cells. The survival of cells following hyperthermic shock depends on both the temperature exposed and length of exposure. Mild heat shock induces the expression of heat shock proteins that function as molecular chaperones that function in cytoprotection, protein folding and synthesis. Heat shock proteins give affected cells thermotolerant properties and allow them to be tolerant to additional stressful stimuli encountered. Severe hyperthermia causes cell death and involves the induction of the apoptotic pathway.Apoptotic pathways are initiated when a stimulus activates an initiator cysteinyl aspartate-specific protease (caspase). The intrinsic apoptotic pathway is characterized by mitochondrial outer membrane permeabilization causing cytochrome c release. Release into the cytosol brings about the formation of an apoptosome, comprised of apoptotic protease activating factor-1 (Apaf-1) and procaspase-9. Formation of this complex allows for activation of caspase-9 and downstream executioner caspases that are involved in the breakdown of cellular proteins. Heat shock induced MOMP occurs via several different pathways. Heat stress activates Bax and Bak directly, two members of the Bcl-2 pro-apoptotic family of proteins. Bax and Bak activation stimulates MOMP activity and induces the release of cytochrome c from the mitochondria. Heat activates caspase-2, causing cytochrome c release from the mitochondria following cleavage of BH3-only protein Bid. Cleavage of Bid is found to sensitize the mitochondrial membrane and allow for induction of MOMP. Heat also activates stress kinases (JNKs) that regulate Bcl-2 family members Bax and Bak induction of MOMP. Heat shock activates an unknown apical protease that mediates MOMP via cleavage of Bid and initiates the cleavage of downstream caspases. The identification of the unknown heat activated apical protease and mechanism of its activation requires further investigation.

Presenting Author: Amy Hathaway

Hennin, Holly, Nicole Barker, David Bradley and Daniel Mennill

Biology, University of Windsor and Biology,

Differences in vocal behaviour between paired versus bachelor Rufous-and-white wrens (Thryothorus rufalbus).

Males who are bachelors may behave differently than males who are paired, exaggerating certain features in an attempt successfully attract females. However, very little research has focused on how male behaviour changes with mating status, or on what features bachelors emphasize in an attempt to attract mates. Using neotropical Rufous-and-white wrens (Thryothorus rufalbus) as a model system, we compared the singing behaviour of paired males to bachelor males. Over a five year period, we collected recordings from 16 males when they were paired and when they were bachelors in the Guanacaste Conservation Area, Costa Rica. We examined how behaviour differed with pairing status, focusing on song rate, intersong intervals, the average number of song repetitions, song switching rate, and evident repertoire size. We found that males as bachelors sing significantly more songs per minute, have shorter intersong intervals, and switch song types less frequently. Surprisingly, bachelors exhibited significantly smaller repertoire sizes compared to when they were paired males. These results clearly demonstrate that males adjust their singing behaviour with pairing status, delivering songs at a higher rate, but with less variety, when they are bachelors. Our results show consistency with previous research that measured similar aspects of song in other species of birds and create a better understanding of alterations in behaviour as a result of pairing status within males.

Presenting Author: Holly Hennin

Hillis, Sonja and Douglas Fudge

Integrative Biology, University of Guelph

Tensile mechanics of draw processed hagfish slime threads.

Human dependence on petroleum-based fibrous materials like nylon has led research towards protein-based fibres as a renewable alternative. Previous research has focused on spider silk, but to date, progress in this area has been disappointing. Here we explore the possibility of using protein threads from the defensive slime of hagfish as a model for renewable protein fibres. Previous work has shown that stretched slime threads adopt a structure similar to spider silk. The purpose of this study was to compare the tensile mechanics of hagfish slime thread fibres to spider dragline silk. Material properties of stretched and control hagfish fibres were determined using a Nano Bionix tensile testing system. Results revealed that the modulus, stress at break, and toughness were all significantly higher in the stretched fibres. There was no significant difference between the stretched and control in the strain at break. Modulus, strain at break, and toughness values for stretched fibres were all comparable to those previously computed for spider silk. Stress at break, however, was only half of the calculated spider silk value.

Presenting Author: Sonja Hillis

Homer, Brad

Molecular and Cellular Biology, University of Guelph

Investigating Initiation of the Trans-Acting PTGS Pathway in Plants.

Transgenes in tranagenic plants are subject to the mechanisms of transcriptional gene silencing (TGS) and post transcriptional gene silencing (PTGS). PTGS mechanisms are additionally involved in endogenous gene regulation, the control of transposable genetic elements and viral resistance. Specific PTGS pathways exist and are dependent upon the generation of short RNA molecules and the subsequent action of proteins they interact with; these pathways are similar with common RNA interacting proteins. Transgenes are silenced through the trans- acting PTGS pathway. Initiation of the trans-acting pathway is dependent upon the recognition of aberrant RNA produced from the transgenic locus and the subsequent association of this aberrant RNA with the RNA dependent RNA polymerase RDR6. Currently it is not understood how aberrant RNA comes into association with RDR6. Recently it was reported in the literature that RDR6 can not recognize aberrant RNAs known to induce PTGS; indicating additional proteins exist to recruit aberrant RNA for RDR6. Recently a novel gene, SDE5, was reported in the literature as being critical to the trans-acting pathway in Arabidopsis. It has been proposed that SDE5 acts to associate aberrant RNA with the RDR6. Here, we propose experiments designed to test the hypothesis that the SDE5 is responsible for recruiting aberrant RNA and associating it with the RDR6. Additional experiments are proposed to characterize the putative interaction of the SDE5 protein with RDR6 and the aberrant RNA

Presenting Author: Brad Homer

Horte, Jared

Molecular and Cellular Biology, University of Guelph

Optimizing DNA extraction and PCR protocols from mixed environmental samples for parallel pyrosequencing applications of DNA barcoding.

Pyrosequencing is a relatively new method for determining the sequences of DNA. The technology is based on the pyrophosphate released when a dNTP is incorporated into a DNA molecule starting a chain reaction that culminates with the release of light. By recording the nucleotide present and the flashes of light, one can determine the sequence of the DNA strand. New generation pyrosequencing-based sequencers can produce up to 250bp from 400,000 DNA strands in parallel. The purpose of this experiment is to utilize the massive throughput of parallel pyrosequencing for use in determining species diversity in mixed environmental samples. Species diversity will be calculated by comparing species-specific gene targets (DNA barcodes) obtained from pyrosequencing of samples to a database of known DNA barcodes from many species. Our early tests with engineered mixtures of DNA samples from 870 species of Lepidoptera, suggested that species resolution can be obtained using short cytochrome c oxidase I pyrosequence reads. However, PCR conditions, especially universality and uniform binding of primers can play a key role in obtaining maximal coverage. Due to contamination of PCR products, the DNA extracted from the mixed environmental samples has not yet been pyrosequenced. It is expected that pyrosequencing should be able to provide an accurate count of species diversity in a mixed environmental sample. This would have great applications in ecology, environmental sciences, and conservation.

Presenting Author: Jared Horte

Hubert, Stephanie and Erin Vandermarel

Biology, Laurentian University

Small mammal diversity in revegetated VETAC stands of different ages in the city of greater Sudbury.

The Sudbury area has been affected by the mining industry since 1888. In 1972, Inco Ltd. built a 381 metre smoke stack, to reduce sulphur dioxide emissions to 0.02 ppm that has resulted in a 90% decrease in sulphur dioxide emissions over the last 25 years. The re-vegetation of 4, 000 hectares began in 1973 with the creation of a committee now known as VETAC. Small mammal diversity of re-vegetated stands of different ages in the Sudbury area was studied. Our hypothesis states that there is greater small mammal diversity in the older re-vegetated stands. Nine sites were chosen; three young, planted 5-8 years ago, three medium aged, 10-15 years ago, and three old, planted more than 20 years ago. One site of each age category was studied over a weekend and all sites were studied over three consecutive weekends. A 150 meter trap line was set at each site and Longworth traps were set every 10 metres. Traps were checked three times a day and species caught were identified, weighed, sexed, and marked by fur trimmings before being released. Tracks were also identified over the trapping weekends. A separate tracking session was performed at all nine sites to determine the species density in a 1500 m2 area along the trap line. Species richness was greatest at a different aged site every weekend. The total species richness observed in all sites was nine. Species richness in the young, medium and old sites was 8, 7, and 6 respectively. Species density in the young, medium and old sites was 6 tracks per 1500 m2, 26 tracks per 1500 m2, and 89 tracks per 1500 m2 respectively . Results show that species richness in the Sudbury area is site specific and not age dependent. Density however seems to be age dependent.

Presenting Author: Stephanie Hubert and Erin Vandermarel

Hurley, Tim and Patricia Chow-Fraser

Biology, McMaster University

A survey of muskellunge nursery habitat: The implications of water level decline.

Nearshore fish habitat is critically important to many Great Lakes fishes. Serving as the interface between the aquatic and terrestrial environments, this habitat is vulnerable to several forms of alteration including anthropogenic disturbance and water level fluctuations. Over the past 26 years (1981-2007), Severn Sound (Georgian Bay) has witnessed substantial shoreline alteration and a decline in water levels of approximately 70 cm. We investigated how muskellunge nursery habitat has changed between the summers of 1981 and 2007. The sampling protocols employed in 1981 were duplicated in 2007 to produce comparative surveys of nearshore fish and plant communities. Despite increased shoreline modifications, there has been no significant change in water-quality conditions between the two sampling years. However, an apparent shift has occurred in the plant communities with a decline in deep water species accompanied by an increase in early successional shoreline species. Pronounced changes were observed in the fish community. Young-of-year muskellunge were absent from their historical nursery areas in 2007. Similarly, black crappie, present in 1981, were not caught in 2007. Yellow perch and largemouth bass represented a significantly lower proportion of the total catch in 2007 than in 1981, with a concomitant increase in the proportion of small minnow species. One such fish, the banded killifish was not found in 1981 but represented nearly a quarter of the total 2007 catch. Furthermore, GIS spatial analysis revealed that a considerable amount of fish habitat has been lost over the past 26 years. Water level decline has altered the geomorphometry of historical muskellunge nursery sites, increasing the exposure of these protected embayments to the effects of wind and wave action. These findings highlight the possible consequences of water-level changes on nearshore fish species and the particular vulnerability of the muskellunge to habitat alteration.

Presenting Author: Tim Hurley

Hyland, Melissa

Human Health and Nutritional Sciences, University of Guelph

Obstacle circumvention strategies used during late-visual cueing in middle childhood aged children.

During daily activities, a child is constantly faced with new obstacles that require them to navigate through cluttered environments. The main purpose of this study was to examine locomotor patterns of healthy children with a constrained base of support (BOS) and limited time allowed to prepare for obstacle circumvention. Subjects (mean age 7.80 years ± 0.51) were asked to perform five unobstructed walking trials followed by randomly ordered avoidance trials, five for constrained BOS (Lead In) and five for normal BOS (Lead Out) turn types. A cylindrical obstacle was located ~ 2 m from the start point of the walkway. Two light bulbs mounted at the children's eye level on either side of the obstacle and connected to a floor mounted light-triggering mat, indicated avoidance direction and resulting turn type. Statistical analyses focused on the obstacle crossing step (OBSTX) and one step prior (OBST-1). An interaction effect was found between turn type and gait cycle event for both step length ($p = 0.02$) and width ($p = 0.0001$). Subjects using "lead-out" strategies increased step width greatly at OBST-1, followed by an increase in step length at OBSTX in order to accelerate the body forward and around the obstacle. When "lead-in" strategies were used, children increased their step width while simultaneously decreasing their step length at OBST-1 to increase BOS in this constrained turn type. Trunk yaw ($p = 0.0001$) and head yaw angular magnitudes ($p = 0.005$) both showed statistically significant increases across gait cycle events. Children appear to use an "enbloc" method to stabilize the head onto the trunk in order to provide stable visual information about their changing position in space. Results from this work suggest that children's locomotor strategies are highly environmental and task-dependent.

Presenting Author: Melissa Hyland

Iles, David

Integrative Biology, University of Guelph

Top-down control of invertebrate communities by waterfowl in the Prairie Pothole Region of North America.

The trophic cascade hypothesis has been tested most thoroughly in freshwater systems involving piscivorous and planktivorous fish removal experiments. However in the Prairie Pothole Region of North America, waterfowl, rather than fish, are the dominant predators of invertebrate populations. In addition, few studies have examined the degree to which compensatory predation by omnivorous zooplankton affects the trophic cascade. In this study, I will test for cascading top-down control of invertebrate and phytoplankton communities as a result of waterfowl predation, as well as the effects of omnivory on the trophic cascade. If ducks imposed top-down control on invertebrates, then invertebrate size and abundance will decrease as duck abundance increases. If the top-down control cascades to the level of phytoplankton, then phytoplankton biomass will increase as duck abundance increases. Finally, if omnivores are dampening the trophic cascade, then the effect of duck predation on zooplankton and phytoplankton will decrease as the abundance of omnivores increases.Prior to this study, invertebrate and phytoplankton samples were collected in mid June 2007 from 36 ponds in North Dakota, and waterfowl abundance on each pond was assessed over the entire breeding season from May until July 2007. The size and abundance of zooplankton will be measured for each pond, and then compared to waterfowl density. Phytoplankton biomass, as estimated by chlorophyll-a analysis, will be compared to waterfowl density. Finally, the relationship between duck abundance and biomass of both zooplankton and phytoplankton will be compared to omnivore abundance to determine if omnivory masked the trophic cascade.The results of this study will fill a gap in current knowledge by providing insight into top-down control of plankton communities in prairie pothole communities, the level to which waterfowl are limited by zooplankton, as well as the effects of omnivorous zooplankton on the cascade.

Presenting Author: David Iles

Jacob, Greg

Molecular and Cellular Biology, University of Guelph

Formylation independent initiation of protein synthesis in pseudomonas aeruginosa and echerichia coli.

Until recently, a formylated initiator methionyl-tRNA (fMet-tRNAfMet) was thought to be necessary for all eubacteria and eukaryotic organelles, chloroplasts and mitochondria, to initiate protein synthesis. Studies done in Pseudomonas aeruginosa however showed that some bacteria have the ability to initiate protein synthesis without a formylated initiator tRNA. Other eubacteria, like Escherichia coli, remain dependent on formylation for protein synthesis initiation. The protein responsible for bringing the initiator tRNA to the 30S ribosomal subunit is Initiation Factor 2 (IF-2). In P. aeruginosa IF-2 was shown to facilitate both formylation-dependent and independent translation initiation. It was expected that the Pseudomonas IF-2, when expressed in a formylation deficient strain of E. coli, could rescue the slow growth phenotype of that strain. When this was shown not to occur, it was hypothesized that a protein acting as a negative regulator must be interacting with the Pseudomonas species of IF-2. To uncover the identity of this protein, the process of Tandem Affinity Purification (TAP) was used. The TAP procedure makes use of two separate purification methods carried out under physiological salt conditions to yield a final eluate containing your protein of interest and any associating proteins. The eluates from the E. coli IF-2-TAP and the Pseudomonas IF-2-TAP were compared and analysed by 2D gels. In the future we hope to compare the co-purifying protein profiles of these two IF-2 species using mass spectrometry. We will also use a bacterial genetics approach to hopefully lead to the discovery of the identity of the putative negative regulator.

Presenting Author: Greg Jacob

Jamal, Alisha

Biology, University of Western Ontario

Micronuclear RNA may regulate post-conjugation macronuclear anlage development in the ciliated protozoan Childonella uncinata.

The purpose of this investigation was to study the regulation of macronuclear anlage development in order to better our understanding of Chilodonella uncinata's unique development after conjugation and to gain insight into its evolutionary relationships with better studied ciliate species. This experiment investigated putative DNA and RNA localization in the ciliated protozoan C. uncinata during post-conjugation macronuclear development. C. uncinata have both a germline diploid micronucleus, and a polyploidy somatic macronucleus, necessitating a unique genome replication process . During conjugation in C. uncinata, a copy of the newly formed diploid micronucleus will develop into the new macronucleus (anlage) of the cell through three distinct stages. Live C. uncinata cells were stained at various points in their life cycle with the fluorescent dye acridine orange, which has differential excitation and emission spectra when bound to double stranded and single stranded nucleic acids. Fluorescence microscopy was used to observe double stranded and single stranded nucleic acid localization using a Green Fluorescence Protein filter and a Texas Red filter respectively. The findings are consistent with the sequence of events that occur during macronuclear development outlined by Pyne et al. (1974) where macronuclear development was studied using the non-differential stain Feulgen; the present results thus generalize those of Pyne et.al. by providing more information about the type of nucleic acid present. The apparent presence of RNA localized in the micronucleus suggests that micronuclear specific RNA could regulate macronuclear development as in the case in the ciliate Tetrahymena thermophila (Mochizuki& Gorovsky, 2004).Pyne, C.K., Ruch, F., Leemann, U., and S. Schneider. Development of the Macronuclear Anlage in the Ciliate Chilodonella uncinata. Chromosoma. (1974)48: 225-238.Mochizuki K, Gorovsky MA. Conjugation-specific small RNAs in Tetrahymena have predicted properties of scan (scn) RNAs involved in genome rearrangment. ¬¬Genes Dev. (2004) 18(17):2068-73.

Presenting Author: Alisha Jamal

Jardine, Catherine and Ryan Gregory

Integrative Biology, University of Guelph

Estimations of genome size in the phylum porifera.

This study provides some of the first estimates of genome size in Porifera. Measurements of a broad range of species encompassing each class of Porifera as well as a wide variety of life history characteristics were preformed. Genome size of both fresh and ethanol preserved materials were estimated using Fuelgen image densitometry analysis. These measurements were used to determine the effect of ethanol preservation on genome size measurment in Porifera. Size estimates were then used to perform comparisons between various groups of sponges based on taxonomy and life history characteristics to suggest possible influences on the evolution of genome size in this phylum. This study acts as a base for further broad scale investigation of patterns of genome size in Porifera.

Presenting Author: Catherine Jardine

Jasra, Sakshi, Jenna Ritchie and Lisa Porter

Biological Sciences, University of Windsor

The role of cortisol and dexamethasone in breast cancer initiation.

There is a great deal of evidence accumulating that stress may affect cancer progression, reoccurrence and patient survival, yet the underlying mechanisms are poorly understood (1). Several clinical studies have demonstrated that levels of cortisol, the primary stress hormone, correlate positively with mortality rate and recurrence of breast cancer (2). Data from our lab illustrates that cortisol stimulates proliferation of multiple breast cancer cell lines in vitro and that this functions directly through the glucocorticoid receptor (GR). Furthermore, using microarray analysis we have isolated a number of interesting downstream targets that may regulate effects of cortisol in the breast. While cortisol appears to mediate proliferation in breast cancer cells it remains puzzling that Dexamethasone, an artificial construct of cortisol, shows opposite effects on breast cell proliferation in vitro and in vivo. Indeed, Dexamethasone is clinically utilized for breast cancer, often being administered prior to the initiation of chemotherapy. It is our hypothesis that small changes in the ligand structure between Cortisol and Dexamethasone dramatically alters GR binding of its DNA response elements. The objective of this study is to compare the effects of Dexamethasone and Cortisol on breast cell growth and on downstream effectors of breast cell proliferation and migration. Herein we demonstrate the results of quantitative analysis of Dexamethasone and Cortisol treatments on a variety of breast cancer or breast normal cells. This project has determined that the GR signalling pathway has the potential to exert opposite effects on cell proliferation in the breast; thereby demonstrating that ligand specificity for cortisol mimetics is critical. Additionally, this project supports that inhibitors of the natural cortisol ligand may provide valuable adjuvant therapies in the treatment of breast cancer patients.(1) Palesh, Oxana, Lisa D. Butler. Journal of Psychosomatic Research 63(2007): 233-239.(2) Sephton, Sandra E, Robert M.Sapolsky. Journal of the National Cancer Institute 92(2000): 994-1000

Presenting Author: Lisa Porter

Jiang, Jenny, Lee-Cyn Ang and Warren Blume

Biology, University of Western Ontario: Pathology, University of Western Ontario: Clinical Neurological Science, University of Western Ontario

EEG patterns of adult patients with cortical dysplasia.

Maximum 300 wordsBackgroundAlthough epileptogenic cortical dysplasias (CD) usually appear as discrete MRI lesions, resective surgery reduces seizures significantly in only 67% (Palmini etal 1991; Bingaman and Catalepe, 2001). To initiate a multipronged investigation into this discrepancy, we review pre-operative EEGs of 50 consecutive patients who underwent resective surgery for CD-related intractable focal epilepsy. MethodThe entry criterion was demonstration of one or more type of CD disclosed by histological review of resective specimens. Sufficiently congruent data for seizure localization from semiology, EEG and MRI were required for surgical candidature. Archived EEG reports, categorised according to our classification system (Lemieux etal 1983), were reviewed for localization of epileptiform (ictal and interictal) and non-epileptiform abnormalities occurring during wakefulness or sleep.ResultsSeveral EEG abnormalities reflected widespread cortical dysfunction 1) independent bi-hemispheric abnormalities (spikes, delta, theta) in 25 (50%) 2) EEGs of 22/25 (88%) had focal spikes in each hemisphere , and 3) 14/50 (28%) had spike-waves (SWs) or other bisynchronous epileptiform patterns. Abnormalities were more widespread in extra-temporal than temporal patients: 1) greater average number of lobes with focal spikes (mean= 3.14 vs.2.14; p=0.02), and 2) higher incidence of SWs. ConclusionsMultifocal and bilateral EEG abnormalities in patients undergoing resective aqsurgery for CD-based intractable epilepsy may underlie less than expected surgical effectiveness.

Presenting Author: Jenny Jiang

Johnstone, Daniel and Jaideep Mathur

Molecular and Cellular Biology, University of Guelph

Demonstrating the uses of the photoconvertable fluorescent protein EosFP in live plant cell imaging.

EosFP is a fluorescent protein that undergoes ~390-405nm irradiation induced photoconversion from 516nm-to-581nm (green-to-red) emission wavelength. Thus, EosFP holds potential for selective photo-illumination during plant live cell imaging. Previously engineered wildtype (tetrameric), monomeric, dimeric, and tandem-dimeric versions of the protein have been transiently expressed in plant cells. Monomeric-EosFP has been fused with the microtubule-labelling MAP4 gene and microtubule positive tip-labelling EB1 gene. Monomeric-EosFP-MAP4 has been transformed into Nicotiania benthamiana stably expressing either GFP-labelled actin or GFP-labelled nuclei and wild type Nicotiania plumbagfolia. These lines are used to produce protoplasts and demonstrate EosFP's use in determining how nuclear-originating microtubules are involved in determining the plane of division prior to cell division.

Presenting Author: Daniel Johnstone

Joshi, Shikha

Molecular Biology and Genetics, University of Guelph

Determination of genes involved in porcine skeletal muscle contraction.

Ractopamine is a ß-adrenergic agonist commonly used in porcine feed to increase lean muscle accretion. Previous studies show that Ractopamine transcriptionally regulates myosin heavy chain isoforms in skeletal muscles. The type 2B isoform of the myosin heavy chain is a key player in muscle contraction and its activity has been found to be increased upon administration of ractopamine. A microarray analysis will be completed using proteins present in skeletal muscle in pigs to determine if there are other proteins involved in the response to ractopamine. RNA will be isolated from pig skeletal muscle using the guanidinium hydrochloride method, and amplified into complimentary DNA using reverse transcriptase. A microarray will then be designed to specifically target proteins expressed during muscle contraction, including myosin heavy chain, myosin light chain, actin, and troponin. The results will allow us to see how muscle contraction occurs through various genes. Further research may involve determination of the promoter region of genes involved in muscle contraction and how they are activated.

Presenting Author: Shikha Joshi

Jun, Youngmin

Molecular and Cellular Biology, University of Guelph

Expression and characterization of human cardiac actin variant M305L.

To date there are 9 recognized actin mutations implicated in cardiomyopathies (heritable cardiac conditions). M305L (Methionine in position 305 becomes Lysine) is one of the 7 ACTC (human cardiac actin) variants that is associated with hypertrophic cardiomyopathy (HCM) rather than dilated cardiomyopathy (DCM). Small change in highly conserved amino acid sequence of the actin can give rise to the change in basic functions such as polymerization, which will result in cardiomyopathies. Overcoming the obstacles in the past that we faced with expressing ACTC variants with bacteria, researchers have found that Sf9 cells from bud-worm has the protein complex that can fold human cardiac actin variants that are transferred by specific recombinant baculovirus into functional protein . To further our understanding of cardiomyopathies, it is necessary to study the structures and functions of the ACTC variants. In order to do that, this research was set up to find out the optimum conditions for ACTC M305L expression in terms of viral concentration and infection time. From this result, further characterization of M305L was possible following affinity purification of the protein. The critical concentration for polymerization was studied by light scattering polymerization assay and ACTC-pyrene actin polymerization assay. Additionally, polymerization spin down assay was performed to verify the polymerization of M305L as well. The actin protein stability was then assayed using DNase-I inhibition assay. Finally, myosin subfragment-1 (S1) induced polymerization assay and myosin S1 co-sedimentation assay were performed to study the myosin S1 and ACTC M305L interaction.

Presenting Author: Youngmin Jun

Kadlubowska, Dorota

Biomedical Science, University of Guelph

siRNA knockdown of alpha-catenin protein expression in human colorectal cancer cell lines and induction of alpha-catenin expression in cells with a premature termination codon.

Human colorectal cancer is believed to be caused by a sequential accumulation of activating and loss-of-function genetic mutations. A defective alpha-catenin tumour-supressor gene, as well as an activating mutation in the K-ras oncogene have both been implicated in colorectal cancer progression. It was hypothesized that knock-down of alpha-catenin tumour-suppressor protein expression might transform colorectal cancer cells and cause an increase in the potential for tumour growth. Four small interfering RNA (siRNA) knock-down constructs, which are specific for human alpha-catenin, were obtained. One of the constructs was used to transiently transfect Dks-8 colorectal cancer cells which are K-ras wildtype. Immunostaining was done to visualize the change in alpha-catenin expression after transient transfection knockdown. To confirm that Dks-8 cells express alpha-catenin, immunostaining was also performed on tumour sections derived from mice that had received sub-cutaneous injections of Dks-8 colorectal cancer cells. Four 55 base-pair oligo-DNA sequences were purchased in order to clone them into a 4.1 p-silencer CMV-hygro expression vector for the in-vivo production of short-hairpin RNA (shRNA) to knock-down alpha-catenin in colorectal cancer cells. Dks-8 cells have not yet been successfully and stably transfected with shRNA for alpha-catenin protein expression knockdown. As an alternative approach, LIIa colorectal cancer cells, which contain a premature stop codon in the alpha-catenin gene, were treated with Gentamycin and Geneticin aminoglycoside antibiotics at a concentration of 125 ug/ml for one week to induce expression of alpha-catenin protein. Aminoglycoside antibiotics were previously shown to induce read-through of premature stop codons. Immunostaining of these treated cells suggests that Geneticin causes alpha-catenin to be expressed. Western Blotting suggests that the expressed alpha-catenin protein is smaller then the expected 102 kDa.

Presenting Author: Dorota Kadlubowska

Kasurak, Ashley and Dennis Higgs

Biological Sciences, University of Windsor

Sensitivity of the round goby (Neogobius melanostomus) to ultraviolet reflectance.

The round goby (Neogobius melanostomus) is an invasive species that is currently a threat to the biodiversity of the Laurentian Great Lakes. While appearing drab in colour to the human eye, the round goby has large patches of ultraviolet reflectance, on both lateral and ventral surfaces. Previous research has shown that an increased area of ultraviolet reflectance is found in more dominant males of some fish species. However, our study appears to be the first study to look at the behaviour of male gobies when exposed to a conspecific reflecting ultraviolet light. Trials were run using a silicone fish model under different light conditions to assess the role of ultraviolet reflectance in behavioural interactions. The model was presented to a male goby in the presence of just incandescent light (400-700 nm) or in the presence of full spectrum light (280-700 nm). This approach was used to assess the response of a resident male to a conspecific with different amounts of ultraviolet reflectance. The behaviours quantified were time to first approach or bite given to the model, time spent outside the shelter, the number of bites and number of approaches to the model. Preliminary results of the model experiment suggest that ultraviolet reflectance may be an important factor in determining the dominance of a conspecific. Once all data are collected, an ANOVA will be used to determine if there is any significance of the behaviour displayed by the resident male gobies under ultraviolet light compared to white light. This study aims to provide further understanding of the role of ultraviolet reflectance on various behaviours to further contribute to our understanding of the round goby.

Presenting Author: Dennis Higgs

Keithlin, Kelly and Nina Jones

Molecular and Cellular Biology, University of Guelph

Characterization of nephrin signaling pathways in the kidney: investigating the role of ShcA at the kidney slit diaphragm.

The glomerular filtration barrier of the kidney is composed in part by a specialized intercellular junction known as the slit diaphragm, which connects adjacent actin-based foot processes of podocytes(1). Nephrin molecules from opposite foot processes interact in the center of the slit to form a central density with pores on both sides. Mutations in human nephrin cause massive proteinuria, absence of the slit diaphragm and neonatal death(3). Nephrin functions as an adhesion molecule, structural component of the slit diaphragm and in signal transduction through its cytoplasmic region, which has a series of conserved tyrosine based motifs that can become phosphorylated(1). ShcA is expressed in the kidney and possess an SH2 and PTB domain both of which bind phosphorylated tyrosine residues(2). ShcA can in turn become phosphorylated on a number of tyrosine residues and recruit other proteins involved in signal transduction. We hypothesized that ShcA interacts with phosphorylated tyrosine residues on the cytoplasmic tail of nephrin to act as a scaffolding protein for signal transduction in kidney podocytes. An immunoprecipitation of wild type nephrin and mutant nephrin (mutated at three conserved tyrosine residues) with wild type ShcA was preformed to determine if ShcA was able to bind nephrin. An immunoprecipitation with wild type nephrin and mutated PTB/SH2 domain ShcA was also preformed to determine which domain of ShcA binds nephrin. Preliminary results suggest that ShcA is able to bind wild type nephrin as well as mutated nephrin, indicating that an additional conserved tyrosine mutation in nephrin needs to be generated and the immunoprecipitation performed. Preliminary results also suggest that both domains of ShcA are able to bind nephrin and this needs to be confirmed with a GST-fusion protein (SH2 or PTB domain) pull down of wild type nephrin.1) Jones, N., I. M. Blasutig, V. Eremina, J. M. Ruston, F. Bladt, H. Li, H. Huang, L. Larose, S. S. Li, T. Takano, S. E. Quaggin, and T. Pawson. 2006. Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. Nature 440:818-23.2) Ravichandran, K.S. 2001. Signaling via Shc family adapter proteins. Oncogene. 20; 6322-63303) Tryggvason, K., J. Patrakka, and J. Wartiovaara. 2006. Hereditary proteinuria syndromes and mechanisms of proteinuria. N Engl J Med 354:1387-401.

Presenting Author: Kelly Keithlin

Kenny, Michelle

Biology- Biodiversity, McMaster University

Change in spring arrival of Neotropical and short-distance migrant birds at Ruthven National Historic Park (1999-2007).

Migrant birds are vulnerable to climatic changes impacting the conditions of North American breeding grounds. Long-term data sets have shown that many avian species are initiating spring migration at earlier dates. The short-distance migrants have been found to arrive earlier in spring to better coincide with changes in insect and plant emergence. Neotropical (long-distance) migrants are more heavily controlled by endogenous rhythms and are less likely to react to climate change than short-distance migrants. We examined the migration patterns of avian species at Ruthven National Historic Park (Haldimand County, Ontario) using the data set collected from 1999 to 2007. This paper focuses on 14 Neotropical migrants and 11 short-distance migrants, all belonging to the insectivorous feeding guild. Using the morning temperature recorded daily at Ruthven, a significant relationship was established supporting temperature warming. However, using the Daily Estimated Total recorded for each species, not a single migrant showed a significantly earlier spring arrival date. Using the first date of arrival, three short-distance migrants are significantly arriving at later dates. Two short-distance migrants are arriving at Ruthven at significantly warmer morning temperatures. The mid-migration distribution, peak arrival date, of two Neotropical migrants significantly support later spring migration trends. The temperature of peak arrival dates is significantly increasing for one Neotropical migrant. The end of spring migration is marked by the date last recorded, with one Neotropical migrant ending migration significantly later. The temperature at migration's end is significantly warmer for 20 of 25 species. There is no evidence supporting the earlier spring migration predictions for short-distance migrants. However, the findings indicate delayed or prolonged spring migration periods for certain species. Our results indicate that data sets spanning longer periods of time are crucial in accurately determining the impact of climate change on the migration timing of bird species.

Presenting Author: Michelle Kenny

Kentner, Jeffrey

Biology, McMaster University

HER-2 cancer vaccine through recombinant Hepatitis B virus-like particles.

HER-2 is type 1 receptor tyrosine kinase (growth factor receptor), whose overexpression is associated with highly aggressive cancers that have a strong tendency for metastasis. Approximately 30% of all breast cancers patients will test positive for HER-2 overexpression and their prognosis is far poorer than those who test negative. In recent years two monoclonal antibody drugs, Herceptin and Omnitarg, have been generated which bind to specific epitopes on HER-2 and block it's function. Another exciting avenue of cancer research is the generation of so called "cancer vaccines" which attempt to redirection a person's own immune to specifically attack cancer tissue. Because of HER-2's atypical overexpression of a specific protein, it is an ideal candidate for this type of therapy. Using crystallographic data from the structures of Herceptin and Omnitarg in complex with HER-2, we have constructed gene vectors which express chimeric Hepatitis B virus-like particles (VLP's) containing fragments of the drug's epitopes in the VLP's protruding spike proteins. Our intention is to create a gene-based vector which can provoke a humoral response against HER-2 cancer cells, through its production of these VLPS and their interaction with B-cells. So far we have designed and constructed several variants of these vectors, transferred them into mice, and are in the late stages of a primary analysis of their immunogenicity. We are also now begging in vitro experiments to determine the anti-tumor potential of the anti-bodies generated in the mice.

Presenting Author: Jeffrey Kentner

King, Christine, Hendrik Poinar and Ben Evans

Biology & Anthropology, McMaster University

DNA barcoding meets metagenomics: investigating the paleoecology of eastern Beringia using permafrost cores.

Beringia refers to the huge, unglaciated Pleistocene subcontinent extending from the Kolyma River in Siberia to the MacKenzie River in Canada. Much of the research in this region has focused on the existence of the Bering Land Bridge and its role as a migration route between Eurasia and North America during the ice ages, although the whole of Beringia is equally intriguing. Macrofossil analyses indicate that Beringia acted as a refugium for a remarkable diversity of megafauna when much of the northern world was covered with ice. An apparent productivity paradox has dominated studies of Beringian paleoecology and the mammoth-steppe biome, with most favouring a mosaic of moist meadows and drier steppe tundra. This research will contribute to the growing body of molecular paleoecological data from the region through metagenomic analysis of Yukon permafrost cores. In order to maximize the number of identifiable sequences obtained from this approach, small regions of chloroplast and mitochondrial DNA have been targeted for analysis using 454 Amplicon Sequencing. To compare biodiversity between climatic extremes, we sampled cores representing stadial (25.3ky), interstadial (80-90ky), and interglacial (740ky) periods of the Pleistocene epoch, and have extracted and amplified DNA from all with age-dependent yields and success rates, respectively.

Presenting Author: Christine King

Kirischian, Nina, Jonathon Stone and Bhagwati Gupta

Biology , McMaster University

Development of a multi-parameter program to detect and locate promoter regions in a DNA sequence.

Each genome, regardless of species, contains genetic regulatory elements known as promoter regions that are responsible for transcription, signal sensoring, and other developmental and metabolic processes. Promoter regions are comprised of complex binding factor sites which are in close proximity to transcription start sites (TSS) and, thus, play an essential role in gene expression, function and transcription initiation. It has been difficult to determine the location and composition of DNA promoter regions, given that they do not contain known coding nucleotide sequences and instead are comprised of various motifs. To date, results from various programs have demonstrated little significance in the successful accuracy of identification of these regions. The purpose of this program design in Mathematica is to incorporate molecular research parameters, resulting in an increase in speed and accuracy of motif identification and leading the user to the promoter region of interest. The development of this program is composed of the following four key milestones: molecular and genetic literature research, design of the multi-parameter algorithm flowchart, design of the multi-parameter optimization program, and simulations of hypothetical and eukaryotic genomes by a multi-parameter program in Mathematica. The Mathematica program will search for conserved motifs, which are significant genomic factors with regional associations. The program has many optimized default parameter filters for adjusting specific promoter searches based on the user's needs; however, the primary search will begin with parameters in relation to an identified TATA box. Proper optimization of the multi-parameter analysis program with appropriate statistical scoring methodology for each search should result in a high probability of promoter region identification. The capability of identifying accurate promoter regions will increase gene identification within the genome and, thus, immensely enhance regulatory knowledge, through the use of computational biology.

Presenting Author: Nina Kirischian

Kita, Elizabeth and Krassimir Yankulov

Molecular and Cellular Biology, University of Guelph

Mutations in Cdc7p enhance the heritability of chromatin state in sub-telomeric regions.

Genes located near telomeres are often repressed by the strong silencing signals emitted from the telomeres, a phenomenon known as "Telomeric Position Effect". The genes are either fully active or completely repressed, but the state is dynamic and switches approximately once every twenty generations in the model organism Saccharomyces cerevisiae. Mutations in replication factors and certain sub-telomeric DNA sequences can alter the heritability of a gene's state. While most mutations in replication factors decrease the chance that a gene will be replicated with a consistent active or silent state, the mutant protein kinase cdc7-1 appears to lock the state of the sub-telomeric reporter gene after any selection pressure is applied. Growth assays were used to test other replication factor mutants with a variety of sub-telomeric reporter constructs for similar behaviour.

Presenting Author: Elizabeth Kita

Kokokyi, Seint and Jurek Kolasa

Biology, McMaster University and Biology,

Deciphering the diversity-stability puzzle.

Past research showed that stability of a community composed of many species increased proportionally with species richness (Tilman 1996, Valone & Hoffman 2003, Lehman & Tilman 2000, Yachi & Loreau 1999). The ecological reason for this pattern was that greater species richness buffers community against environmental change (Yachi & Loreau 1999, Tilman, Lehman & Brisow 1998). The statistical model presented by Loreau and de Mazancourt (unpublished) stated that stability increases with species richness due to the mathematical averaging of covariance, which approaches zero as more species are added. I undertook to test their statistical model for this trend. By using data on abundances and distribution of 70 aquatic invertebrate species collected from 50 coastal Jamaican rock pools over a span of 14 years, coefficient of variation (CV) in species richness was plotted against the number of species per pool to observe the diversity-stability relationship. The results showed that coefficient of variation decreases with species richness – i.e., increase in species richness results in increased compositional community stability. When the average covariance for each rock pool was plotted against species richness, an inverse relationship emerged. It appears that the increase in stability with increasing species richness is not due to the statistical averaging of the covariance as Loreau and de Mazancourt (unpublished) predicted. For future research, manipulation with environmental factors could be taken into consideration to explain the increased stability with species richness.

Presenting Author: Seint Kokokyi

Kolozsi, Edith, Jane Foster, Flourence Roullet and David Rollo

Biology, McMaster University and Psychiatry, McMaster University

Neuroligin and neurexin mRNA involvement in autism.

Autism is a neurodeveopmental disorder characterized by emotional and social deficits. Clinical observations point towards genetic complication that may be involved in autism. Mutation analysis studies by Jamain et al and Laumonnier et al have shown that NLGN3 and NLGN4 mutations are present in a small subset of autism patients. Linkage analysis of patients with autism implicates both synaptic neuroligins (NLGNs) and neurexins (NRXNs). Neuroligin and neurexin genes produce synaptic adhesion proteins important to synapse formation and function. I am examining neuroligin and neurexin gene expression in the VPA maternal challenge mouse model of autism to determine if altered gene expression underlies autistic-like behaviours previously observed in this model. Mouse brain tissue was processed using in situ hybridization and analyzed with densitometry to examine RNA expression of 3 neuroligin genes (NLGN1, NLGN2, NLGN3) and 3 neurexin genes (NRXN1, NRXN2, and NRXN3). Behavioural data previously collected from these mice will be incorporated to determine if behavioural phenotypes are linked to altered gene expression. No significant RNA expression differences have been observed between VPA and control mice in the hippocampus and the somatosensory cortex for NRXN2 and NRXN3. NRXN1 and the neuroligins gene expression still need to be examined. Differences may still be found in the other four genes to suggest that altered gene expression may be involved in autistic-like behavioural phenotypes. However, if no differences are found at this level they may exist at the protein or protein-protein interaction level.

Presenting Author: Edith Kolozsi

Krumholtz, Stacey, Sarah Rosloski and Vojislava Grbic

Biology, University of Western Ontario

Analysis of variation in the insertion allele class of MADS-AFFECTING FLOWERING 2 locus in Arabidopsis thaliana.

Duplication events within a genome, and the subsequent mutation and retention of duplicated genes, give rise to novel gene families. The MADS-AFFECTING FLOWERING or MAF loci in Arabidopsis thaliana are a gene family highly homologous to the major flowering time regulator FLOWERING LOCUS C. MAF1-MAF5 are transcription factors and a mutagenesis experiment identified that MAF2 gene is associated with flowering time. Analysis of natural variation of MAF2 locus uncovered great genetic variability that has been divided into four classes: null, high molecular weight, low molecular weight and insertion. My research focuses on characterization of the insertion allele class. The genetic variation at this locus has been studied through allele sequencing and bioinformatic analysis of the predicted protein products. In addition to studies of genetic variability at MAF2 locus, we also examined phenotypic effects of these natural alleles. Our lab showed that plants homozygous for one of the insertion alleles, MAF2-Ws, exhibit a flowering phenotype similar to the plants lacking MAF2 activity. In addition, MAF2-Ws plants exhibit segregation distortion in favor of the insertion allele. My research focuses on two additional alleles in the insertion allele subclass, MAF2-Sha and MAF2-Tu-1. The phenotype, with respect to flowering time and segregation distortion, will be compared between these allele variants, a MAF2-Ler reference allele and the previously characterized MAF2-Ws allele.

Presenting Author: Stacey Krumholtz

Kwan, Julian and Richard Mosser

Molecular and Cellular Biology, University of Guelph

Cofilin and cell death.

Cofiln is an actin binding protein which plays a role in remodeling the cytoskeleton by depolymerizing and severing actin filaments. Previous work identified non-muscle cofilin as a protein phosphorylated after heat shock in a human lymphoid cell line. Here, the effects of over expression of GFP tagged cofilin and a constitutively active mutant are examined by flow cytometry, fluorescence microscopy, and western blot.

Presenting Author: Julian Kwan

Ladhani, Sadia, Anita Woods and Frank Beier

Biology, University of Western Ontario and Physiology, University of Western Ontario

Role of farnesyl transferases in chondrocyte differentiation.

Endochondral ossification is a developmental process responsible for forming the long bones of the body from a cartilage template intermediate. Proper bone formation requires a precisely regulated balance between differentiation and proliferation of chondrocytes. Final bone length relies on the interaction between three distinct zones within a bones' growth plate, resting, proliferative, and hypertrophic chondrocytes. Signaling pathways regulating the growth plate are unknown in their entirety. Previous data from our lab has shown that the family of Rho GTPases are important for chondrocyte proliferation and differentiation. More specifically, RhoB is highly sensitive to the form of post-translational prenylation for its activity and has been shown to inhibit early chondrocyte differentiation (Woods and Beier, 2006). We were able to inactivate RhoB through inhibition of farnesyl transferases, as RhoB is one of the proteins that require farnesylation for specific cellular localization and activity. Here we examine the role of the farnesyl transferase inhibitor Manumycin A on chondrocyte proliferation and differentiation to hypertrophy. We demonstrate an increase in length of the proliferative zone, along with a decrease in lengths of the resting and hypertrophic zones of growth plates treated with Manumycin A. We confirmed the changes in the growth plate by examining the localization of proteins specific to the zones. Our data suggest a decrease in differentiation to hypertrophy as well as an increase in proliferation of proliferative zone cells in response to inhibiting farnesyl transferases.

Presenting Author: Sadia Ladhani

Langille, Laura and Janet Wood

Molecular and Cellular Biology, University of Guelph

The effects of NEM reactivity with transporter ProP on proline uptake in Escherichia coli.

Osmosensory-osmoregulatory protein ProP of Escherichia coli mediates the uptake of the compatible solutes proline and glycine betaine. As a member of the major facilitator superfamily (MFS) ProP functions as a solute-H+ symporter. Proline transport in the K12 derived strains used in this study was found to be much more sensitive to whole cell exposure to the thiol-reactive compound N-ethylmaleimide (NEM) than the ML 308-225 strain (Janick et al., 1976). This increased sensitivity was attributed to inhibition of respiration in the cells by NEM. A new protocol was developed to allow for the differentiation of proline transport inhibition due to decreased respiration and specific disruption of ProP via reaction with NEM (using strains WG709 (ProP) and WG786(ProP*)). Additionally, the effects of NEM reactivity on ProP were determined in strains WG906 (ProP* A59C), WG1096 (ProP* F55C), and WG1099 (ProP* G58C), and preliminary results were collect in strains WG1100 (ProP* D60C) and WG907 (ProP* S62C). NEM was found to strongly inhibit ProP function in stains WG1096 and WG1099, and decreased proline transport in WG709, expressing wildtype ProP, by approximately 80%. This study aims to further understanding of the transport mechanism of ProP through

Presenting Author: Laura Langille

Larocque, Sarah

Integrative Biology, University of Guelph

Infestation and juvenile production rates with 'natural' and 'artificial' propagations of the endangered kidneyshell mussel (Ptychobranchus fasciolaris).

The endangered freshwater kidneyshell mussel (Ptychobranchus fasciolaris) release conglutinates (packages of parasitic larvae) which increase host encounters. For conservation, artificial propagation infests fish with large densities of parasitic larvae in a confined space to increase host encounters and maximize juvenile production. Regarding kidneyshell mussel propagation, current artificial methods do not use conglutinates and artificial selection may occur. Artificial selection could reduce future survival and fecundity. Thus, the most beneficial propagation strategy was determined by comparing the 'artificial' (individual parasitic larvae) and 'natural' (conglutinates) propagation methods through infestation and juvenile production rates. If both methods have high host encounters, then they would have similar rates. Upon exposing Johnny darter's (Etheostoma nigrum) to either conglutinates or individual parasitic larvae, both methods were found to have similar rates. Since the methods were similar it suggests that future artificial propagation methods should use conglutinates to prevent any artificial selection, and reduce work effort. Artificial selection can not easily be tested for in long-lived mussels and is best to prevent it from occurring. Improving propagation methods by using conglutinates can help conservation efforts while continuing to try to increase survival of natural populations.

Presenting Author: Sarah Larocque

Laurin, Cory and Mitchell Shaw

Biology, Laurentian University

Comparison of macroinvertebrate community structure in naturally recovered lakes versus experimentally neutralized lakes in the Sudbury area.

The lakes in the Greater City of Sudbury region have been severely damaged by atmospheric emissions of acid and metal particulates from area smelters. Over the past 30 years, great environmental improvements have occurred in this region through reductions of SO2 and metal emissions, lake and catchment liming and re-greening projects including seeding. Liming projects were performed between 1973 and 1976 in Lohi Lake, Hannah Lake and Middle Lake. The pH of Hannah and Middle have recovered to greater than the desired pH 6.0 threshold through this manipulation, while Lohi quickly re-acidified. Over time however, Lohi Lake has naturally recovered (pH>6). Clearwater was a reference lake in the early 1970's experiments and was not manipulated but it too naturally recovered. In this study we assessed the current benthic macroinvertebrate fauna in these four lakes and use them as bioindicators to compare the naturally recovered lakes and experimentally recovered lakes in Sudbury. We analyzed the total abundance, the percentage of acid tolerant species (% Chironomids) and acid intolerant species (% EOT) and the diversity of macroinvertebrates across the four study lakes. Five Ekman grabs in the littoral zone at ~ 1 m depth were collected from each lake. Macroinvertebrates were sorted and identified to Order/Family level. Total abundance was not significant among the lakes ($F=2.223, df\ 3,16$ $p=0.125$). The % chironomids was significant higher ($H\ 3, N= 20) =9.767$, $p =0.0207$) in Clearwater Lake than in Middle Lake. The % EOT was significant higher ($H\ (3, N= 20) =8.48$, $p =0.0371$) in Middle Lake than in Clearwater Lake. Both significant differences were between Clearwater and Middle lakes. The highest diversity was seen in the experimentally recovered lakes. Our results show that liming increases overall recovery rate. Recovery from acidification is however a complex problem needing further research.

Presenting Author: Mitchell Shaw and Laurin Cory

LeBlanc, Zacharie

Molecular and Cellular Biology, University of Guelph

A Functional Genetics Approach to Characterizing the Arabidopsis AtIDD1 gene.

IDD genes encode a large family of transcription factors (TF) with zinc finger motifs. Many homologues of the IDD (INDETERMINATE-DOMAIN) genes have been found throughout the plant kingdom. The ID1 gene in maize is the founding member of the IDD gene family; it is a TF which was found to regulate flowering time. In Arabidopsis, 16 IDD genes designated (AtIDD1-AtIDD16) have been found to have great sequence similarity to the IDD first characterized in maize. Of these genes, only AtIDD15 has been functionally characterized to date. Using gene knockouts, generated by T-DNA insertions, the AtIDD15 mutants showed deficiency in lateral shoot branching as well as gravitropic response. Currently little is known of the other AtIDD genes, microarray meta-analyses show that AtIDD1 transcripts are expressed most abundantly in dry seed. Therefore is AtIDD1 involved in seed development and/or germination? The following study makes use of a functional genetics approach to characterize the AtIDD1 gene in Arabidopsis by engineering a line of transgenic plants with constitutive overexpression of the AtIDD1 genes as well as a line of inducible knockouts for the gene. Additional analysis of AtIDD1 expression by RT-PCR will be carried out in order to verify microarray data from online resources. As no T-DNA knockouts currently exist for this gene and the TF itself has yet to be assigned a function, this study should provide valuable insight into AtIDD1's role in Arabidopsis.

Presenting Author: Zacharie LeBlanc

Leone, Danielle

Molecular and Cellular Biology, University of Guelph

The interaction of visual and vestibular sensory input: which is dominant in locomotion?

Understanding how the central nervous system interprets and integrates the sensory information of the head to generate controlled locomotion is of great interest and speculation. The purpose of this study is to probe the current ideology of vision being the dominant sensory input in the control of dynamic whole body movement. Specially, we are interested in investigating how external, investigator applied visual and vestibular manipulations influence the control of body segments during a locomotor task. Six healthy young adults performed a stepping in place task while watching a virtual visual scene that moved as if the observer was walking providing a perception of forward self-motion. While "forward" stepping, the virtual scene would randomly undergo a right or left rotation. Roughly one second ahead of the visual perturbation, galvanic vestibular stimulation (GVS) was applied at three times the subject's individual threshold to elicit conflicting visual and vestibular sensory inputs. Kinematics of the head, trunk, pelvis and feet were recorded and angular displacement (yaw and roll rotation) profiles were used to determine the magnitude and timing of reorientation in response to the visual and GVS cues. Four conditions were randomly applied during the trials. Two perturbations were applied in the SAME direction: left GVS/left vision, right GVS/right vision and two OPPOSITE to each other: left GVS/right vision, right GVS/left vision. Significant segmental movement was observed during both perturbation trails compared to straight trails ($p < 0.05$) at the head, trunk and pelvis segments for both yaw and roll rotations. After both perturbations were applied, subjects moved in the direction of the visual stimulus regardless if the visual and vestibular input was in agreement (SAME) or in conflict (OPPOSITE). Results suggest that while the central nervous system examines all sensory input, visual input is considered the most important information during adapted locomotor tasks.

Presenting Author: Danielle Leone

Leslie, Matthew, Meagan Mojeski and Lucy Mutharia

Molecular and Cellular Biology, University of Guelph

Cloning and expression of rpfA, rpfB and rpfE from Mycobacterium avium subspecies paratuberculosis.

Johne's disease is a chronic, enteric infection that commonly affects domestic ruminant animals. This disease is characterized by diarrhea and weight loss while the animal maintains a normal appetite. The causative agent of Johne's disease is Mycobacterium avium subspecies paratuberculosis (Map). Technical advances have allowed for the identification and isolation of Map from tissues of patients with Crohn's Disease leading to the hypothesis that Map may be involved in Crohn's Disease. Map is capable of entering a viable but non-culturable state (VBNC), requiring the presence of Resuscitation Promoting Factors (Rpfs) to exit this state and form colonies on solid media. Rpfs have been observed to have peptidoglycan hydrolase activity; however their role in the release from the VBNC state is currently unknown. Map contains four Rpfs: RpfA, RpfB, RpfC and RpfE, identified by homology to Rpfs found in other Mycobacterial species. The genes coding for these proteins have been successfully amplified using PCR and the PCR products have been inserted into a cloning vector. This project aims to clone the genes encoding for these four Rpfs into expression vectors and study the expression of the proteins in both E. coli and Mycobacterium vaccae. Once the proteins have been purified they can be further studied in an attempt to understand the role that they play in the exit of Map from the VBNC state.

Presenting Author: Matthew Leslie

Li, Fong-Sue

Biomedical Biology, Laurentian University

Liposomal alpha-lipoic acid: physicochemical characterizations.

Alpha-lipoic acid (LA) is a biological antioxidant which has potential ability to against oxidative stress-induced processes, such as acute respiratory distress syndrome (ARDS). However, free alpha-lipoic acid and its reduced form, dihydrolipoic acid (DHLA) are short-lived in biological environment. Liposome-entrapped lipoic acid enhances intratracheal delivery to the target site and exerts prolonged effect. The objective of my study was to determine and characterize the efficiency of the liposome. Alpha-lipoic acid was reduced to DHLA, following by reacting with monobromobimane (mBBr) to give fluorescence, and the standard curve was determined. The antioxidant was entrapped by freeze-drying method by the phospholipid formulation, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). The lipid capsule was incubated in four different conditions, which including PBS buffer at 4., PBS buffer at 37., human pooled plasma, and rat bronchoalveolar lavage (BAL) fluid over a 24 hour period. The % retention of LA in the liposome for each environment was measured. The entrapment efficiency of DMPC liposome was 34 ± 3.52%. The stability of it at 4. PBS buffer was the most stable of the four conditions, following by 37. PBS buffer, BAL, and the last was plasma. The retention rate of the liposomal lipoic acid in BAL was 73.8 ± 1.72% after incubating for 0.5 hour, indicating a releasing of 26.2% of LA. It maintained a retention rate by which no greater than 2.6% of LA was released in any of the rest of the time intervals. On the other hand, LA showed a constant releasing over 24 hours in plasma. The data suggest that liposomal lipoic acid could be managed for storage at low temperature and able to maintain its prolonged effect in BAL-fluid environment.

Presenting Author: Fong-Sue Li

Lippa, Natalie

Molecular and Cellular Biology, University of Guelph

The Analysis of the Components of Quorum Sensing System 2 in Mannheimia haemolytica A1.

The Analysis of the Components of Quorum Sensing System 2 in Mannheimia haemolytica A1Mannheimia haemolytica A1 is the primary causative agent of bovine pneumonic Pasteurellosis. M. haemolytica possesses a luxS homologue that produces autoinducer-2 (AI-2) like molecules which can induce the quorum sensing system 2 of Vibrio harveyi. A luxS mutant of M. haemolytica has been constructed and has been shown to have altered expression of virulence genes. The other proteins in the quorum sensing system 2 pathway include LuxP, LuxQ and LuxO proteins. To determine if these components of the quorum-sensing system 2 pathway exist in the M. haemolytica genome, a BLAST search was conducted using the V. harveyi LuxP, LuxQ and LuxO amino acid sequence. It was shown that there are luxP, luxQ and luxO homologous genes within the M. haemolytica genome. An in silico analysis was conducted to determine the size, domains and cellular localization of the homologous proteins. It was shown that the LuxPM.h. and LuxOM.h. were similar to the LuxPV.h. and LuxO V.h.. However, the LuxQM.h. showed more similarity to the ArcB protein of Escherichia coli than to the LuxQ V.h.. PCR was used to amplify the luxPM.h.and a recombinant plasmid containing the luxPM.h. gene was constructed. The luxQM.h gene was amplified using a PWO polymerase which was shown to be more effective at amplifying the luxQM.h gene than Taq polymerase. However, the ligation of a lipoprotein promoter to the luxQM.h gene was hindered by the low yield of luxQM.h amplification and the presence of primer dimers. Future studies will involve creating luxQM.h and luxOM.h recombinant plasmids and using the recombinant plasmids to complement luxP, luxQ and luxO mutant strains of V. harveyi. If complementation does occur it will provide insight into the role of quorum sensing system 2 in the regulation of virulence genes in M. haemolytica.

Presenting Author: Natalie Lippa

Livernois, Alexandra

Molecular and Cellular Biology, University of Guelph

Expression and characterization of grape dehydrin.

Dehydrins are glycine rich, hydrophilic and heat stable plant proteins which are induced in response to dehydrative stress. Two grape dehydrins, YSK2 and K2, were cloned into the pET-22b vector using the restriction sites Nde1 and Xho1 which were added by polymerase chain reaction. The YSK2 and K2 proteins were produced using the expression system of Escherichia coli. Purification explored the characteristic high temperature solubility of dehydrins by boiling lysates. The presence of the purified proteins was shown by a high level of purity on a tris-tricine gel. Further purification was attempted, but not attained through affinity and HPLC chromatography. Consequently, site directed mutagenesis was used to add stop codons at the ends of the YSK2 and K2 sequences, preventing their expression with histidine tags. Conditioning, a method used to maximize protein expression from a minimal media, was used to produce proteins. The conditioned expression was successful as was shown by similar expression of the proteins from rich media. Stress testing was used to investigate the ability of the YSK2 and K2 proteins to protect Escherichia coli under cold and desiccation stress.

Presenting Author: Alexandra Livernois

Lomas, Nichelle and John Barta

Integrative Biology, University of Guelph and Pathobiology, University of Guelph

Use of nuclear 18S ribosomal DNA sequences of gregarines infecting earthworms to help resolve the phylogenetic affinities of Cryptosporidium to other apicomplexan parasites.

Growing evidence suggests Cryptosporidium species may not be especially closely related to coccidian, which they had been previously classified. Research based on the similar life cycles, host-parasite interactions and anticoccidial insensitivity suggests that Cryptosporidium is evolutionarily more closely related to the gregarines (Barta and Thompson, 2006). To analyze the phylogenetic relationships between these parasites more needs to be known about the gregarines, especially a wider range of molecular data. To obtain this data, gregarines were isolated from the seminal vesicles of earthworms obtained locally and DNA was extracted by breaking the parasites with glass beads and several ethanol washes. The nuclear 18s rDNA was amplified using gregarine-specific primers designed from known gregarine sequences from GenBank and universal eukaryotic rDNA primers. Products were run on a gel and several amplified fragments appeared to be the correct size, about 1100 or 1200bp. These were cycle sequenced using the same universal eukaryotic rDNA primers that were used to amplify the fragment. Two similar sequences were found and confirmed to be gregarines by comparison with known gregarine sequences. The gregarine amplicons were cloned using the TOPO-TA cloning method to provide a ready supply of sequencing template; additional clones are being analyzed to support the preliminary data. Observation of two similar, but distinct, sequences supported our observations that two morphotypes were visible microscopically. Sequences are being comparison with published sequences of gregarines, coccidia and Cryptosporidium found in GenBank. A phylogenetic hypothesis will be generated for the relatedness of these parasites by creating phylogenetic trees based on maximum parsimony and maximum likeliness. This research may assist understanding of Cryptosporidium and it maybe possible to use gregarines as model species for further research if they are more closely related then previously thought. This will assist in creating effective methods for treatment of cryptosporidiosis in animals and humans.

Presenting Author: Nichelle Lomas

Loo, Tenneille, Joseph Macri and Bernardo Trigatti

Biochemistry and Biomedical Sciences, McMaster University: Pathology and Molecular Medicine, McMaster University: Biochemistry and Biomedical Sciences, McMaster University

Investigating the post-translational modifications of apolipoprotein B in SR-BI-/- mice.

Scavenger Receptor Class B Type I (SR-BI) are receptors found on HDL molecules that are implicated in delaying atherosclerosis through the reverse cholesterol pathway. However, while role of SR-BI in HDL cholesterol has been relatively well studied, there need to be more studies on its effects on other lipoproteins. Bone marrow transplant of SR-BI+/+ into SR-BI-/- hypomorphic apolipoprotein E mice (fed a high fat diet for ether 4 or 12 weeks) are characterized with fatty livers with no noted changes to their lipid profile. It is unknown whether these lipoproteins changes are due to post-translational modifications to the apolipoproteins within the lipoproteins, which may affect the quantity of lipoproteins secreted by the liver or intestine, or overall lipoprotein size. Moreover, it is unknown whether these treated mice have any changes in their apolipoprotein composition of B, E, and A-1, whose changes can also affect lipidation and lipid composition. Given that apolipoprotein B-100 and -48 (apoB-100 and apoB-48) levels correspond directly to the levels of their respective lipoprotein, the levels of LDL, VLDL, and chylomicrons are therefore quantifiable. Furthermore, since post-translational modifications to apoB are known to change the lipoprotein profile, the large apoB-100 (512kda) and -48 (241kDa) were specifically studied to elucidate these issues by using the techniques of 1D and 2D gel electrophoresis, western blotting, and phosphorimager quantification programs. The question that this project attempts to answer is: What are the effects of a bone marrow transplant of SR-BI+/+ into SR-BI-/- hypomorphic apolipoprotein E mice on the apoB structure, and apoB post-translational modification, and apolipoprotein composition of apolipoproteins B, E, and A-1?

Presenting Author: Tenneille Loo

MacIvor, J. Scott, Gard Otis, Jonathan Schmidt and Rob McLaughlin

Integrative Biology, University of Guelph and Environmental Biology, University of Guelph

Dietary influence on pre-mating behaviour and mate choice in large milkweed bugs (Oncopeltus fasciatus).

In Oncopeltus fasciatus, females show a strong preference to mate with males raised on milkweed seeds, their natural diet, opposed to males raised on sunflower seeds. Therefore, male diet influences mating success. However, it is unknown if females assess potential mates during pre-mating behaviour. Two subpopulations of O. fasciatus have been maintained over several generations on either milkweed seeds (Asclepias syriaca) or sunflower seeds (Helianthus annuus). Major elements of courtship in males and females were identified using milkweed-fed females presented with either a milkweed-fed male or a sunflower-fed male. Mating behaviour was analyzed using frame-by-frame video software. An ethogram accompanying time budget was created for each treatment type to determine if male diet influences sexual behaviour. To create males containing different amounts of milkweed-derived compounds, one group of O. fasciatus reared from eggs on milkweed seeds was switched to sunflower seeds upon moulting to 5th instar. Mating trials consisted of two treatments: “high quantity” males (reared completely on milkweed seeds) and “medium quantity” males (those switched to sunflower seeds at 5th instar). It is predicted that sexual behaviour in the first and last mating attempts of milkweed-fed males leads to female acceptance behaviour. Female reluctance behaviour will correlate with behaviour towards sunflower-fed males. Mating success will be greater in males of higher concentrations of milkweed constituents, thus providing evidence for a selective advantage in males that acquire high quantities of milkweed-derived compounds prior to mating.

Presenting Author: J. Scott MacIvor

Macklaim, Jean and Robert Hegele

Biology, University of Western Ontario and Medicine and Biochemistry, University of Western Ontario

Resequencing the LMF1 gene from genomic DNA of patients with severe hypertriglyceridemia.

OBJECTIVE: Hypertriglyceridemia (HTG) is a complex quantitative genetic trait involving many genes and environmental factors. As a result, the genetic basis of the disease is poorly understood and only a small fraction of hypertriglyceridemic patients have a discrete, causative mutation identified. The association of HTG with many other disorders, including metabolic syndrome and diabetes, mean there is an increased risk factor for cardiovascular disease for those with the disease. Very high plasma triglyceride levels also increase the risk of pancreatitis. A recent study in a mouse model found a missense mutation in a novel gene, Lmf1 (Lipase maturation factor 1), to be associated with extreme HTG by causing a lipoprotein lipase (LPL) deficiency. A homologous mutation in the human ortholog of LMF1 in a patient who also exhibits severe HTG and lipase deficiency, suggests that LMF1 is an important candidate gene in HTG. We therefore resequenced the LMF1 gene to search for accumulation of missense mutations in patients with severe HTG compared to normolipidemic subjects. METHODS AND RESULTS: We resequenced genomic DNA from 43 nondiabetic patients with severe HTG, and will determine the prevalence of coding sequence variants compared with 44 age- and sex-matched normolipidemic controls. Most significantly, we have found a heterozygous deletion causing a frameshift mutation and an early truncation of the LMF1 protein (R82G > 162X) in an HTG patient.

Presenting Author: Jean Macklaim

MacMillan, Heath and Brent Sinclair

Biology, University of Western Ontario

The effects of rapid cold hardening on free glucose and phospholipid head group ratios of Drosophila melanogaster.

Exposure of insects to a non-lethal low-temperature stress can result in moderate to high survival of an otherwise lethal subsequent exposure. This phenomenon has been labeled rapid cold hardening (RCH) and the physiological mechanism responsible for its action is currently unknown. The current study investigates two potential mechanisms of the RCH response, accumulation of glucose and membrane phospholipid remodeling in cold-selected lines of the fruit fly (Drosophila melanogaster) using colourimetric assay techniques and thin layer chromatography-flame ionization detection (TLC-FID), respectively. Contrary to previous evidence, glucose concentration does not appear to increase in response to an RCH treatment and was noted to decrease in many cases. Also, phospholipid head group ratios showed no change with respect to an RCH treatment suggesting a mechanistic difference between acclimation and RCH. Selection for cold tolerance appears to affect basal glucose levels but not phospholipid head group ratios in Drosophila melanogaster.

Presenting Author: Heath MacMillan

Mahmood, Tanya

Biological Science, University of Windsor

Role of the spindle assembly checkpoint in drosophila meiosis.

The Spindle assembly checkpoint (SAC) is a cell cycle checkpoint responsible for ensuring proper chromosome alignment before anaphase progression. When the SAC is activated it delays anaphase progression by inhibiting APC (anaphase promoting complex). The APC is responsible for destroying mitotic cyclins, and this is required for the cell to exit mitosis. An APC adaptor, Fzy is responsible for the recognition of cyclin B by APC for destruction in mitosis. Our lab has shown that in Drosophila female meiosis, Cort, a meiosis specific APC adaptor, and Fzy are both responsible for the destruction of cyclin B . We found that in cort mutants cyclin B accumulates specifically at the spindle midzone, implicating the APCCort in local cyclin B destruction. It is not clear why, in meiosis, Fzy does not target this cyclin B for destruction. This could be the result of SAC keeping Fzy inactive at this site. To get some insight into the activity of the SAC in meiosis we are using a combination of genetic and localization studies. We found that cyclin B fails to accumulate at the spindle mid zone when Cort and the SAC component, Zw both are turned off, suggesting that the SAC normally functions to inhibit APCFzy at this site. To confirm the results we are using a triple mutant of fzy, cort and zw to see if this can restore cyclin B on the spindle midzone. We are also looking at the localization of the SAC components and the APC to see if they are localized during meiosis. These studies will allow us to understand how the cell cycle machinery is adapted for the specialized meiotic divisions.

Presenting Author: Tanya Mahmood

Makaji, Emilija

Obstetrics & Gynecology, McMaster University

Effect of Maternal exposure to herbal preparation – Red Raspberry leaf on the drug metabolizing capacity of the offspring.

Although many people share a belief that natural products are safe, there have been many reports of adverse effects from drug-herbal interactions. In addition, fetal exposure to some xenobiotics has been shown to result in irreversible alteration of Cytochrome P450 drug metabolizing enzymes (CYPs), a phenomenon known as imprinting. Red raspberry leaf tea (RRL) is widely used by pregnant women because it is thought to facilitate labour. However, RRL contains a number of polyphenols, a class of compounds known to affect the activity of drug metabolizing enzymes. Therefore the goal of the study was to determine whether maternal exposure to RRL can permanently alter biotransformation of fluorogenic substrates by the CYP enzymes in liver of both male and female offspring.In an animal model, female rats were treated with RRL for the entire duration of their pregnancy. Livers from the offspring were collected at various life stages and microsome preparations were made. Biotransformation of eight fluorogenic substrates from each treatment group was compared to that of control animals. There were marked differences in the biotransformation of some of the substrates between the sexes. However, maternal RRL consumption had no significant effect on biotransformation of any of these substrates by rat liver microsomes in offspring of either gender. These results suggest that RRL does not cause imprinting of CYP enzymes which in turn implies no long term consequences of maternal consumption of RRL on biotransformation of xenobiotics by the offspring.

Presenting Author: Emilija Makaji

Manase, Cristina

Molecular and Cellular Biology, University of Guelph

The impact of microRNA on animal development and its potential use for human therapy.

The discovery of the first microRNA in 1993 has led to the explosion of a new field that has sprouted exponentially in the past decade. It has provided novel understanding of genetic regulation through a process called the RNAinterference pathway. This transcriptional regulation pathway has unique methods of shutting off genes. Work on animal and plant species have detailed the pathway and provided insight for novel therapeutic techniques. Researchers are currently experimenting with this technique in hopes of creating many potential therapeutic applications including cancer treatment. RNAinterference causes the shutting down of genes, and may be used to shut down mutated genes as found in certain cancer patients. Although there have been ethical concerns with the delivery method of microRNA into the human body, experiments on mice, humans and other mammals are currently undergoing. The application of RNAinterference as human therapy is in the process of being perfected for use in the near future.

Presenting Author: Cristina Manase

Mancini, Amanda, Keegan Hicks and Jim McGeer

Biology , Wilfrid Laurier University

Gill accumulation and toxicity in five species of fish in soft water.

The biotic ligand model (BLM) is currently the most advanced tool for predicting metal toxicity in aquatic systems based on site-specific water chemistry. The BLM has recently been adopted by international agencies as a risk assessment tool, however, there are still some uncertainties associated with approach. For example, BLMs for Cu effects on fish (limited to rainbow trout and fathead minnows) in hard water have been published but applicability of these models to other species and in soft waters is unknown. This project is concerned with testing validity of Cu BLMs in very soft waters (representative of those of the Canadian Shield) using native fish species. The relative sensitivity of these species was tested by comparing acute toxicity responses to copper over a ninety-six hour period. Each species was exposed to five concentrations of Cu (plus a control) in flow-through system with soft water (conductivity ranged from approximately 30 to 35 mS). The LC50 values ranged from 3.5 mg/L for brown trout to >22 mg/L for lake trout. Short-term (3-hr) gill binding tests were also performed, in which fish were exposed to Cu and other ions, such as Ca and Na. The gills were sampled, digested and analyzed for Cu accumulation. As expected from BLM, Cu accumulation is inhibited by increased levels of Na+. The degree of protection provided by Na+ varied among species, but increased levels of Ca2+ in exposure medium had no effect on Cu binding. Measured Cu accumulations were compared to BLM predictions of accumulation. The BLM underestimated both Cu accumulation (for the binding tests with increased Na), and toxicity (in terms of the LC50 values). This study will offer insight into the capabilities of the BLM when it is tested in very soft waters and on fish species characteristic of the Canadian Shield.

Presenting Author: Amanda Mancini

Marchand, Kimberly and K. Peter Pauls

Plant Agriculture, University of Guelph

Identification and characterization of a gene involved in microspore embryogenesis in brassica napus.

Previous work in the Pauls laboratory using an Arabidopsis microarray revealed an up regulation of Brassica napus genes from microspores undergoing embryogenesis. One gene, which codes for a protein of unknown function, was expressed 3.05 fold higher and is the focus of this study. Primers were designed for PCR and sequencing of the gene from canola genomic DNA was carried out, revealing new genetic information. Four different lines of mutant Arabidopsis plants (for the gene of interest) were also obtained and grown for observations. These plants showed no distinct developmental or morphological differences from wild type plants. BLAST analysis using the Arabidopsis gene revealed that it may be part of a larger gene family, which could explain why no visible mutant phenotype was observed. Gene and protein bioinformatics analysis was also conducted to try and elucidate hypothetical functions of the Arabidopsis gene. This analysis revealed two possible transmembrane domains as well as other recognized sites involved in various interactions. Furthermore, a domain of unknown function (DUF246), was found within the protein. This domain was also found within the proteins encoded by the two genes found from the BLAST search of the Arabidopsis genome. This domain is found in some rice F-box proteins; however analysis shows that our gene of interest does not have the conserved F-box domain common to these proteins. Further studies regarding the expression levels and patterns at various times during embryogenesis, as well as the possible expression of this gene during other developmental time points in the life of the plant, may provide further information of the function of the gene and it's product.

Presenting Author: Kimberly Marchand

Marini, Andrea

Molecular and Cellular Biology, University of GUelph

Using affinity chromatography to investigate protein-protein interactions involved in peptidoglycan synthesis in pseudomonas aeruginosa.

Maximum 300 wordsLytic transglycosylases cleave the ß 1-4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine residues of peptidoglycan. Penicillin binding proteins catalyze transpeptidation and glycosyltransfer reactions to polymerize peptidoglycan. The purpose of this study is to observe a protein-protein interaction between penicillin binding protein 2 (PBP2) and soluble lytic transglycosylase B1 (SltB1) in peptidoglycan synthesis of Pseudomonas aeruginosa using affinity chromatography. Both proteins were expressed in E.coli and French Pressed, but only SltB1 was purified by High Performance Liquid Chromatography (HPLC) and dialysis. The purified SltB1 was then immobilized in an Econo Pac column by UltraLink resin and sPBP2 lysate was introduced in hopes of an interaction. Affinity chromatography was then used to displace sPBP2 from SltB1 and fractions were analyzed by SDS-PAGE and Western blots.

Presenting Author: Andrea Marini

Marshall, Caroline, Lisa Allen and Frederique Guinel

Biology, Wilfrid Laurier University

Using root-organ cultures to study the development of mycorrhizae.

Arbuscular mycorrhizal (AM) symbiosis is observed in over 80% of land plants; it occurs between the roots of a plant and mycorrhizal fungi of the order Glomales. It is mutualistic as both partners gain from the relationship. E107 is a pea mutant forming few AM associations. By grafting, it was shown that an E107 root and wild-type (WT) shoot graft produces normal mycorrhizal development, while E107 shoot/WT root graft exhibits a low rate of symbiosis. This suggests that the inhibition of mycorrhizal formation in E107 is controlled by its shoot; as it must be a component easily transferred from shoots to roots, this component is likely contained within the phloem sap. We propose that the E107 phloem shoot exudate inhibits AM symbiosis development. To test this, we will isolate phloem sap to apply it to pea root-organ cultures (ROC). ROC is an in vitro system whereby transformed roots grow continuously in the absence of a shoot on sucrose-supplemented agar. The short-term objectives are: 1) establish ROC for both WT and E107 lines; 2) inoculate them with Glomus intraradices to study mycorrhizal development and its potential inhibition by the E107 phloem exudates.ROC are produced via Agrobacterium transformation. Transformed roots are then transferred to Petri plates and challenged with spores of G. intraradices. After successful inoculation of ROC, the isolated phloem exudate is applied to the plates to ascertain whether or not any inhibition of symbiosis is exhibited. The progression of the fungus will be followed with microscopy (dissecting + light) so that a block in the symbiosis can be uncovered. We anticipate that inoculation application of the extract to inoculated ROC will result in an inhibition of mycorrhizal growth. Should the phloem exudate prove to be a successful inhibitor, the phloem sap will then be characterized and its components analyzed.

Presenting Author: Caroline Marshall

Maslikowska, Julia, Heather Freamo, Danielle MacDonald and Elizabeth Boulding

Integrative Biology, University of Guelph: Integrative Biology, University of Guelph: Mactaquac Biodiversity Centre, Fisheries and Oceans, New Brunswick

Morphological differentiation of three endangered inner Bay of Fundy Atlantic salmon (Salmo salar L.) populations.

Atlantic salmon (Salmo salar L.) populations exhibit anatomical, physiological, as well as behavioural differences. However, the extent to which variation in phenotype is driven by differences among habitat is not known. Here, we investigate the degree to which environment, as opposed to genotype, drives morphometric differences among three distinct inner Bay of Fundy (iBoF) salmon populations (i.e., Big Salmon River [BSR], Point Wolfe River [PWR], Upper Salmon River [USR]). If differences in body shape and size among parr specimens from the three populations result from genetics, then morphometric differences will persist in a common environment. To test this prediction, in February 2008, 300 photographs, corresponding to that of offspring that were reared at Mactaquac Biodiversity Facility in Fredericton under identical conditions after being spawned in November 2006, were digitized at the University of Guelph. To achieve this, TpsDig2 software was used in the designation of 12 predetermined landmarks along the lateral side of each fish. Based on landmark data, TpsRegr and TpsUtil were collectively used in the computation of aligned x- and y-coordinates and composite size values. Mean salmon population size and shape were then compared using univariate ANOVA analyses. Specimens corresponding to that of USR were significantly larger than those of PWR which, in turn, were significantly larger than those of BSR. The populations also exhibited differences in shape. Mean snout elevation was significantly higher among PWR fish than among USR fish. In light of the fact that iBoF salmon are endangered, these results suggest that the establishment of population-specific breeding programs would promote the preservation of life history traits unique to each population which may, in turn, improve Atlantic salmon restoration efforts.

Presenting Author: Julia Maslikowska

McClurg, Brian

Integrative Biology, University of Guelph

Effects of climate change on tri-trophic interactions in temperate grasslands: insights from a simple model.

This planet's changing climate threatens more than just the air humans breathe and the water we drink. There are currently more than 970 farms invested into wheat production in Ontario alone (Statistics Canada. 2006). Increases in global temperatures and atmospheric levels of CO2, as well as alterations in precipitation patterns, have the ability to reduce food crop yields by altering the abundance and distribution of harmful crop pests like the cereal aphid Rhopalosiphum padi. Understanding and modelling the interactions of C3 wheat crops, cereal aphid populations and their natural predators, the aphid midge Aphidoletes aphidimyza, will allow predictions about insect population growth responses under climate change. Wheat species, cereal aphid and aphid midge population responses will be measured using a stoichiometrically complete, computer-based model. The model dynamically couples three trophic levels using a water presence sub-model, a wheat plant physiological processes sub-model, a cereal aphid population sub-model and an aphid midge population sub-model, through the use of differential mathematics equations. I will project climate change in southern Ontario for two different emission scenarios [A2 – High emissions and B2 – Low emissions] and compare insect population responses for a baseline time period from 1961 to 1990 with those of the 2020s (2010 to 2039). Climate change data for both emission scenarios are acquired from the Climate Change Scenarios Network (CCSN) Special Report on Emission Scenarios (SRES). It is anticipated under projected climate change that conditions will be made unfavourable for aphid midge populations and their size will decrease, hence the aphid midge populations will be unable to maintain acceptable levels of the crop pest cereal aphids. Pending the results of this study, added emphasis may need to be directed towards additional biological control efforts or other innovative management strategies to ensure the sustainability of Ontario's wheat crops.

Presenting Author: Brian McClurg

McGrath, Kelly

Biology, Laurentian University

PH-dependent expression of phosphatase activity in mycobacteria.

ABSTRACTThe respiratory disease, tuberculosis has taken the lives of many people across our world for thousands of years. It is caused by infection of Mycobacterium tuberculosis in which aerosolized droplets from an active pulmonary tuberculosis patient are inhaled by a recipient. M. tuberculosis is a pathogenic mycobacteria and highly contagious to human hosts. Through DNA-DNA and 16S RNA analysis it has been discerned that Mycobacterium marinum is the closest genetic relative to M. tuberculosis.All pathogenic mycobacteria including M. marinum produce a protein recently identified as secreted acid phosphatase or sapM. This protein is believed to assist mycobacteria in inhibiting phagosome maturation in a host cell by halting the acidification of the bacteria, thereby stalling the cells' natural immune response to the infection. It has been shown in previous research from the same laboratory as this project that sapM production is enhanced when the mycobacteria is cultured under mildly acidic conditions. M. marinum cells were cultured in regular Sauton's media and in Sauton's media containing ammonium chloride. The pH values during growth of the cultures showed increased acidity in the NH4Cl Sauton's sample. After culturing, RNA was extracted from both samples using a unique RNA extraction protocol method. Quantification of sapM mRNA was performed using quantitative real time polymerase chain reaction technique. RT-qPCR showed a definitive increase in the amount of sapM produced in the more acidic culture of NH4Cl Sauton's media. Thus, the assumption that M. marinum grown in acidified media produces a larger amount of sapM protein was proven true.Future research must verify increased sapM production in M. tuberculosis in mildly acidic conditions. The importance of this protein and whether it is responsible for the pathogenicity of tuberculosis will assist researchers in their development of improved treatment and control of this respiratory disease.

Presenting Author: Kelly McGrath

McGregor, Caroline

Molecular and Cellular Biology , University of Guelph

Fluorescence Resonance Energy Transfer Investigations of the Interactions between Carboxysomal Shell Proteins of Thermosynecoccus elongatus BP-1.

Thermosynechococcus elongatus BP-1 possess large protein complexes called carboxysomes. These polyhedral protein bodies are responsible for carbon dioxide fixation, by encapsulating enzymes such as RuBisCo and carbonic anhydrase. Carboxysomes are composed of several shell proteins including CcmK1, K2, K3, K4, CcmO and CcmL. Studies have shown that the shell proteins organize themselves into hexagonal monomers, forming a sophisticated tiling patterns. We hypothesize that the hexagonal protein monomers have preferred interaction partners that specify the tiling pattern of the carboxysomal shell. To test this hypothesis, our experimental approach is to label purified T. elongatus BP-1 carboxysomal shell proteins with thiol reactive fluorophore dyes for analysis by fluorescence resonance energy transfer (FRET). In preparation for FRET, constructs encoding CcmK4 and CcmK1Q71C have been generated. Preliminary over-expression and purification experiments of CcmK1 and CcmK4 have been completed and analyzed by SDS-PAGE.

Presenting Author: Caroline McGregor

McRae, Margaret

Biology, McMaster University

Environmental Dependant Phenotypic Plasticity in Echinoderms.

The echinoid species Arbacia punctulata transforms from a bilaterally symmetrical larva into a penta-radially symmetrical juvenile. The imaginal rudiment is a group of cells that form on the left side of the larva and develop into the body of the adult sea urchin. The growth of the larvae is plastic, being influenced by factors in the environment. One of these factors is the quantity of food available which determines when the rudiment is formed and its rate of growth. The question addressed in this experiment is whether the rudiment can be resorped during times of nutritional stress. If this is true and sea urchin larvae containing a rudiment are subjected to starvation conditions, then the process of rudiment resorption will occur, delaying development of the juvenile form. This outcome would suggest phenotypic plasticity in the ability of the larvae to retain or discard the rudiment. Furthermore, if recommencing feeding can induce the larvae to form a second rudiment then the phenotypic plasticity may be adaptive. The larvae could stop rudiment development when food were scarce and reinitiate rudiment development when more resources were available. As the digestive system of the larva is reconfigured during metamorphosis, the new juvenile must depend on nutrients stored in the larva for a period of time. Therefore, having larger nutrient reserves in the larvae before they undergo metamorphosis could increase their chances of survival as young juveniles.

Presenting Author: Margaret McRae

Melas, Marisa

Biology, McMaster University

The effect of blood cell tnf-alpha on the progression of sandhoff disease in mouse models.

Sandhoff lysosomal storage disease is caused by a defect in the hexb gene that results in ganglioside accumulation, leading to neurodegeneration and death in children of 2 to 5 years of age. Inflammation of the central nervous system (CNS) plays a major role in the progression of Sandhoff disease. During inflammation, invading blood cells excrete cytokines to regulate the immune response. Tumor necrosis factor alpha (TNFa) is one such cytokine whose negative role in Sandhoff disease progression has been demonstrated in mouse models; tnfa -/- hexb -/- double knockouts (DK) mice have improved life expectancy and neurological function (Igdoura lab, unpublished). It remains unclear if the majority of TNFa observed in the CNS is a product of endogenous cells or if the invading blood cells of the inflammatory response mediate the incursion of TNFa. In an effort to elucidate the origin of TNFa contributing to disease pathology, two a bone marrow transplants (BMT) were conducted. In the first, BM from hexb -/- mice was transplanted into the hexb -/- tnfa -/- double knockout (DK). The reintroduction of TNFa into solely blood cells was hypothesized to mimic characterized disease course rather than aforementioned improvements of DK mice if blood cells play a role in TNFa increase. The second BMT involves transplantation of hexb-/- tnfa-/- DK BM into hexb-/- mice. This results in wild type TNFa gene being present in all cells of the body except the blood cells. If blood cells play an important role in TNFa overexpression then it would be expected that these mice would have ameliorated disease pathology. In addition, behavioural tests were conducted on a wide array of genotypes in order to establish standard disease pathology. Preliminary results suggest that blood cells do in fact play a role as distributors of TNFa during Sandhoff disease course.

Presenting Author: Marisa Melas

Miao, Qianqian Doreen

Molecular and Cellular Biology, University of Guelph

Characterization of Enzymes WbpK and WbpV from Pseudomonas aeruginosa serotypes O5 and O6.

Pseudomonas aeruginosa is an opportunistic pathogen which infects cystic fibrosis patients and compromised individuals. Lipopolysaccharide (LPS) is an important virulence factor. The O-antigen polysaccharide is the outer-most domain of LPS and N-acetyl-D-fucosamine (FucNAc) and N-acetyl-D-quinovosamine (QuiNAc)L are O antigen residues in serotypes O5 and O6 respectively. WbpM is a UDP-N-acetyl-D-glucosamine (UDP-GlcNAc) 4,6-dehydrogenase which synthesizes a 4-keto sugar-nucleotide product. wbpM is present in both serotypes O5 and O6. We hypothesize that WbpK (from serotype O5) and its homolog WbpV (from serotype O6) reduce the 4-keto sugar-nucleotide intermediate and produce the UDP-FucNAc and UDP-QuiNAc O-antigen precursors. WbpK and WbpM His6-tagged fusions Lwere co-expressed in E.coli. Since both proteins are associated with the membrane fraction, purified E. coli membranes containing these proteins were incubated with UDP -GlcNAc and NADH. The results were analyzed by Capillary Electrophoresis (CE). No WbpK activity was observed, possibly because the putative WbpK co-factor, NADH, was oxidized rapidly, probably by the NADH oxidase associated with E.coli. membranes. Several detergents were tested and Triton X-100 and n-Octyl-ß-glucopyranoside Lwere found to solubilize WbpK. Current work in progress is to purify the solubilised, His6-tagged WbpK using immobilized Ni2+ ion affinity chromatography. The same experiments used to characterize WbpK will be used in the characterization of WbpV.

Presenting Author: Qianqian Doreen Miao

Miller, Ashley, Jacob Robinson and Jim Ballantyne

Integrative Biology, University of Guelph

Incorporation of exogenous ammonia into amino acids in Nile tilapia (Oreochromis niloticus) on high and low protein diets.

Recent research has found that low levels of exogenous ammonia can promote growth in rainbow trout juveniles. Another study on the ureogenic abehaze goby (Mugilogobius abei) and a mudskipper (Periophthalmus modestus) revealed that exogenous ammonia is incorporated into the amino acids of these fish. This study aims to determine if incorporation of exogenous ammonia can occur in ammonotelic fish and if nitrogen limited fish incorporate more ammonia than non-nitrogen limited fish. Juvenile tilapia were housed in the Hagen Aqualab, University of Guelph, Guelph ON and were maintained on two different diets: a high and a low protein diet. Eight fish from each group were then exposed to 1mM of 15N-Ammonium chloride and six fish were exposed to water as controls. Both groups had daily water changes. The whole fish were analyzed to determine the proportion of 15N incorporated into the free amino acid pool and the protein amino acids. There appeared to be 15N incorporation in the free amino acid pool of the low and high protein groups. Water samples from the final 24 hours of exposure were stored at -80*C until analysis. The concentration of ammonia in the water samples were measured along with the proportion of 15N remaining in the water. Enhanced growth in the presence of ammonia may be useful in an aquaculture context.

Presenting Author: Ashley Miller

Miron, Corey and C.L. Milligan

Biology, University of Western Ontario

The influence of swim velocity on recovery of swim performance after exhaustive exercise in juvenile Rainbow Trout (Oncorhynchus mykiss)

Research has concluded that following a bout of exhaustive exercise, fish are able to recover muscle metabolites and swim performance more efficiently in a current than in still water. The objective of this study was to determine if an optimal current for recovery exists in juvenile rainbow trout (Oncorhynchus mykiss). Fish were exercised to exhaustion and were allowed to recover in either 0.5 body lengths per second (BL/s) current or 2.0 BL/s current. Recovery was monitored after 0.5, 1, 2 or 4 hours. At 2.0 BL/s the number of individuals recovering swim performance begins to decline after recovering for 4 hours (from 69% to 44%). To uncover the possible cause of the decrease in percent recovered after 4 hours, muscle lactate and inorganic phosphate were measured. These metabolites do not appear to be correlated to the drop in percent of individuals recovered. However, lactate did appear to decline in concentration after recovering in current compared to levels immediately following exhaustion. Inorganic phosphate did not show a correlation to recovering in current over any length of time.

Presenting Author: Corey Miron

Monette, Nicole

Biology, Laurentian University

The effects of waterlogging on fine root morphology, production and turnover rate of a wetland grass, Calamagrostis canadensis (Michx.) Beauv. in the fall season.

Root structural and functional aspects were assessed in response to soil waterlogging.The effects of waterlogging on root structure and root turnover were investigated using a wetland grass characteristic for Northern Ontario, Calamagrostis canadensis (Michx.) Beauv. The experiment was conducted outdoors in an experimental garden. The grass was grown in 10L pots filled with top soil for 16 weeks under equal soil moisture conditions, at which point the treatments were imposed for 17 weeks. Waterlogging was achieved by immersing, to the rim, the pots in pools of water. The other treatment consisted of freely draining pots, being supplied with sufficient water to avoid drought stress. Root production was measured for a 6-week interval between August 31st and October 26th using ingrowth cores. Waterlogging had no effect on root production, the average for both treatments being 47 g/m2. Root mortality was assessed by staining with triphenyl tretrazolium chloride. Most of the roots remained alive until the end of the experiment. On October 26th, only 12% were dead, and by December 16th this percentage had increased to 30%. Specific root length (SRL) was not affected by waterlogging, but increased by 23% from the first to the second collection. Root fineness, diameter and density were assessed during the second collection and were not affected by waterlogging. Lastly, cross-sections of a few waterlogged roots showed the presence of aerenchyma. I conclude that that C. canadensis is well adapted to waterlogged conditions. The lack of any effects of flooding on root structure and turnover indicated that root structure is constitutively adapted to low oxygen conditions. However, more detailed studies would be necessary to assess the detailed effect of flooding on the carbon budget of this species.

Presenting Author: Nicole Monette

Mroz, Caitlin, C. David Rollo, G. McClelland and Vadim Aksenov

Biology, McMaster University

Assessment of left ventricular hypertrophy in transgenic rat growth hormone mice.

Laws of Allometry see that larger animals have proportionately larger organs. Transgenic rat growth hormone mice have chronically elevated levels of GH and insulin-like growth factor-1 (IGF-1). Prelimary research has observed TG mice to have disproportionately larger hearts when compared to normal mice. Cardiac hypertrophy is characterized by an enlarged heart and enlargement of the left ventricle. It is a physiological response to extracellular stimuli such as growth factors, prolonged excess workload and mechanical and oxidative stress. LV hypertrophy is one of the earliest signs of heart failure and is an independent risk factor for cardiovascular diseases. Recent research shows a connection between excess growth hormone (GH) and LV hypertrophy. LV hypertrophy was compared between normal mice and TG mice. Heart morphology was standardized by body weight and tibia length to assess degree of hypertrophy. As expected, the absolute value of the heart weight and left ventricle weight were larger in transgenic mice. When standardized by body weight and tibia length there was a significant difference compared to the standardizations of normal mice. Other morphology analysis included right ventricle weight, LV wall thickness and intracavity length. This enlargement of the LV is thought to be caused by an increase in the total number of myocyte nuclei and non-myocyte nuclei. GH and IGF-1 has been linked to increase the total volume of the myocytes and connective tissue as well as increasing the total length of myocytes. IGF-1 has been found to enhance cardiac ventricular hypertrophy in rats. In addition to high levels of GH and IGF-1, TG mice have also been observed to have high levels of free radicals in the heart. This oxidative stress could be another cause for LV hypertrophy in TG mice. Future research could include antioxidants in the prevention and treatment of LV hypertrophy in TG mice.

Presenting Author: Caitlin Mroz

Naaum, Amanda

Molecular and Cellular Biology , University of Guelph

Characterization of glycosylation of beta-1 integrin in a breast cancer model.

Integrins are a major family of cell surface receptor proteins. ß1 integrin has been implicated in the invasive processes of many types of cancer. N-acetylglucosaminyltransferase V (GlcNAc –TV) catalyzes the addition of an N-acetylglucosamine sugar residue to ß1 integrin via a ß1,6 linkage. Much research has shown that upregulation of GlcNAc –TV is indicative of metastatic cancer cells. Matrigel is a synthetic basement membrane suited for the culture of cells in three dimensions. When breast cancer cells are spatially organized as a result of contact with basement membrane, signaling pathways involving ß1 integrin have been found to become coupled and bi-directional. This does not occur in two dimensions, therefore a three-dimensional analysis may prove to be more physiologically relevant in the study of ß1 glycosylation. This study aims to characterize the levels of ß1,6 branched ß1 integrin in the genetically matched MCF10 breast cancer model in both two and three dimensions. The levels will be determined for normal, transformed and metastatic cells in an attempt to determine if ß1,6 branching is correlated with a metastatic phenotype in the MCF10 model, and to determine if three-dimensional interactions play a role in glycosylation of ß1 integrin.

Presenting Author: Amanda Naaum

Naik, Hetanshi and Jack Wang

Biology, McMaster University and Pathology and Molecular Medicine, McMaster University

Delineating the extent of deletions and a potential mechanism in 3q interstitial deletions.

Objective: Interstitial deletions on the long arm of chromosome 3 are particularly rare with only 27 cases previously reported. This study presents three new cases. Specific objectives include delineating the breakpoints, comparing the breakpoints with gene densities, determining if previously identified mechanisms can be applied here and identifying candidate genes responsible for observed phenotypes.Subjects: All patients were unrelated with de novo deletions. Patient 1 del(3)(q29q29), gait ataxia, cleft lip and palate, mild deafness, nearsightedness and delayed motor skills. Patient 2 del(3)(q25.1q25.32), dysmorphic features with apneic spells. Patient 3 del(3)(q22.3q24), classified BPES Syndrome. Methods: Fluorescence In Situ Hybridization was used to delineate the breakpoints. Several databases were utilized; WTSI was used to analyze gene density, HORDE and TCAG databases allowed identification of OR clusters and CNVs, NCBI allowed a search of LCRs by BLASTing breakpoints against each other and UCSC and NCBI were used to find genes within the deleted regions.Results: The breakpoints were narrowed to 2 BAC clones for patients 1 and 2 while a distal breakpoint is still being analyzed for patient 3. The deletions were of varying size with different breakpoints and the average deletion size was 6.37Mb. Potential mechanisms were then studied; we found Olfactory Receptor (OR) gene clusters and Low Copy Repeats (LCRs) were not involved in these rearrangements. Several Copy Number Variants (CNVs) were found at the breakpoints of patients 1 and 2 with the significance yet to be determined. Several promising candidate genes were found for each patient.Conclusions: Most deletions occur in regions of moderate-high gene density. Mechanisms mediating 3q interstitial deletions need further research, however CNVs were found with appropriate positioning to be involved in patients' 1 and 2 deletions. Several genes of interest were also identified for each case with further research necessary to determine exact correlations.

Presenting Author: Hetanshi Naik

Nash, Brady

Molecular and Cellular Biology, University of Guelph

The role of callose and callose synthase in Nicotiana benthamiana during susceptible and resistant responses to pathogens.

Callose is a 1,3-ß-D glucan that plays an important role in a plants response to biotic and abiotic stresses. It can be found in pollen tubes, at the cell plate, around the plasmodesmata, in sieve plates as well as around sites of pathogen attack and wounding. It is deposited by callose synthase, a 1,3-ß-D glucosyl transferase, which is believed to be encoded by the GLUCAN SYNTHASE-LIKE (GSL) genes in plants. Plants were challenged with Pseudomonas syringae pv. tabaci (PST), Colletotrichum orbiculare (Cgm) or Colletotrichum destructivum (N150) following induction with Agrobacterium, benzothiadiazole (BTH) or butane-diol. Leaf samples were then checked for callose deposition using the Aniline blue stain under UV illumination. Butane-diol appears to have had the greatest impact on priming Nicotiana benthamiana plants following pathogen induction, with a noticeable increase in callose deposits compared to its isomer control, and a decrease in the level of diseased tissue. Callose synthase was able to be amplified from genomic DNA using degenerate primers based on conserved regions of callose synthases from different solanaceous species. These results show that callose deposition may play a partial role in disease resistance in Nicotiana benthamiana.

Presenting Author: Brady Nash

Nguyen, Trinh , Jiamila Maimaimti, Maher El-Masri and Lisa Porter

Forensics, University of Windsor: Biology, University of Windsor: Nursing, University of Windsor

Detecting the viral penetrability of used condoms.

AIDS continues to present a serious public health threat that infects an average of 2500 Canadians every year (1). In the absence of vaccination, the use of condoms has become the most effective preventive tool against HIV infection. Given that condoms are subject to perforation, it is important that their durability be carefully examined. Although condom companies test the durability of condoms when they are manufactured, little is known about the extent to which they perforate during use in sexual intercourse. Based on user reports of visible perforation, the breakage rate of latex condoms during sexual intercourse ranges from 0.3% – 1.2% (2-7). However, user reports of visible breaks are not sensitive enough to detect microscopic perforations that may allow the transmission of HIV. In fact, studies that examined microscopic perforations that occurred during laboratory simulation of sexual intercourse showed higher breakage rates than those reported by condom users via visual inspection (2, 3, 5-11). To date, condoms have not been tested for microscopic perforations after use in real-life sexual intercourse. Given the serious health related consequences that can occur as a result of condom perforation, it is important that the microscopic durability of condoms be examined after having been subjected to the stress of actual sexual intercourse. Thus, the long-term goal of this study is to compare the microscopic perforation rates of un-used condoms and those used during sexual intercourse. The objective of my project was to set up a laboratory feasibility protocol for measuring viral penetrance of latex condoms. Here we utilized a surrogate bacteriophage fX174, having a diameter of 0.027 U compared to HIV, which has a diameter of 0.10 U (12). We have successfully designed an in lab procedure to accurately detect microscopic perforations with a diameter of 254 U with no false positive results. Future work will utilize this model to test used condoms as well as condoms of varying materials for their ability to protect against viral infection.

Presenting Author: Trinh Nguyen

Niewiadomski, Stella and Baozhong Meng

Molecular and Cellular Biology, University of Guelph

Exploring the subcellular localization of the Triple Gene Block protein 3 of Grapevine Rupestris Stem Pitting-Associated Virus.

Triple Gene Block (TGB) proteins are responsible for the intracellular and intercellular movement of Grapevine rupestris stem pitting associated virus (GRSPaV). This study was performed to further examine the subcellular distribution of TGBp3 by biolistically bombarding constructs into Nicotiana tabacum BY-2 cells. TGBp3:CFP has previously been shown to be associated with the ER network for GRSPaV. When TGBp3:CFP is co-expressed with the non-tagged TGBp3, aggregates of fluorescence were seen, suggesting that the large autofluorescent tag may interfere with the activities of TGBp3. TGBp3 was fused to a small immunofluorescent myc tag to avoid the potential interfering effects of the autofluorescent tags. When immunofluorescence was performed on myc-tagged TGBp3, the patterns produced resembled that of the cobombardment of TGBp3:CFP with TGBp3 more closely than that of TGBp3:CFP alone. To determine if TGBp3 colocalizes with TGBp1, GFP:TGBp1 and TGBp3:mRFP were combombarded. However, the fluorescence of TGBp3:mRFP when co-expressed with another construct was too weak to be detected. The mRFP gene was mutated (Q66 to T66) in order to increase the intensity of the fluorescence of mRFP. Both the mRFP(Q66 to T66) and the TGBp3:mRFP(Q66 to T66) appear to have increased fluorescence in comparison to their non-mutated counterparts. No data has been gathered yet from the cobombardment of TGBp3:mRFP(Q66 to T66) and GFP:TGBp1. The mutated TGBp3-mRFP(Q66 to T66) and the myc-tagged TGBp3 will be used in the future to examine potential colocalization of TGBp1 and TGBp3. Confocal laser scanning microscopy will be used to further confirm these results.

Presenting Author: Stella Niewiadomski

Oiamo, Tor

Biology, University of Western Ontario

Effects of stanniocalcin in the nucleus of the solitary tract on arterial pressure.

Stanniocalcin-1 (STC-1), a 50 kDa glycoprotein hormone that regulates calcium/phosphate homeostasis within cells, is expressed in central neurons and the choroid plexus and has been shown to be protective against hypercalcemic damage to neurons by decreasing cytosolic calcium. A decrease in calcium availability to neurons of the nucleus of the solitary tract (NTS) results in decreases in arterial pressure (AP). Therefore, the possibility exists that STC-1 may function to regulate the availability of calcium to NTS neurons resulting in changes in AP and heart rate (HR). In urethane-chloralose anesthetized rats, microinjection of STC-1 (1.76-176 nM; 20 nl) into the caudal medial NTS elicited a dose-related decrease in AP and a small associated bradycardia. On the other hand, neither similar doses of STC-1 given intravenously (iv) nor microinjections into NTS of STC-1 conjugated to GTP elicited cardiovascular responses. The STC-1 evoked depressor responses were due to sympathoinhibition as they were not affected by the iv administration of atropine methyl bromide, but were blocked following iv hexamethonium bromide. Finally, microinjection of an STC-1 primary antibody (1:1000 dilution; 100 nl) bilaterally into STC-1 responsive sites in the NTS elicited a long lasting increase in AP. Taken together these data suggest that endogenous STC-1 in the NTS is involved through its receptor mediated effects in the regulation of calcium availability to NTS neurons that function as components of neuronal systems controlling AP.

Presenting Author: Tor Oiamo

Osmun, Anneka, Nicole Barker and Daniel Mennill

Biology, University of Windsor

Comparison of male and female territorial behaviour in the rufous-and-white wren (Thryothorus rufalbus).

Female territoriality is an understudied topic in the biological sciences. However, the study of female territoriality can help us learn more about conflict and cooperation between the sexes. This study examined sex differences in territoriality in a socially monogamous Neotropical passerine, the Rufous-and-white wren (Thryothorus rufalbus). Both males and females sing in this species and we compared the areas defended with song by both sexes using an Acoustic Location System (ALS). An ALS is a sophisticated array of interconnected microphones that uses sound recordings to accurately obtain the position of birds each time they sing. We used an ALS to monitor territoriality in 14 pairs of wrens living in the Guanacaste Conservation Area of Costa Rica. Using Geographic Information System (GIS) software, we created territory maps for each sex by drawing convex polygons around all of their song posts. When considering all songs, male territories were significantly larger than female territories. However, when we controlled for the fact that males sing more frequently than females in this species, territory size showed no significant difference between the sexes. Similarly, the area that a bird occupies outside of the territory shared with its partner (“territory excess”) was significantly larger for males when all songs were considered, but not when controlling for song output. This study demonstrates that males and females defend similar territory spaces in Neotropical Rufous-and-white wrens and adds to our understanding of female territoriality and conflict between the sexes.

Presenting Author: Anneka Osmun

Patel, Reena

Biology, McMaster University

Maintenance of shell coiling dimorphism in snail populations: twisting by the gene pool.

In snails, shell coiling is a dimorphic trait, with one phenotype being dextral (right-handed shell coiling) and the other sinistral (left-handed shell coiling). Usually, snail populations are fixed for either one of the morphs, due to physical incompatibilities encountered by individuals in opposite shells in copulating. In addition, frequency dependent selection results in speciation; that is to say, if a snail with left-handed shell were to occur in a dextral population, then it would either die due to its inability to mate with right-handed individuals, or potentially create a new species were it successful in finding another left-handed individual. In populations of the snail subgenus Amphidromus, both dextral and sinistral individuals coexist and are capable of interbreeding. This interbreeding allows for the exchange of alleles, resulting in the production of both phenotypes through a maternal effect, thereby maintaining dimorphic populations. Unlike the majority of snail species, Amphidromus snails are capable of inter-chiral mating due to chirality of the male spermatophore and the female reproductive tract. Such inter-chiral mating has been shown to yield greater fecundity. The focus of my thesis was to investigate factors that are involved in maintaining a dimorphic population. I used computer simulations that closely mimic the situation in nature to analyze how variations in parameter (chirality genotype) affect the dynamics of a dimorphic population. In particular, I looked at the chirality locus in snails to study the proportionate occurrence of dextral and sinistral in subsequent generations. I first revised the Evolution with Twist (ET) computer program so that the initial demes contained equal proportions of dextral and sinistral snails. Each deme differed from the others in terms of its genetic make-up. This modification allowed us to investigate the effect of the gene pool in maintaining dimorphism within a population. This study provides insight into why selection on traits that hold the potential to lead to reproductive isolation and create a new species has yet to result in speciation.

Presenting Author: Reena Patel

Patel, Shruti and P. Josephy

Molecular and Cellular Biology, University of Guelph

Expression of the histidine-tagged human cytochrome P450 1A2 in Escherichia coli.

The human cytochrome P450 1A2 or CYP 1A2 is a hepatic microsomal enzyme mainly involved in drug and xenobiotic metabolism. It exists as a heme protein that requires an accessory flavoprotein called P450 reductase to metabolize its substrates. P450 1A2 participates in the activation of aromatic amines, a group which includes mutagens and carcinogens. Thus, these proteins are of significant interest due to their association with cancer-causing chemicals. P450 1A2s have been heterologously expressed in Escherichia coli, and the function of these membrane-bound enzymes has been analyzed using strategies such as N-terminal sequence modification. The goal of this project is to study and compare the expression of the histidine-tagged P450 1A2 against the conventionally modified P450 1A2 using a LacZ reversion mutagenicity assay, and to obtain a purified form of the protein. The histidine-tagged protein will be detected by a western blot technique using a HisDetector(TM) that utilizes nickel conjugates . In addition, a spectroscopic analysis will be performed in order to verify the presence of the protein at 450nm, the wavelength at which P450s display an increased absorbance.

Presenting Author: Shruti Patel

Peltsch, Heather

Biology, Laurentian University

Immunoprecipitation of B1 protein from malignant and non-malignant breast and ovarian cells

A novel protein, the B1 protein, was discovered during research on PCNA, which is involved in genome maintenance. An antibody was developed against the interdomain connector loop of PCNA and as a result of the homology between the B1 protein and PCNA, both are detected by this antibody, now known as the B1 antibody. The B1 antibody detected differences in expression of the novel B1 protein between malignant and non-malignant samples This observation suggested the potential for the B1 protein to play a key role in the maintenance of normal cell proliferation. To confirm the identification of the B1 protein, I prepared protein lysates from malignant breast cell lines, a malignant ovarian cell line, and an immortalized ovarian cell line. Immunoprecipitation experiments were performed to isolate the B1 protein. Immunoprecipitates were focused using isoelectric focusing techniques, resolved by 2D SDS-PAGE and detected by staining. Protein spots were cut, digested with trypsin and analyzed by MALDI-TOF to determine peptide fingerprints for searching in peptide databases. The three cell lines that the B1 protein was isolated from resulted in an identification of cytoplasmic actin, which exists in beta and gamma forms. Analysis of B1 spectra recognized a potential phosphorylation site observed to be phosphorylated in all cell lines according to peptide masses. Immunoblotting with beta-actin antibodies support that the B1 protein is an isoform of cytoplasmic actin, however the relationship between the expression of malignant compared to non-malignant cells did not demonstrate a clear pattern as shown in previous work with other cell lines. Despite this, the B1 antibody appears to only bind to a sub set of actin isoforms. Identification of differences in which isoforms of actin are present in relation to malignant and non-malignant cells may present the potential for this protein to be a biomarker for the detection cancer.

Presenting Author: Heather Peltsch

Prévost, Micale and Thomas Johnston

Biology, Laurentian University

Reproductive life history variation in Great Lakes naturalized rainbow trout populations

Rainbow trout have been introduced into the Great Lakes since the late 1800s and naturalized populations are now well established throughout the basin. We hypothesized that reproductive characteristics among adults within these populations would vary with both ontogeny and energetic status, and that reproductive characteristics among populations would have diverged to match the particular environments they inhabit. We examined variation in reproductive traits of rainbow trout spawning stocks across the Great Lakes to test the predictions that: i) reproductive investment is positively related to both adult size and body condition within populations, and ii) reproductive investment declines across the Great Lakes from southern (Erie, Ontario) to northern (Superior) populations. Within populations, gonad size (gonadosomatic index, GSI) was positively related to adult size but not to body condition in both males and females. Neither ovary nor testes lipid contents were significantly related to adult size or body condition. Egg size was positively related to female size but not to body condition. Among populations, there was significant variation in both GSI and gonad lipid content in both sexes, and in egg size in females, but the pattern of this variation did not follow the north-south gradient we had predicted.

Presenting Author: Micale Prévost

Prince, Neil, Derek Hillis, Paul Sibley and John Klinoromos

Integrative Biology, University of Guelph and Environmental Biology, University of Guelph

The effect of a veterinary antibiotic on plant rhizobia growth and interaction.

The purpose of this research is to evaluate the effects of general purpose antibioticson the plant biota in the soil, as well as the rhizosphere. It will be run over thecourse of eight weeks at the University of Guelph, Guelph campus in the Phytotron. Theantibiotic in question is Tetracycline, an antibiotic commonly given to animals tofight bacterial infection. Rhizobia will be observed to see if their ability to formthe symbiotic relationship with the plant species is affected, and if they show signs ofreduced growth. The hypothesis is that Tetracycline affects organisms such as rhizobiaat the molecular level, disrupting ribosomal actions, which would lead to an impairedability to grow. If the rhizobia show signs of reduced growth then the plant may elicita response in the form of reduced growth as well. This may mean that the antibiotic hasin fact compromised the rhizobia in some way and that there could be potential dangersto soil and plant health if antibiotics continue to find their way into soils.

Presenting Author: Neil Prince

Pufall, Erica

Molecular and Cellular Biology, University of Guelph

Dog Zhangfei as a potential model for neurodegenerative diseases: polyglutamine tract expansion causes differential subcellular localisation and disrupts protein interactions.

Polyglutamine disorders are a class of neurodegenerative disease mediated by gain of function mutations. They are characterised by an expansion of the already existing glutamine tract in the protein-encoding region of the DNA, with more repeats causing more severe conditions. Zhangfei (ZF), a basic region leucine zipper transcription factor, was found to have a homolog in dogs (dZF) that contained a polyglutamine tract of naturally-variable size. This, coupled with the knowledge that ZF is located in neurons and is part of the unfolded protein response pathway, which is known to be involved in certain neurological conditions, led to the hypothesis that dZF could be a potential molecular lesion for polyglutamine disorders. To date, an expanded CAG tract has only been identified in vivo in humans, so artificially expanded CAG tracts were created in dZF, transfected into Madin-Darby Canine Kidney cells and evaluated by immunostaining to determine subcellular localisation. A CAG tract expanded past the critical number of repeats changed dZF localisation from the nucleus to the cytoplasm. Next, the interactions of normal and transformed dZF with its cellular partners were assessed via immunostaining. A CAG expansion in dZF disrupted the localisation of its cellular partners ATF4 and HCF-1, implicating a possible mechanism of disease-causation. Finally, the presence of an expanded CAG tract in dZF in vivo was assessed via PCR analysis of DNA extracted from tissue samples of dogs suffering from neurological disorders. This showed that while the dZF CAG tract was expanded in vivo in certain conditions, it did not reach the critical number of CAG repeats identified by the in vitro transfection data. However, these preliminary data suggest that dZF is a potential molecular lesion for neurodegenerative disorders and that it may eventually be possible to use it, and dogs, as a model for these conditions in humans.

Presenting Author: Erica Pufall

Qureshi, Samir, Chad Harvey and Hugh MacIsaac

Biological Sciences - GLIER, University of Windsor

Detection of invasive species when rare: Are we chasing an exotic goose?

The establishment and spread of non-indigenous species (NIS) is a growing global concern. The detection of new NIS before they become established is vital in combating this problem. In this study, we estimate the sampling effort required to detect a new NIS when it is still rare. We sampled Hamilton Harbour, Lake Ontario, for the fishhook waterflea (Cercopagis pengoi). The unique life history of C. pengoi makes it the perfect and ethical model species. C. pengoi is parthenogenetic and overwinters as eggs in lake sediment. Individuals that emerge in spring are morphologically distinct from subsequent generations of asexually-produced individuals and thus readily identifiable as the initial generation. This biphasic seasonal morphology allowed us to target sampling to the initial generation animals only, which, when first hatching from eggs, would be rare, analogous to a new invader. One hundred vertical plankton hauls, spaced 100m apart, were taken on six dates in the spring/summer of 2007 across the emergence period. C. pengoi presence/absence data were used to construct sample-based rarefaction curves for each sampling date. Capture rates ranged from 0 to 92%, with a mean of 0 to 2.17 C. pengoi per sample. Rarefaction curves demonstrated a positive association between the probability of detecting C. pengoi and the number of samples taken. Rarefaction curves, based on the probabilities found using each data set, indicate a minimum of 30 samples in a single habitat to detect NIS at rare densities. Furthermore, the typical standard of 3-5 samples per lake would be sufficient to detect this NIS only at peak density. These results suggest that a much higher sampling intensity is required to detect colonizing NIS than standard performed protocols. Because newly colonizing NIS can only be detected with intense sampling effort, sampling protocols must be augmented above those typically used for routine monitoring.

Presenting Author: Samir Qureshi

Racine, Danielle

Biologie, Universite Laurentienne

Application de la methode de Polymerase en chaine pour le typage des PVH16 et 18 (amplification des oncogenes E6 et E7).

Le virus du papillome humain (VPH) est l'infection virale la plus transmise au monde. La plupart des personnes ont contracté le VPH un moment durant leur vie. Les VPH infectent spécifiquement les épithéliums de la peau ou des muqueuses. Ces virus ne présentent généralement aucun signe ou symptômes. Mais, ils peuvent induirent des lésions bénignes (verrues palmaires et plantaires par le VPH 1 et 2) et malignes (cancer du col utérin chez la femme par MTS/ Maladies Transmises Sexuellement) . Des VPH spécifiques, les types de VPH 16 et VPH 18 sont retrouvés dans des biopsies de cancers du col utérin et ils causent 75% des cancers du col utérin. De plus, la prévention et la détection sont devenues très populaires depuis ces derniers dix ans. Si une femme passe un test de détection VPH régulièrement, elle baisse ces chances de développer le cancer du col utérin par 90%. Ce projet s'est concentré sur l'analyse des virus papillomes humain de type 16 et 18. Nous avons utilisé la méthode de Polymérase en chaîne (PCR ou Polymerase Chain Reaction) afin d'isoler et d'amplifier l'ADN de VPH 16 et VPH 18 dans les lignées cellulaires cancéreuses HeLa et SiHa. Nous avons amplifier un produit de 450 pdb qui sont les oncogènes E6 et E7 des PVH16 et 18, nous avons utilisé les amorces MYO9/MYO11 pour la technique du PCR. Ensuite, les produits du PCR ont été mis sur un gel d'électrophorèse avec une échelle de poids moléculaire afin de confirmer l'amplification des oncogènes E6 et E7 à partir des amorces MYO9/MYO11.

Presenting Author: Danielle Racine

Radford, Devon and Lewis Lukens

Molecular and Cellular Biology, University of Guelph and Plant Agriculture, University of Guelph

Genome-wide analysis of transposon mediated expression in Arabidopsis thaliana.

Transposons are known to regulate the expression of nearby genes in plants. By examining the genome sequences, genes were found associated with transposons in structures indicative of transposon regulated expression. The complete Arabidopsis thaliana genome was analyzed for transposons with the correct position relative to nearby genes to identify possible transposon mediated genes. Based on these analysis 1036 genes have been identified as potentially under transposon mediated expression. These are further grouped into six classes based on the type of transposon mediating expression. In total 12 LTR bound genes, 536 LINE associated genes, 177 Mutator element associated genes, 35 Spm associated genes, 237 Helitron associated genes, and 61 hAT related genes were identified. Some of these genes show association to more than one transposon. Of genes with known segmental or tandem duplicates, eleven showed transposon association in the duplicate, suggesting the transposon mediation of these genes may have arisen after duplication of these genes. One hundred thirty-one duplicate groups have no conservation of transposon association. Various conditions of stress were analyzed to detect significant changes in gene expression in the transposon linked genes. Compared to unassociated genes, certain classes of transposon associated genes significantly depressed variability in specific tissues under certain stresses. Mutator and Spm associated pairs exhibited significant decreases in average mean and standard deviation of expression ratios in root and shoot tissues under genotoxic stress and under drought stress in root tissue. Helitons significantly reduced variability of expression in roots under genotoxic stress. LINE elements and Mutators significantly depressed variation of expression under drought stress in shoots. Genes associated to hAT elements did not cause significant effects on variability of expression. Expression data was unavailable for LTR bound genes under the conditions examined. Thus, under stress these transposon classes reduce variability of expression genome-wide.

Presenting Author: Devon Radford

Rodriguez, Adrian and Steffen Graether

Molecular and Cellular Biology , University of Guelph

Expression and purification of wildtype Type IV Antifreeze protein from the longhorn sculpin (Myoxocephalus octodecemspinosus).

Through evolution, organisms have adapted to live in a wide array of environmental conditions. For fish living in polar environments, freeze avoidance and tolerance is achieved by different mechanisms including the secretion of antifreeze proteins (AFP). The structure of Type IV AFP has only been hypothesized and the purpose of this project is to optimize its expression and purification conditions in preparation for structural and activity studies. Using a bacterial system, the protein has been successfully expressed in LB media using a pET22b+ plasmid vector containing a pELB leader sequence for periplasmic localization. Endogenous signal peptidases are responsible for the cleavage of this leader sequence, but SDS-PAGE reveals the presence of two forms of the protein suggesting that the signal peptidase responsible for this cleavage is not fully functional. The presence of the protein in the media suggests that the protein is being secreted, possibly because of the pELB leader sequence. The presence of a His6 tag has allowed for the purification of the cleaved and uncleaved forms of the protein via nickel affinity chromatography. In preparation for structural studies using nuclear magnetic resonance (NMR), an attempt to express the protein in M9 minimal media has so far been unsuccessful. It becomes important to elucidate the structure and activity of this and other antifreeze proteins in hope that they can be used in biomedical applications.

Presenting Author: Adrian Rodriguez

Ross, Brent

Biology, Laurentian University

Effects of climate variation on structural and community components for galls of Diplolepis ignota (Hymenoptera: Cynipidae)

The cynipid wasp, Diplolepis ignota (Osten Sacken) induces coalesced galls on the underside of leaves of Rosa arkansana Porter in the driest parts of southern Alberta and Saskatchewan. Abundance of galls and size appears to vary from season to season, presumably in relation to the availability of moisture influencing the condition of the host plant. Populations of galls at a typical site near Lethbridge, Alberta were collected in the spring of 2003 and 2007, and in the fall of 2007 and returned to the laboratory for analysis and emerging of inhabitants. Galls size, mass, and the number of chambers per gall is correlated with precipitation in the months prior to gall harvesting. Other inhabitants associated with galls include a cynipid inquiline of the genus Periclistus and chalcid parasitoids of the genera Eurytoma, Pteromalus, Aprostocetus, Torymus, Eupelmus, and Ormyrus, along with an ichneumonid of the genus Orthopelma. The abundance of inquilines and parasitoids is correlated with gall size.

Presenting Author: Brent Ross

Ruzzini, Antonio and Janet Yee

Chemistry, Trent University

Studies of the cell cycle in an unusual waterborne parasite, Giardia lamblia.

Cell reproduction begins with the duplication of the cell's contents, followed by distribution of those contents into two daughter cells. This process, called the cell cycle, can be divided into different stages such as DNA synthesis, chromosome segregation, and cell division. In order for a cell to progress smoothly through these stages, different sets of genes need to be activated to produce the proteins that are required at specific times. Giardia lamblia is a protist found in freshwaters worldwide, and is the most common cause of parasitic diarrhea in humans. Giardia is an unique eukaryote as it has two nuclei that are identical in their size, DNA content, and transcriptional activity. Its binuclear feature and relatively short doubling time offers an opportunity to study the cell cycle in this unusual eukaryote. I began by using flow cytometry, a technique that gives a profile of the distribution of cells in the different stages of the cell cycle within a sample, to monitor the ability of different drugs and treatments to synchronize Giardia cultures. My results showed that aphidicolin, a reversible inhibitor of DNA polymerase, was the most effective at achieving synchronization, while other drugs acting on the mitotic spindle and other cellular targets were much less effective. In a time course experiment, RNA was extracted at regular intervals from a Giardia culture synchronized by aphidicolin treatment. These RNA samples were used with primers designed for Giardia homologues of genes that are expressed at different stages of the cell cylce in other eukaryotes. These results and their significance, as well as future research directions will be discussed in my presentation.

Presenting Author: Antonio Ruzzini

Santala, Kierann

Biology, Laurentian University

Influence of heavy metal stress on plant water relations in Betula papyrifera

Betula papyrifera seedlings were exposed to a drought treatment in addition to an elevated metal treatment to observe if heavy metals impaired seedlings ability to endure drought. Spring-germinated seedlings were grown in an outdoor experiment which began in mid-July. Two heavy metal treatments were produced by mixing slag with sand so that 2.5% and 0.5% of the sand-slag mixture volume contained slag. Slag is known to be high in Ni and Cu. Pure sand was used as a control. The drought treatment consisted of half of the pots no longer receiving water following August 20th until they were harvested in mid-November. Due to reduced plant size at the 2.5% metal level, soil moisture was higher and therefore influenced the drought treatment. Due to the impact of the metal treatment on soil moisture, soil moisture was analyzed as continuous data. The plants growing in the 2.5% heavy metal treatment were found to have reduced total dry mass, root mass ratio, stem diameter, stem cell number and cell size in the annual ring. Similarly, drought had a reducing effect on these characteristics; however, only in the absence of slag. At the highest slag level, soil moisture had no clear effect on any of the measured characteristics. Therefore the response to drought was more pronounced in the absence of slag. Despite the fact that metals in this study slightly reduced the severity of drought, the data strongly indicates that the decreased response to drought in 2.5% metal treatment is the result of the direct impact of metals on plant water stress regulation mechanisms. Such impacts could have been the result of disruptions to the functioning of water channel proteins, mitosis or premature lignification of cell walls as a result of heavy metal stress.

Presenting Author: Kierann Santala

Sashaw, Jessica, Sara Thomps and Pat Wright

Integrative Biology, University of Guelph

The effect of chronic ammonia exposure on ammonia excretion in rainbow trout (Oncorhynchus mykiss) embryos.

Ammonia is the primary excretory product of aquatic animals. Salmonid embryos can be exposed to slightly elevated levels of ammonia during development, either in redds or in aquaculture. This study aims to examine ammonia excretion rates and ammonia tissue concentrations in rainbow trout (Oncorhynchus mykiss) embryos in response to chronic (4 day) low and high external ammonia exposure at 15oC. Embryos were kept in the Hagain Aqualab from the day of fertilization until the end of the experiment (16 days post fertilization (dpf)). At the "eyed-up" stage of development (11dpf), the embryos were divided into three groups, where each received different concentrations of external ammonia: control (0uM NH4HCO3), low (70uM NH4HC03) and high (225uM NH4HCO3). Flux experiments were conducted at time 0h, 24h and 4 days for ammonia excretion rate determination and tissue samples were collected for analyses of ammonia tissue concentration. When first exposed to the treatment conditions (time 0-4h), excretion rates were initially depressed in the low external ammonia group and reversed in the high external ammonia group compared to control. Excretion rates were re-established by time 24h in the low external ammonia condition and by day 4 in the high external ammonia condition. The ammonia tissue concentrations of the embryos were compared for all time points between groups. Though the current model for ammonia excretion is by simple diffusion across the membrane, recent research has suggested a role for ammonia transporters. At the 24 hour time point, mRNA expression of protein transporters involved in excretion of nitrogenous wastes were examined. It is important to understand the physiological mechanisms through which embryos respond to elevated external ammonia since embryos are faced with this challenge naturally, in aquaculture and through anthropogenic sources like sewage runoff, industrial emission and fertilizers which continually increase the ammonia levels in the water.

Presenting Author: Jessica Sashaw

Shanmuganathan, Sangitha

Molecular and Cellular Biology, University of Guelph

Respiratory infection of chickens with Marek's disease cell-free virus, RB1B.

Marek’s disease (MD) is a lymphoproliferative disease of chickens caused by an alpha herpesvirus. Although the disease occurs via the respiratory system naturally, the disease has been mainly studied by subcutaneous injection of the virus due to lack of respiratory infectious model. The aim of this study is to build an infection model to investigate the infectious ability of very virulent cell-free RB1B strain of Marek’s disease virus (MDV) in vivo and in vitro. Twenty day old chickens were infected with cell-associated RB1B intra-abdominally. On day 30 post infection, cell-free RB1B was extracted from skin and feather tip. Aerosols of size 1.9 µm of the extracted cell-free RB1B was produced in the built infection model using a nebulizer and an air compressor and was used to infect 3 day old chickens for 5, 10, and 15 minutes as well as chicken kidney cell cultures for 5 and 10 minutes. On day 12 post-infection, DNA extracted from feather follicle epithelium of all aerosolized chickens as well as one contact chicken was positive for infection of MD. By day 18 post-infection, the internal organs of chickens aerosolized for 5 and 10 minutes contained signs of lesions. Chicken kidney cells aerosolized with RB1B was positive for infection, containing 6 and 8 plaque forming units (PFU) for 5 and 10 minutes of aerosolization, respectively. In conclusion, results show a successful respiratory model to study MDV infection via natural route in the future.

Presenting Author: Sangitha Shanmuganathan

Shapiro, Noah

Biology, University of Western Ontario

Detecting single nucleotide polymorphisms (SNPs) for use in population genetics studies of butterflies.

Butterfly movement is known to be limited by the structure of landscapes, which in turn may be changing due to global warming. For example, trees are slowly shrinking mountain-top meadows in which the Apollo butterfly (Parnassius smintheus) resides. Pathways between meadows are disappearing as well, effectively ceasing gene flow. One method for studying movement and gene flow in this species across a changing landscape is to introduce individuals with a novel molecular marker into a study population and track the spatial locations of the progeny of those individuals over time. To this end, a marker is needed that occurs at a high abundance in the source population but is absent from the study population. This study attempted to discover single nucleotide polymorphisms (SNPs) that would be present at a high frequency in a population of P. smintheus from Banff National Park (Banff) but absent from a study site approximately one hundred km away in the Kananaskis region. Six mitochondrial DNA (mtDNA) loci were amplified via the polymerase chain reaction (PCR) in both Banff and Kananaskis individuals, and products were sequenced to detect such potential SNPs. No individual mutations that were abundant in Banff but absent in Kananaskis were detected. Too few genes were surveyed in hopes of finding usable unique SNPs for directly tracking gene flow. Furthermore, mtDNA may not have been as beneficial as originally pondered. However, results suggest that mtDNA haplotypes rather than individual mutations may occur that are present in Banff but absent from Kananaskis. Further sequencing would be required to confirm this.

Presenting Author: Noah Shapiro

Smyth, Eric and Patricia Chow-Fraser

Biology, McMaster University

Factors that govern pumpkinseed distribution in coastal wetlands in the Great Lakes.

The pumpkinseed, Lepomis gibbosus, is one of the most common fish in coastal communities of the Great Lakes, and is an important part of the food web, being predator to numerous arthropods and gastropods, and also prey to many piscivores. Despite its ecological importance, very little is known about factors that control pumpkinseed distribution within the Great Lakes shoreline. Based on literature of inland lakes, we determined that water quality, presence of certain macrophyte species, and abundance of competitors and predators may control pumpkinseed abundance. Because we are dealing with coastal communities, additional factors include the shape and size of the wetland, as well as degree of exposure. We used data collected from 111 coastal wetlands that had been sampled with paired fyke-nets (set overnight in parallel orientation to shoreline) between 2001 to 2007, inclusive. Pumpkinseed abundance was significantly correlated with abundances of two competitors (golden shiner and bluegill), but not with predators (largemouth bass, northern pike and smallmouth bass). The Geomorphic Index (GI), an index of degree of protection from the lake, was found to have a significant positive effect on pumpkinseed abundance in sites with poor water quality, but a significant negative effect in sites with unimpaired water quality. This suggests that pumpkinseeds can tolerate degraded water quality as long as wetlands have a large opening to the Great Lake, whereas connection to the Great Lake may inhibit the presence of pumpkinseeds when water quality is not degraded. The average size of pumpkinseeds increased with species richness of floating macrophytes, with latitude, and with the Wetland Exposure Index (accounts for both GI and fetch). Our results indicate that pumpkinseed abundance in coastal wetlands can be predicted by habitat features such as site location, geomorphology, water quality, and macrophyte species.

Presenting Author: Eric Smyth

Standeven, Kyla and Thomas Johnston

Biology, Laurentian University

Sex-based divergence in mercury bioaccumulation by northern fishes.

It is well known that concentrations of bioaccumulative contaminants, such as mercury (Hg), are positively related to the age and/or size of fish. But, how might this relationship be influenced by the sex of the fish? Sex-based differences in feeding ecology, habitat use, growth, energetics, and reproductive allocation could lead to divergent rates of Hg bioaccumulation. We examined muscle total Hg concentrations in mature males and females of 20 boreal fish populations, comprising 7 species. We predicted that the net effect of all ecological and physiological differences between the sexes would result in higher Hg bioaccumulation in females than males at a given body size. We found significant differences in Hg concentrations between the sexes in some populations but not in others. Furthermore, females had significantly lower mercury concentrations than males in some walleye populations, contrary to our predictions. Reanalyses of these data following adjustment for both body size and growth rate indicated that some of the sex-based differences in Hg concentrations were attributable to differences in growth rates. Our results have implications both for setting fish consumption guidelines and for understanding contaminant dynamics in aquatic systems.

Presenting Author: Kyla Standeven

Stecjuk, Ashley and Joseph Colasanti

Molecular and Cellular Biology, University of Guelph

Analysis of the interaction of Zea mays INDETERMINATE 1 and ARGONAUTE 4.

INDETERMINATE 1 (ID1) is a nuclear-localized transcription factor first identified as an important regulator of flowering time in maize (Zea mays). The id1 mutants flower much later than wild type plants and produce more leaves before finally making the transition from vegetative to reproductive growth. When mutant plants do flower, they usually lack female inflorescences (ears) and produce aberrant male inflorescences that have vegetative characteristics. The ID1 protein is characterised by four zinc finger domains, of which only two are involved in DNA binding. The activity of many transcription factors has been shown to be regulated through interaction with other proteins. This leads to the hypothesis that the two zinc fingers of ID1 that do not bind DNA may mediate interaction with other proteins. A yeast two hybrid screen of a maize immature leaf cDNA library with ID1 protein as bait yielded a number of potential interacting proteins. One such protein was a partial sequence of Zea mays ARGONAUTE4 (ZmAGO4), a homolog of the Arabidopsis thaliana ARGONAUTE4. This finding suggests that ID1 function may be regulated by a novel RNA-directed gene silencing mechanism. We used in a yeast two hybrid swapping experiment and Bimolecular Fluorescence Complementation (BiFC) to confirm interaction of ID1 and ZmAGO4. The full length ZmAGO4 was cloned into a bait vector and ID1 was cloned into a prey vector for the yeast two hybrid experiment. Onion cells were transformed with two vectors, one containing ID1 fused to the N-terminal of a Yellow Fluorescent Protein (YFP) with the other vector containing either the partial or full-length ZmAGO4 fused to the C-terminal of a YFP. Preliminary results provide the first evidence of the interaction of ID1 with the full-length ZmAGO4.

Presenting Author: Ashley Stecjuk

Stepanowicz, Justin

Molecular and Cellular Biology, University of Guelph

Random mutagenesis of the endoglucanase CenA, to lower its pH optimum.

In an attempt to shift the pH optimum of the cellulolytic enzyme CenA from Cellulomonas fimi to an acidic pH, the error prone PCR (epPCR) method was employed. Before this can be done the mutation rate of the epPCR reaction must be optimized. To accomplish this, preliminary epPCR reactions were carried out, varying the relative amounts of components in the reaction mixtures. Problems were encountered when attempting to ligate and transform epPCR product into a competent host strain of Esherichia coli. Initially epPCR products were digested and ligated with a pET30a vector and transformed into CaCl2 Competent BL21 cells, however, no transformants were recovered. A variety of methods were attempted in the troubleshooting process, including the use of electro-competent DH5a and Top-10 E. coli for transformation via electroporation and switching to the pGEM T-easy Vector System I for ligations. Transformants were recovered, but sequencing results of the products from these reactions were unavailable at the time of this presentation. Simultaneously, a method of screening for CenA mutants with low pH activity was developed using an adapted version of the carboxylmethyl cellulose screen. Cellulolytic activity is observed in this method through zones of clearing in overlay agar, which contains carboxylmethyl cellulose. Test trials were carried out with different compositions of overlay agar, using 3 cultures of Escherichia coli, which possessed genes for the wild-type CenA enzyme, a CenA mutant with reduced activity and a CenA mutatant with no activity. It was determined from these trials that when screening for CenA mutants with a low pH optimum, an overlay agar containing 1% carboxylmethyl cellulose, 250 mM sodium phosphate buffer, pH 4.5 should be used. In future studies, once an optimal mutation rate has been established, this overlay agar will be used to screen for CenA mutants with a low pH optimum.

Presenting Author: Justin Stepanowicz

Stosic, Alix, Julie Marentette and Sigal Balshine

Psychology, Neuroscience and Behaviour, McMaster University

The impact of contaminants on activity and predator avoidance in the round goby.

Environmental pollutants have the potential to alter the natural steady-state of an organism, ultimately causing behavioural changes, which can result in reduced survival and fitness. We investigated the effect of contaminant exposure on activity and response to simulated predator attack in the round goby, Neogobius melanostomus, an invasive species in Hamilton Harbour. We compared frequencies of behaviours during the day and night between fish from contaminated sites and a cleaner site. Round gobies were more active at night, and at night contaminated fish were less active than cleaner fish. Females showed higher levels of activity than males, but again this sex difference was only significantly at night. Contaminated fish were more likely to respond to simulated predation by swimming vigorously away, while fish from cleaner sites were more likely to bury in the substrate. The results suggest that exposure to contaminants influences activity and cognitive processes such as risk perception.

Presenting Author: Alix Stosic

Stryker, Lauren

Integrative Biology, University of Guelph

The effects of increased temperature on nitrogen storage in the central mudminnow (Umbra limi), an air breathing teleost.

The central mudminnow (Umbra limi) lives in environments where immense diurnal and seasonal fluctuations in temperature and water availability can be coupled with high concentrations of toxic ammonia. It is critical to understand physiological adaptations of air-breathing teleost fishes such as Umbra limi against temperature changes to better understand the impacts increased temperature will have on aquatic systems in the future. In normal environmental conditions most aquatic organisms reduce ammonia accumulation via enhanced excretion. The detoxification of ammonia to urea has been observed in lungfishes and some teleost species from environments where ammonia excretion can be hindered. It was hypothesized that Umbra limi would express adaptive responses to avoid endogenous ammonia build-up during exposure to a higher temperature (31 C) compared to a low temperature (15 C). From October 2007 to March 2008 white muscle tissue of Umbra limi was analyzed for urea and ammonia content at the University of Guelph. Ammonia and urea stores in white muscle tissue were evaluated in comparison to nitrogen end-product excretory responses and metabolic rates over increased temperature (P. Wright, S. Curne, B. Baggatto, unpublished data). Differences in tissue urea content (umol/g) were not significantly different at 15 C or 31 C compared to fish in the control condition immersed at 24 C. Although, a fish immersed at 31 C expressed a large difference in muscle tissue urea content. Fish species possess variable responses to ammonia toxicity and this could result in drastic changes to aquatic assemblages if ammonia exposure is coupled with environmental factors such as warmer habitat temperatures.

Presenting Author: Lauren Stryker

Styles, Erin

Molecular and Cellular Biology, University of Guelph

Analysis of vitis cbf transcription factors for differences in transactivation capabilities and salt stress tolerance.

The cold acclimation signal transduction pathway in grapevines is known to be responsible for the production of cryoprotectants that serve to protect the plant cells from the effects of freezing damage. A crucial component of this pathway is a transcription factor known as the CRT Binding Factor (CBF). Within the grapevine family, CBF1, CBF2 and CBF3 are hypothesized to have functionally similar roles whereas CBF4 is thought to be functionally divergent. This is due to a difference in expression timing, as well as differences between the activation of CBF1 and CBF4 from grape species Vitis riparia, as determined by transactivation assays (Xiao et al. 2007 and 2008). The salt tolerance of the Vitis CBF paralogs was tested through plating transgenic Arabidopsis seeds on a medium containing sucrose only, as well as a medium containing both sucrose and salt. Four separate lines over-expressing VrCBF1, and three lines over-expressing VrCBF4 were used for this experiment. Preliminary research indicates that compared to wildtype Arabidopsis plants, seedlings over-expressing a CBF gene grow better. What's more, lines over-expressing VrCBF4 typically grow better than those over-expressing VrCBF1, indicating that the two CBF genes are functionally divergent in terms of salt stress tolerance. Future research will involve testing the transactivation capabilities of CBF1 as compared to CBF4. Effector and receptor constructs are being prepared for such an assay that will take place through the agroinfiltration of Nicotiana benthamiana. The effector plasmid will contain VrCBF4 or VrCBF1 coding sequence downstream of a constitutive 35S promoter. The receptor plasmid will be constructed to contain two luciferase sequences, RiLUC behind a minimal 35S promoter with a 4xCRT CBF binding domain, and, as a control FiLUC behind a 35S promoter.

Presenting Author: Erin Styles

Subedi, Sanjeena, Jaideep Mathur, Michael Emes and Ian Tetlow

Molecular and Cellular Biology, University of Guelph

Ultra structural characterization of amyloplasts using 14-3-3 proteins.

Starch is the major carbohydrate storage product in plants and is the major dietary source of energy. Although the core pathway of starch biosynthesis is known, important details regarding the regulation and coordination of the pathway are still unclear. A better understanding of the development of the starch granule can be achieved by visualization of amyloplast using fluorescent proteins. Previous work suggests that 14-3-3 proteins may play an important role in starch granule synthesis. Therefore, mRNA from wheat (Triticum aestivum) was extracted for the isolation and amplification of a plastidial 14-3-3 sequence. 14-3-3 sequence was then cloned into the 5' multiple cloning site of the vector eYFP containing a yellow fluorescent protein tag and the construct was then shot into onion cells and wheat endosperm using a biolistic gun. Our study suggests 14-3-3 localization to discrete organelles of about 1-1.5 µm in diameter. The study opens up avenues for dissecting the causes of differential localization of 14-3-3 protein and linking them to specific functions in different organisms.

Presenting Author: Sanjeena Subedi

Sullivan, Ashleigh, Olaf Berke and Prabhat Jha

Molecular and Cellular Biology, University of Guelph: Population Medicine, University of Guelph: Public Health Sciences, University of Toronto

Spatial analysis of regional HIV-1 prevalence in southern India from 2002 to 2005.

Objectives: To assess spatial patterns in regional HIV-1 prevalence in southern India from 2002 to 2005. The relationship between different surveillance methods and patterns are also investigated over time. Methods: Surveillance data for three HIV-1 sentinel surveillance systems in place across southern India were obtained from the Centre for Global Health Research which is affiliated with St. Michaels Hospital and the University of Toronto. The presence of disease clustering was investigated using spatial and spatio-temoral scan tests. Isopleth maps were created through kriging of smoothed prevalence estimates, and were used to identify high-risk areas. Differences in spatial distribution over time and across the surveillance methods were also assessed using semi-variograms and Moran's I coefficient of spatial correlation. Results and Conclusion: The surveillance data obtained from Sexually Transmitted Infection Clinics showed no spatial correlation and was eliminated from further investigation. This is likely due to the limited number and large distances between clinics. Clustering of HIV-1 cases was detected in every year and surveillance system. The cluster locations appear to be both spatially and temporally persistent. Other results are pending.

Presenting Author: Ashleigh Sullivan

Taves, Matthew, Julie Desjardins, Sandeep Mishra and Sigal Balshine

Psychology, Neuroscience and Behaviour, McMaster University

Dominance establishment elevates male and female androgens in a highly social cichlid fish.

In most vertebrates, aggression and dominance are facilitated by high circulating testosterone. Fish, however, have two major androgens (testosterone, T and 11-ketotestosterone, 11KT) that influence aggression and dominance. To date, very few studies have compared the effects of aggressive contests and dominance establishment on androgen levels in both males and females of the same species. To investigate sex differences in androgens we staged 14 female and 10 male size-matched resource contests and examined androgen levels in emerging dominants and subordinates. Newly established dominant females had higher plasma T but equivalent 11KT to subordinate females. In contrast, newly established dominant males had higher 11KT but equivalent T to subordinate males. Females had higher T than males but no sex difference was found in 11KT. In all fish 11KT was positively correlated with relative gonad investment. These findings provide the strongest support to date that different androgens play an equivalent role in the effects of female versus male dominance establishment.

Presenting Author: Matthew Taves

Tennenhouse, Erica

Biology, University of Western Ontario

Song rate and complexity in song sparrows: do good singers flaunt what they've got or sit on their laurels?

In passerine birds, song conveys information to females about male quality, and females subsequently use this information to select mates. However, different features of song could interact with one another in the process of signaling. This study looks at whether the level of performance of song rate, which is thought to represent current condition in song sparrows (Melospiza melodia), is adjusted to compensate for song complexity, which represents condition early in life. It also examines the repeatability of song rates of individual birds over time. Song rates and repertoire sizes were measured on a population of song sparrows in eastern Ontario during May of 2007. I found that there was a strong positive association between song rate and repertoire size, which means that compensation between these song features does not occur. Song rate and complexity might communicate similar information to females about male quality. It is also possible that males with highly complex songs have more to gain from singing than males with less attractive songs. Alternatively, the conditions in early life that affect song complexity in song sparrows could have carry-over effects on song rate in adulthood.

Presenting Author: Erica Tennenhouse

Thompson, Sarah, Jessica Sashaw and Patricia Wright

Integrative Biology, University of Guelph

Effects of chronic exposure to varying ammonia concentrations on growth, total protein and nitrogenous waste excretion in rainbow trout (Oncorhynchus mykiss) embryos.

Embryos of rainbow trout (Oncorhynchus mykiss) in both natural and aquaculture environments may experience increased ammonia either due to external pollutants or increased embryo density. Ammonia has historically been classified as a toxicant, causing many adverse side effects in fish including death. Yet, recent studies found that when juvenile rainbow trout are exposed to a low external ammonia concentration (70 µmol·L-1 total ammonia) growth is increased instead of suppressed (Linton et al., 1998; Reid et al., 1998; Wood, 2004). I tested the hypothesis that exposure to low external ammonia will 1) accelerate growth and 2) will stimulate the conversion of ammonia to urea in O. mykiss embryos. At 11 day post fertilization (dpf) embryos were exposed for 4 days (15oC) to one of three treatments; control (0 µmol·L-1 NH¬4¬HCO¬3¬), low external ammonia (70 µmol·L-1 NH¬4¬HCO¬3) or high external ammonia (225 µmol·L-1 NH¬4¬HCO¬3¬). Nitrogenous waste excretion rates and tissue concentrations were measured by sampling water and tissue at the following times: 0 hours, 24 hours and 4 days. Mass and total protein measurements were taken on embryonic tissue before ammonia exposure and then subsequently at 24 hours and 4 days. Growth was not significantly different between the three exposure groups at any exposure time. Urea excretion increased over the developmental period, however it was not significantly different between the three exposure groups at any time. At the time of writing this abstract, data on urea tissue concentrations and tissue protein levels was still being analyzed. It is possible that the exposure time period was not long enough or that not enough time points were sampled to determine an initial stimulatory effect of ammonia on growth. Therefore future studies need to be performed to confirm the stimulatory effect of ammonia on growth and define its duration.

Presenting Author: Sarah Thompson

Tschirhart, Janine

Molecular and Cellular Biology, University of Guelph

The expression and purification of putative virulence factors belonging to the poly(ADP-ribosyl)transferase (pADPRT) family.

The poly(ADP-ribosyl)transferase (pADPRT) family of enzymes catalyze the repeated transfer of single ADP-ribose units from NAD+ to itself, and/or other proteins. This post- translational modification of proteins is found only in eukaryotic cells and is involved in DNA repair and programmed cell death. All members of this family of enzymes display limited primary amino acid sequence identity, yet have a signature secondary structure fold. The genome of the bacterium, Microscilla marina, was examined using data mining techniques and was found to contain a putative pADPRT that we have called Mmar, which may potentially be the first pADPRT found in prokaryotic cells. After cloning into an inducible expression vector, soluble Mmar protein was produced in E.coli, and verified by western blot analysis. Mmar was found to be soluble in a high salt buffer and purification of the protein using IMAC was successful, however, complete purity has not been achieved. Unpurified Mmar has shown enzymatic activity, as illustrated by an eEF-2 biotinylation assay. Attempts to further purify the protein in order to facilitate a detailed kinetic analysis are underway.

Presenting Author: Janine Tschirhart

Turgeon, Zachari and Rod Merrill

Molecular and Cellular Biology, University of Guelph

Discovery and characterization of putative ADP-ribosyltransferase toxins as targets for 1-8-naphthalimide, a potential broad-spectrum toxin inhibitor.

The emergence of antibiotic resistant microbes poses an enormous challenge in the pursuit for new antibacterial compounds and threatens human health worldwide, increasing the need for alternative drug discovering techniques. A yeast growth-defective phenotypic screen was utilized in an attempt to overcome the current dilemma. The assay utilized the yeast Saccharomyces cerevisiae, previously shown as a useful model for bacterial infection in humans, to characterize the in vivo activity of ADP-ribosyltransferase exotoxins from various bacterial strains. The assay allowed for the characterization of the in vivo cytotoxicity of 12 toxins, one of which served as a control toxin. From the eleven putative toxins, seven were shown to be active ADPRT toxins and four had no measurable cytotoxicity. Site-directed mutagenesis was performed on predicted catalytically important residues for five of the active toxins. Mutations of putative catalytic Glu residues severely or completely abolished cytotoxicity in four of the five toxins. Furthermore, the ability of 1-8-naphthalimide, in functioning as a broad-spectrum ADPRT inhibitor was tested against two of the active putative toxins as well as against the control toxin. The assay showed that 1-8-naphthalimide protected yeast cells against the cytotoxic activity of all three ADPRT toxins. These results suggest that bacterial toxins can be characterized using in vivo screening techniques, without the prior requirement of purified protein. Combining this assay with bioinformatics is a powerful tool in the discovery of novel toxins and may potentially be used to discover other virulence factors. Furthermore, it was shown that 1-8-naphthalimide can function as a broad-spectrum ADPRT toxin inhibitor and shows promise as a small molecule prophylactic drug against a wide array of ADPRT toxins and may function against the pathogens that produce them.

Presenting Author: Zachari Turgeon

Turko, Andy and Patricia Wright

Integrative Biology, University of Guelph

Differences in air emersion behaviour between individuals of clonal mangrove killifish, Kryptolebias marmoratus.

The mangrove killifish, Kryptolebias marmoratus (Poey), is a self fertilizing hermaphroditic fish native to the mangrove forests of the tropical West Atlantic. These fish can tolerate prolonged air exposure, and will emerse in response to aggression, poor water quality, or harsh environmental conditions. However, the emersion patterns of individual fish vary widely, even between members of the same clonal lineage. In the present study, we sought to quantify differences in emersion behaviour between genetically identical K. marmoratus and hypothesized that fish prone to air emersion would demonstrate associated morphological features such as gill area reductions and epidermal thickening. To quantify air emersion behaviours, 24 K. marmoratus were individually filmed for a 7d period and emersions were recorded to the nearest minute. Each recorded fish was then sectioned sagitally through the left gill and transversely at the level of the pectoral fins. We were able to define three groups of fish – frequent, moderate, and infrequent emersers. Furthermore, fish were able to be classified based on the average length of emersion and their preferred time of emersion (nocturnal vs. diurnal). Histology results were unavailable by the abstract submission deadline.

Presenting Author: Andy Turko

Turner, Jimmy

Genetics, McMaster University

The evolution of leg lifting behaviour and territory marking in mammals.

Evolutionary models have been developed to explain the existence of many aspects of the biological world. Using molecular and historical data, it is now simple to determine the origins of a species, when and where it first was seen on our planet. It is also possible to discover the relationship between any two species and to find the most recent common ancestor. By comparing physical and genetic traits, the time since the split between two species can be traced backwards and be accurately estimated. The techniques available today can be used to trace the origins of individual characteristics of a species. The finest details of an animal can all be explained through the use of evolutionary models and phylogenetic research. The origins of many different types of behaviour can be found in the roots of phylogenetic trees. One type of behaviour that will be looked at in this project is the leg lifting behaviour of mammals and the territory marking through the means of urination with which it goes along. Many modern mammals exhibit this behaviour today. But territory marking is not observed across the entire mammalian class. The research into the origins of this behaviour will start by determining which mammals do indeed exhibit this behaviour. Once these species have been mapped on a phylogenetic tree, an evolutionary pattern will be found. This pattern will enable the location in time of a common ancestor to all the modern species, an ancestor who possesses the leg lifting behaviour and was the first to possess this behaviour. Once this research is complete, the origins of another aspect of animal life will be better understood, giving everyone a more complete understanding of the process of evolution and how it has shaped the species that exist today.

Presenting Author: Jimmy Turner

Turrin, Natalie and Hugh Henry

Biology, University of Western Ontario

Interactive effects of warming and nitrogen addition on plant senescence in a temperate old field.

Higher air temperatures and increased nitrogen deposition are key attributes of global climate change. Changes in the duration of the growing season, as observed in phenological records and by the normalized difference vegetation index (NDVI), indicate that plant phenology is sensitive to climate change. Though the timing of phenological processes like senescence is constrained by genetic and photoperiodic cues, increased temperature and nitrogen deposition may cause a shift in the duration of the growing season. To test this hypothesis, I tracked the fall senescence of four temperate old field species in a long-term warming and nitrogen addition field experiment in London, Ontario. The site is comprised of ten replicate blocks experimentally warmed by 2-3ºC and deposited with additional nitrogen. I tracked the most prevalent species at the site, Cirsium arvense, Poa pratensis, and Bromus inermis, by tagging three plants per species with yarn in each replicate plot. Plants were assigned a senescence score between zero and 100 based on the percent greenness of the plant. I used a FieldSpec® Pro UV/VNIR spectroradiometer to observe overall plant greenness at the plot level based on NDVI readings. The mean percent greenness of Bromus inermis was highest in plots that were warmed all year ($P<0.05$) and plants which received additional nitrogen ($P<0.05$) indicating that senescence had been postponed by the treatments. There were no significant delays in plant senescence in Poa pratensis, or Cirsium arvense. Spectroradiometric data were compared to the senescence scores and did not demonstrate a delay in senescence, reflecting the fact that no effects were observed in the majority of species. The results reported have implications for productivity, retranslocation of nutrients, and potential for frost injury for plants in which senescence is postponed.

Presenting Author: Natalie Turrin

Tuziak, Sarah, Hooman Moghadam and Roy Danzmann

Integrative Biology, University of Guelph

The characterization and expression of the ornithine decarboxylase (ODC) gene and antizymes in salmonids.

Ornithine decarboxylase (ODC) is a key 'signature' enzyme that functions in regulating cell growth and anabolic processes in organisms. It is involved in the biosynthesis of polyamines, specifically putrescine, spermidine and spermine, which are used for growth and differentiation of cells. Due to multiple whole genome duplication (WGD) events in vertebrates (with 3 and 4 WGD events identified in teleost fishes [i.e. 3R and 4R]), multiple ODC copies may exist in these fishes. Duplicated chromosome affinities that have been identified in salmonids (4R), and model teleost species (zebrafish and medaka) (3R) were used to investigate the genomic location of the ODC genes (i.e. the main ODC1 gene and two antizyme genes [OAZ1 and OAZ2]). Genomic locations were related to quantitative trait loci (QTL) for early life history traits, such as growth and development, and the evolution of these genes with respect to their ancient vertebrate ancestor. PCR products for the ODC gene complex were cloned, sequenced, and the genetic variation ascertained within multiple gene copies using mutation detection polyacrylamide gel techniques. Genomic locations for the genes were ascertained in two mapping panels of rainbow trout (Oncorhynchus mykiss) (Lot 25 and 44). Duplicate copies of these genes have been detected in rainbow trout. Two copies of OAZ2 are in the O. mykiss genome mapping to linkage groups RT-8 and RT-9. An allelic variant mapping to the short arm of RT-9 exhibits a high degree of segregation distortion. The RT-9p is also of interest as it exhibits a high degree of ancestral mosaicism with respect to 3R linkage group affinities. These duplicated genes are related to QTL regions associated with growth, condition factor, body weight, and other life-history traits.

Presenting Author: Sarah Tuziak

Vance, David and George van der Merwe

Molecular and Cellular Biology, University of Guelph

Vid24p, a component of the Vid30 complex , participates in the regulation of the tyrosine permease Tat1p in Saccharomyces cerevisiae.

Yeast fermentations are used in the production of alcoholic beverages and bio-ethanol. Understanding the adaptation of Saccharomyces cerevisiae, the commonly used fermentation yeast, to the changing environment of alcoholic fermentations will provide insight into the mechanism(s) used by the yeast to tolerate fermentation stresses. During the course of a fermentation the yeast is exposed to various nutrient stresses, osmotic pressure and increasing ethanol concentration. The yeast senses and adapts to varying nutrient conditions by altering its transcriptional profile and the post-translational modification and cycling of its proteins. The Vid30 complex (Vid30c) is needed to degrade the gluconeogenic enzymes Mdh2p and FBPase when cells are shifted from a glucose starved environment to glucose rich conditions, and to degrade the high affinity hexose transporter Hxt7p when nitrogen becomes limiting. Several high affinity amino acid transporters, including Tat1p, Tat2p, Hip1p, are regulated in response to changing nutrient conditions. Little is known about the regulation of Tat1p, a tyrosine permease. It is shown here that Vid24p, a component of the Vid30c, plays a role in regulating Tat1p at a post-transcriptional level. These findings implicate the Vid30c in the regulation of amino acid metabolism in yeast.

Presenting Author: David Vance

Villegas, Sylvia, Rebecca Meagher, María Díez-León and Georgia Mason

Integrative Biology, University of Guelph and Animal and Poultry Science, University of Guelph

Habituation to novel stimuli: do stereotyping mink pay attention?

Captive animals sometimes persist in repetitive, invariant and apparently functionless 'stereotypic behaviours' despite changes in the environment aimed at interrupting or eliminating them. Anecdotally, it has been suggested that performing stereotypic behaviour may reduce an animal's ability to pay attention to its environment, but this hypothesis remains untested. This study tested the attention paid by mink to a novel visual stimulus using a habituation paradigm. Habituation of the orienting response (a decrease in interest or approach behaviour with repeated stimulation by a non-signal stimulus), requires attention to ensure no important events are contingent on the stimulus. With reduced attention, it is expected that responses would be weaker and habituation would take longer. Farmed mink were repeatedly exposed to the moving point of a laser beam either when they were performing a stereotypic behaviour (n = 10) or during normal activity (n = 16), and the duration of the orienting response was recorded over consecutive trials. As in previous studies, stereotyping mink showed a weaker initial response to the stimulus compared to normally active mink ($U = 20$, $p = 0.002$). However, there was no significant difference in subsequent habituation rate. In a counterbalanced probe trial, the stimulus was presented once more in either the same or opposite condition to that in which they were habituated. Mink showed weaker responses to the probe while stereotyping, regardless of prior treatment: animals previously stimulated while stereotyping did not treat the probe as more novel or 'surprising' than animals previously stimulated during normal behaviour. The present results suggest that mink pay as much attention when stereotyping as during normal behaviour, although this study requires replication on a larger scale. Furthermore, the question remains as to why responses elicited during stereotypic behaviour are weaker than in normal behaviour.

Presenting Author: Sylvia Villegas

Walsh, Scott

Molecular and Cellular Biology , University of Guelph

Investigation of luman activation and the related unfolded protein response in pancreatic beta cell Lines subjected to glucose stress.

Type 1 diabetes mellitus is a chronic disease characterized by the spontaneous death of the insulin producing pancreatic ß-cells and elevated blood sugar levels. Currently, the most reputable theory describing the molecular pathogenesis of type 1 diabetes (the mechanism causing ß-cell death) is autoimmunity, though this has not been verified. Recent studies have found evidence that cellular stress and the Unfolded Protein Response (UPR) are actively involved in the molecular pathogenesis of type 1 diabetes. The Akita mouse strain spontaneously develops type 1 diabetes 8 weeks after birth and this has been linked to a mutation (Ins2C96Y) of the Insulin 2 gene that disrupts a stabilizing disulphide bond of the insulin 2 protein. The aberrant conformation of the Ins2C96Y protein has been linked to an upregulation of apoptosis related UPR proteins, such as CHOP, and the development of diabetes. Luman is a transmembrane protein of the ER and is involved in the UPR. This study found that Luman is activated in C6 cells (rat glial cells) and is localized in punctates under hyperglycemic culture conditions. Luman expression in the rat ß-cell line, INS-1, was to low to detect Luman protein activation. Also, CHOP expression is induced in C6 cells and INS-1 cells treated in hyperglycemic culture conditions. Expression levels of BiP, a protein involved in sensing global ER stress in the cell, was not affected by hyperglycemic culture conditions in both cell lines, except by the 500mM glucose concentrated media at which point evidence of apoptosis was observed. These results suggest that the a non-global ER stress signal is activated in these cell lines under elevated glucose stress conditions causing low level Luman activation and induction of CHOP related apoptosis in C6 and INS-1 cells. This has important implications in explaining the development of diabetes and diabetic neuropathy.

Presenting Author: Scott Walsh

Whalen, Dan, Ryan Mailloux and Vasu Appanna

Biology, Laurentian University and Chemistry, Laurentian University

A green technology for the production of biofuels.

The past several decades have been demarcated by growing concerns about climate change, a global condition attributed to the emission of green house gases (GHG). The mitigation of this global threat has been met by the development of green technologies and alternative fuel sources in an effort to diminish GHG production. One of the most promising technologies currently being developed is the conversion of agricultural biomass into alternative fuel sources. Indeed, the conversion of cellulose into alternative energy sources has become a cornerstone of green technologies. However, the development of efficient methods for the use of hemicellulose, a major component of woody materials, in the production of alternative fuels remains elusive. In the present study, we developed a microbial system capable of converting xylose, the dominant structural component of hemicellulose, into value-added products. A microbial consortium grown under various nutrient conditions allowed the efficient metabolic conversion of xylose into hexoses. Microbes cultured in a standard mineral medium were found to have the highest conversion rates of xylose to glucose. In contrast, microbes exposed to nutrient stress preferentially generated mannose. The proficient metabolism of xylose was attributed to an NADP-dependent xylose dehydrogenase. Intriguingly, NADP-dependent xylose dehydrogenase displayed a lower activity profile in the nutrient stress. Thus, we have developed a microbial system capable of converting xylose into fermentable hexoses. The optimization of this process will enable the production of fuels in a sustainable manner.

Presenting Author: Dan Whalen

Williamson, Samantha

Molecular and Cellular Biology, University of Guelph

Regulation of myogenesis and its potential implications for muscle regeneration.

Myogenesis is the formation of muscle cells. Throughout adulthood it occurs by way of satellite cells augmenting existing tissue. Skeletal muscles of vertebrate embryos are somite derived. Initiation of myogenesis occurs by the delamination of muscle progenitor cells in the dermomyotome, expressing the myogenic determination factors Myf5 and Mrf4 (helix-loop-helix transcription factors). The cells move under the dermomyotome and rapidly differentiate. In mature vertebrate myotome, cells expressing Pax 3 and Pax 7 are derived from the central dermomyotome. These satellite cells proliferate but express no muscle determining factors and can give rise to skeletal muscle cells if Myf5 and MyoD are activated. In adult myogenesis, quiescent skeletal muscle satellite cells can become activated and the resulting myoblasts, express Pax7:Pax3, Myf5 and MyoD. Once committed to differentiation, myoblasts stop cycling and lose expression of Pax7, Pax3, and Myf5. Differentiating myogenin expressing myocytes will then align and fuse to form myofibers. Regulation of myogenesis as it pertains to the reprogramming of stem cells to regenerate skeletal muscle tissue is a current area of intense study due to the potential medical implications of such knowledge. The current literature regarding myogenesis regulation is examined. Potential areas of new research are highlighted. Taking cells from a patient afflicted with a muscular disorder, converting them to stem cells and inducing healthy myogenesis in those cells is the research postulated here. Those cells will be transplanted back into the individual in hopes of damaged muscle tissue regeneration. The practicality, ethics and longevity of such treatments will also be addressed.

Presenting Author: Samantha Williamson

Wills, Melanie

Molecular and Cellular Biology, University of Guelph

Domains of attraction: mapping protein interactions in a ShcD-mediated neural cell signaling cascade.

The ShcD protein has been characterized as an intracellular phosphotyrosine adaptor belonging to the Src homology and collagen (Shc) family. These scaffolding molecules facilitate receptor-mediated signal transduction through their ability to selectively recognize and bind phosphorylated tyrosine residues in the cytoplasmic tails of a variety of activated receptors. Shc proteins then recruit other factors to the membrane that participate in mitogenic signaling, notably elements of the MAP Kinase cascade. ShcD is most similar in form to ShcA, however unlike the latter, its expression in the mouse is localized to the brain and skeletal muscle. Consistent with this observation, we have found that human ShcD is able to bind the TrkA neurotrophin receptor, which is responsible for potentiating survival and differentiation signals in neurons. Here we characterize the domains and residues that influence this interaction and contribute to ShcD-mediated signal transduction.

Presenting Author: Melanie Wills

Winegardner, Amanda , Andrea Allen, Ingrid Ng and Karl Cottenie

Integrative Biology, University of Guelph

The role of local and regional processes in the community structure of sub arctic rock pools.

The influence of local environment and dispersal limitations as determined by spatial relationships on community structure remains an active area of research in community ecology. The zooplankton communities of rock pools in Churchill, Manitoba are an ideal system for a metacommunity study. Zooplankton samples from rock pools on three separate rock bluffs in the Churchill area, collected in July 2007 along with environmental and spatial data was used to evaluate the contributions of environmental variables and dispersal limitation to the species composition and distribution of this community. Zooplankton was identified and quantified and redundancy analyses were used to explain any variation found in the communities. We tested hypotheses suggested by the results of a previous study that faced problems related to sample size, bluff replication, and confounding spatial and environmental variables: dispersal between pools is limiting between bluffs but not within each bluff. We found surprisingly strong differences in community composition between the bluffs that were not related to differences in environmental variables, suggesting dispersal limitation between bluffs. In addition, we also found evidence for both dispersal limitation and efficient dispersal within different bluffs. The results of this unambiguously confirm the previous study, and provide a complete survey of zooplankton diversity of the area; detailed information on environmental gradients present in small and temporary pools of this northern ecosystem and evidence that it is crucial to study dispersal limitation in zooplankton metacommunities at different scales to incorporate the full complexity of metacommunity dynamics.

Presenting Author: Amanda Winegardner

Wood, Janet

Biology, Laurentian University

Blanding's turtle (Emydoidea blandingii) movements and habitat selection in an urban landscape.

Habitat fragmentation has been identified as a major global threat and key factor in declines of animal populations, including populations of turtles. Blanding's turtles (Emydoidea blandingii), are a Threatened species found in urbanized areas such as the Greater Toronto Area (GTA), and may be profoundly impacted by habitat loss and fragmentation. Six Blanding's turtles were studied in the urban landscape of the Rouge Park, Toronto to describe home range size, daily distances moved, macrohabitat selection, microhabitat selection, and possible basking site limitations to determine if habitat fragmentation was having an effect on these population parameters. Blanding's turtles in the GTA exhibited larger home ranges in general than turtles in more natural, unfragmented landscapes because turtles in fragmented landscapes must travel further in search of resources that are further apart and separated by areas that contain unsuitable habitat. However, daily distances moved by Blanding's turtles in the GTA did not differ from those of turtles inhabiting less fragmented landscapes. At the macrohbaitat scale, Blanding's turtles in the GTA spent the same amount of time in man-made habitats as they did in natural habitats, indicating that habitat creation is a viable management action in urban areas. Variation existed in macrohabitat selection among Blanding's turtle populations regardless of the level of fragmentation of the habitats. AIC modeling indicated that Blanding's turtles in the GTA selected specific microhabitat characteristics: shallow, warm water with deep mucky substrates and dense aquatic vegetation. Basking sites in the GTA were a limiting resource to Blanding's turtles, possibly because basking structures were being removed by humans for "aesthetic" purposes. My study is important because it provides data to assist in the design of conservation plans for at-risk turtles in an urban landscape.

Presenting Author: Janet Wood

Wright, Michael and Mihai Costea

Biology, Wilfrid Laurier University

Cuscuta salina (Salt marsh dodder; Convolvulaceae): a phenetic analysis of morphological variation.

The dodders, Cuscuta (Convolvulaceae), are a cosmopolitan array of obligate plant parasites dependent on their hosts for nutrition. Considerable taxonomic confusion exists within this genus due to the reduction and loss of almost all vegetative characters. The salt marsh dodder, Cuscuta salina, exhibits a significant morphological variation, reflected in the description of three varieties: var. salina, var. major and var. papillata. Recent molecular evidence has given new clarity to the evolutionary relationships among these taxa, but it further requires a resolution at the morphological level. Principal components analysis, discriminant analysis and canonical variates analysis were performed on a dataset of 40 morphological characters scored on 60 specimens of the three currently recognized varieties of C. salina. The phenetic analyses and accompanying herbarium studies suggest that only two lineages are distinguishable from morphological, ecological, and biogeographical points of view. These lineages correspond to C. salina var. salina and var. major which could alternatively be treated at the specific rank, provided that more characters are found in the future. Cuscuta salina var. papillata is not a valid taxon because the presence of papillae on the corolla and/or calyx, which was previously considered the only exclusive characteristic of this variety, is also shared by the varieties salina and major.

Presenting Author: Michael Wright

Wyszynski, Emily

Biology, Laurentian University

Assessing population demography of Eastern White Cedar (Thuja occidentalis) in the greater Sudbury region.

There has been a long history of disturbance within the Greater Sudbury region and it is likely due to consequences of the mining industry. For years this area was exposed to tons of sulfur dioxide as well as other heavy metals such as copper and nickel, which resulted in large scale acid precipitation. The health of most forest systems were severely threatened and only began to recover with the introduction of new emission control measures such as the super stack in 1975. Studies in the past may have examined the recovery of certain vegetation types, but no research has been conducted on the population health of Thuja occidentalis, more commonly known as Eastern White Cedar. This species is of particular interest to the First Nations people in this area who use it as a traditional material, and more importantly in their medicines. Using the Roberts-Pichette & Gillespie (EMAN biodiversity permanent monitoring plots) protocol, two cedar stand communities located in the vicinity of two smelter sites, were surveyed. Cedar seedlings, saplings and mature individuals were measured, and an overall assessment of surrounding ground vegetation was completed. Mature trees were examined in terms of height (m), dbh (cm), abundance and physical condition. This preliminary study indicates that there is no significant difference between the two cedar populations. This study will be part of a larger one which will include the establishment of permanent biodiversity monitoring plots in non-smelter affected regions as well as on First Nations lands, in order to better understand the current and future health of cedar stands in Northeastern Ontario.

Presenting Author: Emily Wyszynski

Yagi, Katharine

Integrative Biology, University of Guelph

The effect of active season length on the growth rate of the Spotted Turtle, Clemmys gutatta, in a southern Ontario population.

The spotted turtle (Clemmys gutatta) is an endangered species in Ontario due to habitat loss, habitat fragmentation and illegal trade. The length of the active season or length of the turtle's aestivation period may affect turtle growth rate. Increasing seasonal temperatures, due to global warming might alter the timing and length of aestivation, a behavior of which is observed during high summer temperatures. If turtle growth rate depends on the length of the active season, and increasing ambient temperature shortens the active season, then turtle growth rate will decrease with increasing seasonal temperatures. Ontario Ministry of Natural Resources (OMNR) collected mark-recapture data on spotted turtles from a southern Ontario population from 1998-2005 and tracked 14 turtles using radio telemetry. Data was collected on body size, temperature, behavior, and habitat from 2000-2005 for telemetry and mark-recapture turtles. Using linear regression analysis, an insignificant negative correlation was found between cumulative maximal number of active season days and the average growth rate ($R2= 0.946$, $p=0.173$). A significant negative correlation was found between the cumulative maximal number of aestivation days and the average growth rate of the turtles ($R2= 0.988$, $p=0.043$). These relationships suggest that longer aestivation periods decreases turtle growth rate. However, sample size was low, therefore additional research is warranted. Also, further research is needed to determine if habitat quality affects turtle growth since reptile thermoregulation depends greatly on habitat. Lab research on growth rate would provide more control on determining the rate of growth in individual age groups. Understanding how temperature affects the behavior and population dynamics of this species at risk can help with future conservation planning.

Presenting Author: Katharine Yagi

Yavno, Stan and Lynda Corkum

Biological Science, University of Windsor

Visual and chemical signaling in the round goby, Apollonia melanostoma.

Chemical communication between sexes of a given species may enhance the reproductive success of individuals. Males of the invasive fish, the round goby, release a sex attractant that lures females to their nest. In the laboratory, gravid females orient their movement and increase their swimming speed to conditioned water from reproductive males (RM). Our lab has demonstrated that sex steroids are released in the urine of RM. Using a lab flume, we determined if gravid female round gobies showed an increased attractiveness to RM urine compared with non-reproductive male (NRM) urine in the presence of physical models (made of silicone) of each male type. We also conducted a dose response experiment to determine if different concentrations of NRM and RM urine released from a RM model exhibited similar responses from gravid females. Females spent more time at a nest with black (RM) rather than mottled (NRM) models ($F1,8 =9.293$, $P=0.016$), whereas urine ($F1,8 =4.776$, $P= 0.06$) and the model (with urine) ($F1,8 =4.790$, $P=0.06$) affected the time spent by females at the nest to a lesser degree. There was no significant difference in the relationship between time spent at a nest with RM model regardless of the RM urine concentration. Female attraction to a RM model declined with decreasing concentrations of NRM urine ($F1,2.=33.64$, $P=0.029$, $r2 = 0.94$). Our findings suggest that the use of physical models and odours (urine) will trap gravid females in the field. Field studies are needed to confirm the usefulness of these stimuli to control this invasive species in selected areas.

Presenting Author: Stan Yavno

Yebio, Bethale and Abdel Omri

Biology, Laurentian University

Antimicrobial activity of co-incorporation gentamicin and gallium into liposomes

Objectives: Quorum sensing-regulated expression of virulence factors is a mechanism pathway utilized by bacteria to form and preserve biofilms, tolerate conventional antimicrobials, as well as the host innate immune system. We sought to construct liposomes that would increase bacterial susceptibility of P. aeruginosa (PA), disrupt the communication pathways by which biofilms form, and exhibit minimal toxicity. Methods: The Dehydration-rehydration method was used to encapsulate gentamicin (Gen) and gallium (Gal) into the liposomes. These liposomes were prepared from a mixture of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-[phospho-rac-glycerol] (DMPG) (10:1). Gal and Gen were quantified by atomic absorbance spectrophotometry and by microbiological assay, respectively. The in-vitro stability of liposomal-gentamicin-gallium (LGG) was examined over a 48h period in PBS (4°C), plasma (37°C), and BAL (37°C). The MICs and MBCs of LGG and free-Gal-Gen (FGG) were assessed by broth dilution. Bactericidal activity of LGG and FGG on biofilms was assessed by MBEC assay. QS-expression within PA, was examined by an AHL plate assay. We assessed Gal toxicity by treating a human lung cell line (A549) with both encapsulated and free Gal. Cell viability was assayed by the Trypan Blue exclusion method. Results: Liposomes were stable in all fluids except plasma. Liposomes retained 57% of the initial encapsulated antibiotic over 48 h in plasma. The MICs of LGG were lower than that of free formulation in all used strains. The MICs of LGG were 3-4 folds lower than that of the free formulation. Quorum sensing between planktonic strain (PA 48913) was inhibited when treated with LGG (0.078µM, 0.94mg/L). Viability of human lung cells (A549) incubated with liposomal-Gal was greater than the controls. Conclusion: Overall, these results indicate that LGG may serve as a useful tool in controlling pulmonary infections associated with P. aeruginosa.

Presenting Author: Bethale Yebio

Yehia, Adham

Biology, McMaster University

The role of Telemerase Repeat Binding Factor 1 and phosphorylation in cell cycle regulation.

Telomeres are specialized protein/DNA structures found at the end oflinear chromosomes that play a key role in maintaining genomicstability and preserving chromosomal integrity during the cell cycle.Telomerase Repeat Binding Factor 1(TRF1) is a telomeric protein thathas been shown to be associated with telomeres in a cell-cycleregulated manner with the lowest association being in S phase. TRF1binds double stranded telomeric repeats as a dimer and acts as anegative regulator of telomerase dependent telomere elongation. Thecentral dimerization domain (TRFH) found on TRF1 has been shown to benecessary for dimerization and TRF1 interaction with TIN2, apoly(ADP-ribose) polymerase modulator that, along with TRF1, is acomponent of the Shelterin complex. TRF1 has been shown to bePoly(ADP-ribose)ylated by Takyrase1 which in turn causes TRF1dissociation from telomeres. Two putative phosphorylation sites on theTRFH domain (Threonine137 and Serine249) have been identified by massspectroscopy. We set out to study the effects of the phosphorylation ofthese sites on TRF1 dimerization and it's interaction with TIN2 andpropose a mechanism for the cell cycle regulation of TRF1 and itsimpact on telomere dynamics.

Presenting Author: Adham Yehia

Yu, Darrick

Molecular and Cellular Biology, University of Guelph

Genes Involved in the synthesis of core oligosaccharides in Pseudomonas aeruginosa.

Pseudomonas aeruginosa is a gram negative bacterium and opportunistic pathogen. A major component of the cell walls of gram negative bacteria is a macromolecule composed of both lipids and carbohydrates called lipopolysaccharide (LPS). LPS is made up of three main parts: lipid A, the core region, and O antigen. A number of different genes catalyze the synthesis of LPS in P. aeruginosa strain PAO1. Among them are migA and wapR, two genes involved in the biosynthesis of the LPS core region. Previous studies have shown that MigA catalyzes the addition of a-1,6-linked L-rhamnose to the outer core region of LPS, whereas WapR catalyzes the addition of a-1,3-linked L-rhamnose to the outer core region. This report describes attempts at determining the cellular localization of the products of these two genes. In addition, the function of several genes located within the core oligosaccharide biosynthetic gene cluster, and PA1014, a homologue of the migA gene, was investigated with respect to core region LPS phenotype. Preliminary findings suggest that PA5001, PA5002, and PA5003 mutants have a similar outer core structure, while a PA1014 mutant might possess an altered outer core structure. Further investigation is needed to validate this finding.

Presenting Author: Darrick Yu

Zimmer, Zoey

Molecular and Cellular Biology, University of Guelph

The analysis of SNAP23-protein interactions in mammalian cells.

Cell adhesion and migration are essential processes in multicellular organisms. They are required for many physiological events, from embryonic development to tissue homeostasis. When cell adhesion or migration is disrupted disease can result, for example arthritis, developmental disorders and tumor progression. During tumor progression, cells exhibit irregular abilities to adhere and migrate in their environment. Before we can fully understand how tumor progression results from aberrant cellular adhesion and migration, we must first elucidate the molecular mechanisms involved in normal cellular motility. Recently, it has been shown that soluble NSF attachment receptor proteins (SNAREs), such as SNAP23, are involved in mediating cell adhesion and migration through their participation in membrane trafficking. SNAREs regulate the fusion of vesicles with target membranes, which results in the delivery of cargo to different compartments within the cell. The transport of proteins is necessary for proper cell adhesion and migration. For example, matrix metalloproteinases (MMPs) and integrins, whose respective functions are to degrade the extracellular matrix (ECM) and attach the cell to the ECM, are trafficked in a SNARE dependent manner. In order to further characterize the roles of SNAREs in mediating cell motility, we are examining SNARE-protein interactions within mammalian cells. Specifically, analysis of SNAP23 interactions will provide information concerning vesicle trafficking by this SNARE and its role in mediating cell motility. Mammalian cells were transfected with a GFP-tagged SNAP23 construct. Subsequently, immunoprecipitation was performed with the lysate from these transfected cells using an anti-GFP antibody. The eluent was immunoblotted for a variety of proteins involved in vesicle trafficking: cortactin, dynamin2, and myosin. Furthermore, immunofluorescence was used to examine the co-localization of SNAP23 and potential interacting proteins. Following these experiments, it will be possible to purify SNAP23 from migrating cells and further elucidate the mechanisms by which it interacts with these proteins to regulate vesicle-membrane fusion.

Presenting Author: Zoey Zimmer

Zou, Shicong and Shiva Singh

Biology, University of Western Ontario

Characterization of potassium channel Kcnj10 and its role in ethanol preference determination: expression and SNP analyses in C57BL/6J and DBA/2J mice.

Comprised of seven subfamilies, inwardly-rectifying potassium (KIR) channels play a crucial role in maintaining the cell's resting membrane potential and K+ homeostasis. In the mouse, Kir4.1 is encoded by the Kcnj10 gene which maps to both a known seizure susceptibility locus and a quantitative trait locus associated with the voluntary alcohol consumption (VAC) phenotype. Furthermore, microarray studies have found Kcnj10 to be downregulated in the brains of human alcoholics suggesting that, like other KIR channels, Kcnj10 and/or Kir4.1 may be targets of ethanol action. Therefore, expression and regulation of this gene are critical in gaining an understanding of the complex phenotype of VAC. Expression studies were conducted using eight different tissues (brain, liver, lung, heart, spleen, kidney, testes, and muscle) from male mice of the alcohol-preferring, seizure-resistant C57BL/6J (B6) strain and the alcohol-avoiding, seizure-prone DBA/2J (D2) strain. Semi-quantitative RT-PCR revealed significantly higher expression in the brain, spleen and kidney of both strains when compared to other tissues from the same strain. There were no significant differences in expression between strains except in the testes. Previous studies had identified a nonsynonymous SNP between the B6 and D2 strains that changes amino acid 262 from threonine (B6) to serine (D2). F2 mice of B6 and D2 reciprocal crosses were tested for ethanol preference, with the top and bottom 10% being classified as high- and low-drinkers, respectively. The presence of the high-drinking B6 allele correlated with the high-drinking phenotype. There was no significant correlation of the low-drinking D2 allele. These results point to a contribution (direct or indirect) of the B6 allele on ethanol preference.

Presenting Author: Shicong Zou