RE Peter Biology Conference Abstracts Booklet

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Abstracts

Both Poster and Oral Presentations

1 Function of Meis1b In Zebrafish Development

Thomas Chou, Lyndsay Selland, Andrew Waskiewicz

Generation of a segmented body plan is central to the development of many organisms, ranging from Drosophila to humans. In order for each segment of the body to acquire the correct identity, they must express the correct hox genes (a subset of homeotic genes), which will in turn drive the expression of downstream genes. Binding of hox transcription factors to appropriate targets within the genome requires the formation of heterotrimeric complexes composed of Hox, Pbx, and Meis proteins. Pbx (Pre-B cell leukemia transcription factor) and Meis (myeloid ecotropic viral integration site) proteins are co-factors which increase the binding specificity of Hox proteins. We have generated a meis1b knock out containing a 49 base pair deletion which will result in a premature stop codon in the early region of the protein that lead to a loss-of-function. Previous work in our lab has postulated an important role for meis1b in hindbrain development, however loss of meis1b function does not have significant effect on hindbrain segmentation and generation of segment specific neurons. Further examination of meis1b mutant morphology instead reveals two novel phenotypes. Beginning at 3 days post fertilization (dpf), there is decreased circulation and blood pooling in the tail. Circulation in the anterior part of the body remains functional. Secondly, at 7 dpf, meis1b mutants fail to inflate the swim bladder, resulting in a loss of balance. In order to determine the cause of the blood pooling phenotype, we will use the Tg (kdrl:GFP) line to examine blood vessel structure. To follow up on the relationship between Meis1b and blood vessel formation, we will examine VEFG (vascular endothelial growth factor), which is proposed to be regulated by Meis1b and is required for vascular endothelial formation.

2 Growth of Methylocystis sp. Strain Rockwell in Assorted Nitrogen and Carbon Sourced Media

Jordon Chan

Methane, a compound which has a small percent composition of our atmosphere happens to have a profound effect on our global environment. Methane monooxygenase is a special enzyme utilized by bacteria called methanotrophs which are crucial in oxidizing methane. The utilization of this carbon dissimilatory pathway can contribute to biotechnological advances in bioremediation, single cell proteins, and sustainable bioprocessing. Polyhydroxybutyrate (PHB) production is a popular target for biotechnological companies as it is a sustainable substitute to petroleum based plastics as the polymer is biodegradable, however the cost of up-scaling the production of PHB prevents the usage of such a resource. With the abundance of methane in our atmosphere, the price of PHB production can be greatly reduced with the use of methane as a cheap substrate to create an economical resource. Methylocystis is a genus of bacteria that are capable of methanotrophy and many strains of this genus have the genes for PHB biosynthesis. While Methylocystis sp. Rockwell has the genes for PHB, it is unknown what is necessary for the expression of these genes in this strain. In order to elucidate whether or not PHB production can be induced in Methylocystis sp. Rockwell, Methylocystis sp. Rockwell was put under several growth conditions with various nitrogen sources and carbon sources with a limitation on the nitrogen source to see if PHB production could be induced. Growth of Methylocystis sp. Rockwell was established under various conditions, however PHB analysis of Methylocystis sp. Rockwell grown in these conditions has yet to be determined.

3 Factors Driving Moose (Alces alces) Vehicle Collisions in Alberta

Benjamin Sperling, Milou Geven, and Mark S. Boyce

Moose-vehicle collisions (MVCs) are a widespread issue found across Canada, Alaska, and Scandinavia. They incur millions of dollars in insurance costs and bring harm to both humans and moose alike. MVC frequency within Alberta, Canada was observed relative to the following factors: population density, vegetative cover, hunting pressure, and time of year. The relationships uncovered among these factors revealed regions most vulnerable to MVCs. Winter was prone to the highest frequency of MVCs compared to other seasons with collision risks peaking at night. Within 48 identified MVC hotspots, the cover types found in the highest abundance were: shrub wetlands, treed wetlands, broadleaf forests, and herbaceous. Hunting pressure and MVC frequency had a positive correlation with each other. The above information is meant to provide an index for mitigating future MVCs.

4 Understanding the role of kcnv2b gene in regulating photoreceptor morphology

Tehreem Khalid and Jennifer C. Hocking

Photoreceptors(PRs) detect light and are required to perceive vision. There are two main types of PRs, called rods and cones, that allow us to see under dim-light and differentiate between colors, respectively. These PRs have different morphologies and gene expression, but both have hugely expanded and specialized cilia called outer segments, which have stacks of photopigment-laden membranous disks. Abnormalities in PRs often lead to cell death and functional blindness, and zebrafish can be used as a model organism for studying PRs. Previous work found that loss of the early patterning gene gdf6a in zebrafish leads to abnormal PRs, with shrunken cone morphology and expanded rod outer segments. An RNAseq experiment identified a severe downregulation in kcnv2b, a potassium channel subunit involved in modulating the PR activity. KCNV2 human mutations lead to a disease called 'Cone-rod dystrophy with supernormal rod response', a phenotype which aligns well with the aberrant PR morphology in gdf6a mutant fish. We designed CRISPRs to target the zebrafish kcnv2b gene through CRISPR-Cas9 mutagenesis. Two different sgRNAs were injected into one-cell stage zebrafish embryos together with Cas9 protein. Genomic DNA isolated from injected embryos was analyzed by high resolution melt temperature(HRM) analysis to screen for mutations. We confirmed by sequencing that

CRISPR 1 was cutting the DNA and inducing mutations, and many injected embryos are currently being raised. Additionally, an overexpression construct was created for the kcnv2b gene using gateway cloning, and includes a cone-specific promoter and a red fluorescent tag. Both the kcnv2b mutant and the overexpression construct will be used to study how modulating photoreceptor activation can affect the morphology and function of photoreceptors. This research will help us understand the mechanisms of PR-related eye diseases.

5 An examination of troodontid tooth anatomy and the implications of functional morphology on diet.

Mark Powers, Angelica Torices and Philip J Currie

Troodontids have had a convoluted history since their discovery, and have been identified as lizards, coelurosaurids, thescelosaurids and pachycephalosaurids. These different taxonomic assignations have created a great deal of confusion regarding their paleoecology and behavior. However, they are known to be a sister taxon to Dromaeosauridae, and are one of the more specialized families of theropod dinosaurs. Ziphodonty and the presence of denticles on the carinae are characters shared by carnivorous animals like theropod dinosaurs, crocodiles, early archosaurs and varanids. However, because troodontid teeth are somewhat unusual, some authors have considered them to be omnivores based on the coarseness of the denticles. Coarse denticles are present in herbivorous animals like thescelosaurids, pachycephalosaurs and even some modern herbivorous lepidosaurs. The apparent similarity between the serrated teeth of pachycephalosaurids and troodontids is why they were considered to be the same animals for many years. However, the enamel structure of these taxa is very different, and the shape and structure of a troodontid denticle is more characteristic of a highly carnivorous diet. Troodontid denticles are relatively large, but are also widely spaced, which provides greater cutting efficiency than the closely spaced serrations of herbivorous forms. Interesting structures that appear within the inter-denticle areas of troodontids, tyrannosaurids and other theropods are razor-sharp enamel ridges that form very narrow slots for efficiently cutting muscle fibres. The sharpness of these ridges is protected by the thickness of the serrations themselves. We have analysed the presence of these structures in theropod and ornithopod dinosaurs from Alberta. The presence of enamel ridges on the midlines of troodontid denticles, but not on the serrations of herbivorous dinosaurs, suggests they may be specialized to cut meat fibers. These in turn demonstrate strong support for a more carnivorous diet, and add to the repertoire of predatory adaptations of troodontids.

6 Regulatory Links or Cross-Talk Between CpxRA and ArcAB Two Component Regulatory Systems

Jamie Stark*, Randi Guest, Tracy Raivio

Bacteria possess adaptive mechanisms that enable adjustment to environmental changes via modification of gene expression. Among these are the Arc and Cpx two-component signal transduction systems. Each system is comprised of an inner membrane sensor kinase, which receive external signals, and a response regulator, which regulates gene transcription in the nucleus, allowing the cell to adapt. The separate functions of both systems are well established. Arc responds to quinone oxidation state and regulates genes for transport, energy metabolism and cell structure. Cpx responds to protein mis-folding from environmental stressors and regulates genes for cell-envelope integrity. At a glance, the regulatory roles of these systems seem independent, but an overwhelming accumulation of data suggests an interaction between the two occurs. However, this has never been explicitly confirmed, nor is the molecular mechanism by which the interaction transpires, known. In this study I aim to confirm the occurrence of cross-talk and characterize the molecular nature of the regulatory links between the Arc and Cpx two-component response systems. This is investigated by determining each pathway's activity when the other is stimulated, using luminescent reporter plasmids to monitor the activity of promoters regulated by ArcA and CpxR in various E. coli mutants. A luminescent reporter for the Arc regulon has been constructed to analyze how an ArcA regulated promoter's activity is affected by mutations in either response pathway. Initial results support regulation of the Arc reporter by Cpx, suggesting a link. Future directions will include using a CpxR luminescent reporter in the same way. Both Cpx and Arc responses contribute to virulence in enteropathogenic bacteria. Comprehension of how Cpx and Arc signals are integrated to allow bacteria's comprehensive adjustment to changing hostile surroundings in the gut may lead to discovery of a novel target for treatment of certain infections.

7 A Road Runs Through It

Caitlin Willier, Scott Nielsen, Kevin Devito

When a road is build through a peatland with horizontal water flow, roads can act as dams; as a result, trees on the "upstream" side of a road can become waterlogged and either die or become stunted, whereas on the "downstream" side of a road, a prolonged drop in the water table can cause the trees to root deeper and grow taller than what is average in the wetland. Interestingly, this phenomenon does not occur consistently. In fact, the same road constructed through two different wetlands may disrupt tree growth patterns in one wetland whereas the other location may appear unaffected. This study examines the conditions that maintain wetlands: landscape position and soil substrate in wetlands with road disturbances and compares the magnitude of stand structure and vegetation changes among different peatland types. We used LiDAR derived tree canopy height and cover from 96 peatlands and vegetation field sample plots from 48 peatlands in North Eastern Alberta. We analyzed the data using Generalized Linear Mixed models to explain the variation in canopy height and cover. Results from this study are anticipated to help guide management decisions for building roads through areas with complex forested wetlands by identifying peatland and substrate types that are more susceptible to road impacts.

3 Minute Thesis Only:

8 Mosasaur maelstrom

Hallie P. Street

Mosasaurs are a group of extinct aquatic squamates that inhabited the world's oceans during the Late Cretaceous (100-66 Ma). The first mosasaur fossils known to the scientific community were discovered in the Netherlands during the 1760s and 1770s and sparked several decades of debate about what organism the specimens represented and what it should be called. Subsequent discoveries revealed the diversity of the clade, but the systematics of the type specimen and its closest relatives has remained largely unrevised. The purpose of my thesis was to examine the morphology of the species currently assigned to Mosasaurus in order to compare them to the type species M. hoffmannii. I also performed the most inclusive phylogenetic analyses for the group to assess the relationships between the various species. The interpretations from the morphological analyses were supported by the results of the phylogenetic analyses: Mosasaurus should be restricted to the type species and three morphologically similar species within a monophyletic clade of related mosasaurs that lived during the last ~12 million years of the Cretaceous.

Oral Presentations:

9 The role of ancestral nrl in rod development- mechanism for the evolution of mammalian rod-dominated retinas

A. Phil Oel, Keon Collett, W. Ted Allison

"Rod and cone photoreceptors mediate vertebrate vision; rods work in dim light, while cones enable high-acuity and colour vision in bright light. The ancestral vertebrate retina was likely cone-dominant, but early mammals that diversified into extant lineages were nocturnal and adopted a rod-dominant character that persists today. The mechanisms of this adaptation remain mysterious. We hypothesize that the pro-rod transcription factor nrl mediated this change; mammalian NRL is critical to rod development, and when ectopically expressed, converts cones to rods. We used zebrafish, a cone-dominant vertebrate, as an outgroup to mammals to explore the ancestral role of nrl, testing the hypothesis that ectopic expression of ancestral nrl in cone photoreceptors converts developing cones to rods, perhaps facilitating the shift to rod-dominant retina in early mammals. We also explored an alternative hypothesis– that protein sequence changes in NRL of early mammals account for the shift to a rod-dominant retina.

We drove ectopic expression of zebrafish nrl in a subset of cones via transgene, and depleted nrl during development using knockdown/knockout tools (morpholino/CRISPR). By thus modulating nrl expression, we assessed its role in a cone-dominant vertebrate (zebrafish). As in mammals, nrl depletion appears to have reduced rod generation. Moreover, cones expressing ectopic zebrafish nrl express rod markers, indicated a conserved role for non-mammalian nrl in the rod development program. We also assessed the effects of expressing mouse NRL and chicken MAFA (closest homolog) in similar contexts.

Whereas our lineage tracing work provides no evidence that rods derive from cone precursors in zebrafish, our collaborators show this does occur in mice (Kim et al., 2016 Dev Cell). Here we investigated the mechanism by which early mammalian retinas became rod-dominant, departing from the ancestral cone-dominant state. Our preliminary results suggest that ancestral NRL could influence the development of cones."

10 The evolutionary making of biting jaws in vertebrates

Tetsuto Miyashita

"A developing vertebrate head is a whole of many serial structures: hindbrain rhombomeres, neural crest streams, pharyngeal arches and pouches, placodes, and others. Although no single scheme of segmentation can explain all, it remains a puzzle whether these serial structures originated at once or independently. A long-standing challenge to test this is that the serial patterns appear to be shared by all known vertebrates.

I provide fossil and developmental evidence that this is not the case — early-branching vertebrates do depart from the commonly accepted serial patterns in the pharynx. Comparison between cyclostomes (hagfish and lampreys) and gnathostomes (jawed vertebrates) reveals that the mandibular region does not have a typical pharyngeal arch organization in cyclostomes. A diffuse boundary between ‘premandibular’ and ‘mandibular’ anlagen does not persist. The ‘mandibular’ ectomesenchyme occupy posterior positions in the head of cyclostomes that would only emerge as developmental defects in gnathostome embryos. Extinct lineages of jawless vertebrates have similar muscular and skeletal patterns to cyclostomes in general, but also suggest that diffuse boundaries around the ‘mandibular’ elements independently evolved in hyoid or hypobranchial positions in some lineages.

A synthesis of the evidence indicates that the mandibular arch assimilated a pattern of gill arches to give rise to a jaw. This assimilation occurred as the mandibular arch lost peripheral differentiations and became spatially confined. This Mandibular Confinement Hypothesis proposes an evolutionary scenario reverse to the one presented in textbooks: not that a gill arch was modified into a jaw, a labial portion of the pharynx mimicked the gill arches to create one more segment at the anterior end of the pharynx, which became the much feared biting apparatus."

11 Monitoring temporal trends in polar bear (Ursus maritimus) foraging ecology using stable isotope analysis

Amy C. Johnson, Andrew E. Derocher, Nick J. Lunn, Evan S. Richardson, and Keith A. Hobson

"Polar bears (Ursus maritimus) are an important top predator in the Arctic ecosystem and they rely on sea ice for hunting their main prey, ringed seals (Pusa hispida) and bearded seals (Erignathus barbatus). However, sea ice is declining and this is associated with reduced polar bear body condition, reproductive success, survival, and population abundance. Further sea ice declines are predicted for the future, which will reduce access to prey and affect the persistence of polar bear populations. This indicates the importance of monitoring the influence of climate change on polar bear ecology to understand population responses to changing habitat conditions. The objectives of this project are to: 1) Determine temporal trends in foraging ecology of the Western Hudson Bay population; 2) Analyze variation in foraging ecology within the population; and 3) Examine the relationship between foraging trends and climate over the past two decades.

Stable isotope analysis (SIA) on polar bear guard hair samples will be used to examine foraging ecology dynamics. Temporal trends in foraging ecology will be determined by examining dietary composition from long-term (1993-1994 and 2004-2017) SIA data. Preliminary results indicate differences among recent years in polar bear dietary composition and future analysis will determine longer-term trends in foraging ecology. Secondly, variation in foraging ecology within the population will be investigated by examining the relationship between individual polar bear characteristics (age, sex, reproductive status, body condition) and diet (from SIA). Lastly, the relationship between long-term foraging trends and climate (sea ice and climate indices) will be examined. This project will describe how polar bear foraging ecology is responding to changing climate conditions over time and how this may vary within the population. This research will be useful for monitoring the influence of climate change on polar bear ecology in the rapidly changing Arctic ecosystem.

12 Diet composition and prey selectivity of gray wolves (Canis lupus) in Prince Albert National Park, Saskatchewan

Justin Shave, Andrew Derocher, and Seth Cherry

The gray wolf (Canis lupus) has been studied to examine both the direct and indirect effects of predation on other species. Most studies have focused on wolf-ungulate predator-prey systems, yet few studies have investigated the direct role of wolves in predator-prey dynamics with the plains bison (Bison bison bison). Understanding predator-prey dynamics of wolves and plains bison will allow for better management plans to be implemented to monitor and conserve plains bison populations in Canada, which are listed as threatened under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). This project aims to study wolf predation on plains bison and other ungulates in the southwest corner of Prince Albert National Park (PANP), Saskatchewan. The Sturgeon River Plains Bison (SRPB) population is the only wild population of plains bison in their historical range in Canada, and have experienced a decline of around 50% in the last six years. We investigated to what extent wolves are contributing to the decline of the SRPB population using direct observation of kill sites. Kill sites were determined via spatially- and temporally-clustered data points obtained from GPS-collared wolves over three consecutive winters (2014, 2015, and 2016). Overall, white-tailed deer (Odocoileus virginianus) were consumed most frequently, followed by moose (Alces alces) and plains bison. Our next step in this study will be to perform stable isotope analysis on wolf hair and blood tissue, to assess whether the proportion of prey species consumed changes in comparison to kill site investigations. In addition, we will assess the influence of prey body condition and stress physiology on prey selection by wolves, through marrow fat and cortisol analysis. The results of this study will quantify the impact that wolves are having on the SRPB population, and help to ensure the long-term viability of plains bison in this region.

13 Assessing polar bears (Ursus maritimus) use of the Hudson Bay and Beaufort Sea flaw lead polynyas

Erin Henderson, Nick Lunn, Evan Richardson, Andrew Derocher

"Sea ice is important habitat for polar bears. Adult female polar bears select for different sea ice features throughout the winter, but optimize prime foraging features and sea ice conditions for energy conservation and safety. Reproductive status affects habitat selection due to differences in the energy expenditures and movement capabilities of the groups. Polynyas are areas of open water within sea ice and are important for a large number of species, including polar bears prey. The importance of polynyas to polar bears, however, has not been examined. Sea ice in the Arctic is significantly declining due to climate change and negatively impacting polar bears.

This study assesses polar bears use of the polynya in Western Hudson Bay and the Cape Bathurst polynya in the Beaufort Sea. This is achieved by measuring the amount of time polar bears spend near the polynyas, and how polynya size affects polar bears movements. These trends will then be compared between study sites, as well as throughout the winter, between years, and between reproductive groups. Global positioning system (GPS) collars deployed on adult female polar bears from 2004-present measure polar bears locations and movements throughout the ice-covered period (January-ice breakup). The polar bears locations, when compared to the locations of the polynyas, measure the amount of time polar bears spend near the polynyas, their movements relative to the polynyas, and how these vary between study sites, throughout the winter, between years, and between reproductive groups. This research is important for preserving biodiversity in the Canadian Arctic by testing whether changes in polynya size or abundance due to melting sea ice will affect polar bear habitat selection or inhibit polar bears movements throughout the Arctic."

14 Taz interactions with Wnt Signaling regulates brain ventricle morphogenesis

Lyndsay G. Selland and Andrew J. Waskiewicz

Taz (wwtr1; WW domain containing transcription regulator) is a transcriptional co-activator, commonly associated with the Hippo signaling pathway. Previous work by the Piccolo lab has provided evidence that taz may also interact with components of the Wnt signaling pathway. Specifically, Taz is incorporated into the β-catenin destruction complex in murine and cell culture models, where it recruits the E3-ubiquitin ligase, β-TrCP, which results in the proteasomal degradation of both β-catenin and Taz. Despite this, the role of taz during development is poorly understood. We have created mutants for taz containing a 29 bp deletion. Examination of taz-/- mutants for developmental defects finds that they display midline separation defects in the hindbrain ventricle. To determine if taz is interacting with Wnt signaling to regulate ventricle morphogenesis, we assayed for the expression of the Wnt ligand, wnt1 in the hindbrain. Taz-/- mutants have disorganized wnt1 expression at rhombomere boundaries. This suggests that the loss of taz results in disregulation of wnt1 transcription and Wnt signaling in the hindbrain. To determine if Taz is involved in the β-catenin destruction complex we used a pharmacological inhibitor of Wnt signaling, XAV939, which acts to stabilize the β-catenin destruction complex. If Taz is incorporated and degraded by the complex, then stabilization will result in constitutive degradation of Taz, and a phenotype similar to that seen in taz mutants. Treatment with XAV939 does phenocopy the ventricle defects observed in taz-/- mutants. Conversely, inhibition of the β-catenin destruction complex using SB216763, should prevent Taz from being degraded and result in stabilization and accumulation Taz protein. Treatment with SB216763 resulted in increased Taz protein localization throughout the hindbrain. Together, our results suggest that Taz interacts with the β-catenin destruction complex in zebrafish and this interaction plays a novel developmental role in the regulation of ventricle morphogenesis.

15 Manipulation of key eye dorsoventral axis patterning genes, vax2 and tbx2b, affects the closure of a novel optic fissure

Kevin Yoon, Jennifer C. Hocking, Jakub Famulski, Sonya Widen, Sophie Koch, Ordan Lehmann, Andrew J. Waskiewicz

Congenital ocular coloboma is a genetic disorder that affects 10% of blind children worldwide. It is typically observed as a cleft formation in the inferior aspect of the iris due to the failure of the choroid fissure in the optic cup to close during eye development. Recently, identification of individuals with colobomata in the superior aspect of their iris led to the discovery of a novel, second ocular fissure in the dorsal eye, which we refer to as the “superior fissure”. The established understanding of eye development does not account for a transient fissure in the dorsal aspect of the developing eye. Its role is not yet fully understood, although previous research in our lab has found that, similar to the choroid fissure, the superior fissure plays a role in vasculature formation during eye morphogenesis. Previous research in our lab has shown that disruption of dorsoventral axis patterning can affect proper superior fissure closure, revealing that a decrease in BMP signaling can delay the closure of the superior fissure, while a global decrease in Shh signaling can rescue this phenotype. Using patient-derived exome sequencing data, we discovered mutations in key eye axis patterning markers, VAX2 and TBX2. To assess the roles of these genes in superior fissure formation and closure during eye development, we have begun studying vax2, tbx2a, and tbx2b using zebrafish. Our findings suggest that overexpression of vax2, a Shh-induced regulator of Wnt signaling in the ventral eye, can lead to delay in superior fissure closure. Conversely, we have found that decrease in tbx2b expression results in superior fissure closure delay. We are now using a CRISPR-Cas9-mediated knockout approach to investigate the effects of vax2 loss during eye development. In addition, we will study the effects of TBX2 overexpression in zebrafish eye development.

16 Utility of wing length in sexing three Albertan songbird species

Rowan French, Amélie Roberto-Charron, Kenneth Foster

Conservation, ecological, and behavioural research relies upon accurate identification of male and female individuals in the field. In many breeding songbirds, sex-specific feather colours or breeding characteristics aid sex determinations. Other species that lack distinct male and female plumage morphs cannot be sexed using these means, if captured outside of the breeding season. An alternative method to identify sex in these species may be to measure wing length. In his Identification Guide to North American Birds: Part 1, Pyle (1997) bases sex- and species-specific wing chord ranges on study skins of varying age and locality. This guide is widely used by field ornithologists across Canada and the United States, yet the validity of its wing chord ranges for live birds and individual populations has been little explored. To determine the accuracy of wing chord ranges in Pyle (1997), we analyzed wing chord data collected from aged and sexed Clay-colored Sparrow (Spizella pallida), Myrtle Warbler (Setophaga coronata coronata), and Least Flycatcher (Empidonax minimus) captured at two Alberta field sites. Wing chord ranges developed from Alberta data varied marginally from those in Pyle 1997, which correctly sexed >90% of Myrtle Warblers and Clay-colored Sparrows, and 81.4% of Least Flycatchers. For each species, Fisher's exact test found a significant relationship between sex classifications based upon Pyle (1997) wing chord ranges and those based upon external morphology ($P < 0.001$). However, variations in the reliability of these ranges exist within and among species. We suggest a role of wing morphometrics in tentative identification of sex in songbirds, but caution that wing chord reference ranges published in Pyle (1997) are best used in conjunction with other sexing criteria.

17 Novel regulation of C. briggsae ovotestis development

Katharine Pelletier, Dave Pilgrim

The evolution of novel traits relies on heritable changes in gene content or gene expression, but the processes by which these occur is not always clear. Sex determination is a particularly interesting trait with which to model these processes because its regulation seems to be subject to rapid evolution. Androdioecy, or a species ability to make an ovotestis in an otherwise female animal, has independently evolved three times in the *Caenorhabditis* nematode clade from a dioecious ancestor. By comparing the genetic regulatory network allowing for androdioecy in two species, *C. elegans* and *C. briggsae*, to their dioecious close relatives, we hope to understand how the novelty arose. Sex determination is well understood in *C. elegans*, and we have been using similar molecular and genetic tools to understand hermaphroditism in *C. briggsae*.

Forward genetic screens allowed genetic identification of the *C. briggsae* orthologs of *C. elegans* genes (*tra, fem*) required for somatic and gonadal sex determination, but unlike in *C. elegans*, none of the feminizing mutants blocked spermatogenesis in the female ovotestis. A second screen, looking for suppressors of masculinizing mutants identified several phenotypes not seen in *C. elegans*. Many of these suppressors permit the development of XX hermaphrodites and XO males in a masculinized *tra-2* background, in contrast to *C. elegans* feminizing mutants where XO animals are feminized. Mutant alleles with this phenotype have been identified both within know sex determination genes, such as *fem-3*, and in novel regulatory loci. Through examination of these alleles, we will gain key insights into differences in *C. briggsae* sex determination in both the soma and the germ line. We will present our current understanding of somatic and germ line sex determination in *C. briggsae* and compare that to what is known in *C. elegans.* This illustrates how we can use comparative genomics within the *Caenorhabditis* clade to better understand the evolution of genetic regulatory network.

18 Characterizing the masculine phenotype in novel C. briggsae fem-3 alleles

Sabrina Fox, Katharine Pelletier, Dave Pilgrim

"Sexual reproduction is essential to metazoan development. However, a high variation of mechanisms for determining an animal's ability to produce either male or female gametes exists. This high degree of variance indicates that sex determination mechanisms are rapidly evolving in metazoans. Hermaphroditism, the ability to simultaneously produce male and female gametes, independently evolved in C. elegans and C. briggsae from a common female ancestor. In both species, XX animals develop as hermaphrodites with ovotestis, and XO animals develop as males that produce exclusively sperm. By comparing the regulation sex determination in these two species, we can better understand how this signalling networks have been modified through evolution. The signalling pathway for sex determination in Caenorhabditis elegans is very well understood, and the same methods used to study C. elegans can be applied to study C. briggsae.

Through forward genetic screens, C. briggsae orthologs of C. elegans sex determination genes were characterized. Specifically, mutations in the fem genes were found to feminize XO animals. In C. briggsae, all fem mutants developed as XX and XO hermaphrodites, whereas all C. elegans fem mutants developed as XX and XO females, indicating that C. briggsae spermatogenesis is controlled downstream of fem1/2/3. However, unique alleles of Cbr-fem-3 (ed34 and ed44) are capable of supressing masculinizing tra phenotypes but otherwise develop as normal XO males and XX hermaphrodites, a phenotype not observed for fem mutants in C. elegans. I will present our understanding of the genetic interactions of these unique alleles with other members of the C. briggsae sex determination pathway and compare this to what is known about fem regulation in C. elegans. Using these comparisons, we will better understand how genetic signalling pathways can be modified to produce novel traits."

19 MazG affects phage production in Burkholderia cenocepacia

Fatima Kamal and Jonathan J. Dennis

Burkholderia cepacia complex (BCC) is a group of opportunistic gram-negative bacteria that infect cystic fibrosis (CF) and immunocompromised patients. Of the 18 species that comprise BCC, Burkholderia cenocepacia are found to be most associated with CF patients and are problematic due to their high innate resistance to antibiotics. A proposed alternate treatment strategy for antibiotic resistant bacterial infections is phage therapy, which involves the use of bacteriophages to specifically treat the infection. Some bacteria produce a molecule called MazG under stringent response which allows survival. The goal of this research is to investigate the role of a phage encoded mazG homolog on phage production, as the MazG protein is thought to provide a propagation advantage to phage when the bacterial host faces nutrient limiting conditions. To investigate this, we cloned mazG from two BCC phages KL1 and AH2 into B. cenocepacia C6433 and K56-2 and studied the production of phage KS14. A ten-fold increase in the number of phage produced was observed over controls with no mazG. Since MazG has also been associated with virulence in some bacteria, we tested the mazG carrying strains in the duckweed and Galleria mellonella models. An increase in virulence was observed for both C6433 and K56-2 in the presence of MazG. We are currently working on deleting the mazG gene from the phages KL1 and AH2 to study the effect on phage infection and production.

20 Ice-period migration dynamics of polar bears (Ursus maritimus) in the western Hudson Bay

Alyssa Bohart, Nick Lunn, and Andrew Derocher

Migration cycles of animals are often predictable with seasonal changes as they depend on ephemeral resources such as food. Polar bears (Ursus maritimus) migrate onto sea ice as it forms because they depend on ice to hunt their prey. This seasonal feeding ensures their survival during their fasting period when ice and prey are absent. Climate change is altering the seasonal ice within the western Hudson Bay, resulting in the break-up season occurring sooner and the freezing season becoming later, decreasing the overall ice period. The objective of my research is to determine if bears demonstrate different migration strategies and if these patterns have changed with altered ice-periods, as no previous studies have examined these aspects of movement in a migration context in polar bears. I will use satellite-linked collar movement data of female polar bears in the western Hudson Bay collected between 2004-2017 to determine migration patterns. By comparing the different rates of migration, distance travelled and tortuosity of movements, I anticipate to find groups of bears that exhibit similar strategies. I will use ice cover data collected via remote sensing to investigate the effect of ice dynamics on migration strategies. Different rate movement patterns have been shown in polar bears during the ice-free period in Alaska. Thus, it is expected that differences in migration rate patterns in Hudson Bay will be evident. Previous studies have found that male and female polar bears demonstrate differences in tortuous movements related to their sex-specific behaviours, suggesting that similar patterns are expected in migration movements. Within the Hudson Bay, distances travelled have declined as ice has diminished, so it is expected that migration strategies will change with ice dynamics. Determining how ice dynamics have influenced polar bear migration will give insight into future conservation and management strategies to ensure this species survival.

21 Genetic compensation complicates disease modeling when using gene editing in the zebrafish

Francesca Jean and Dave Pilgrim

"Zebrafish have become a powerful tool for disease modeling, due to its ease of manipulation, rapid development, accessibility and transparency. Studies out of many labs, on diverse diseases (cancer to hearing loss to vision) has validated that progression often follows similar stages. A key difference is that an extra genome duplication in the teleost lineage has led to a two-to-one paralogy compared to mammalian gene copies. While this can assist disease modeling (each mutant paralogue may only affect a subset of tissues compared to mammals), this also increases the possibility of genetic redundancy and/or compensation, which can complicate disease modeling.

I am using zebrafish to model a cone-rod dystrophy caused by mutations in the human unc119 gene. The orthologous gene in zebrafish, unc119b, shows expression in the photoreceptor layer and antisense-mediated gene knockdown results in truncated photoreceptors. To assay whether genetic mutations result in disease formation, I used CRISPR-Cas9 technology to create deletion mutations in the orthologous zebrafish gene. I induced a 45,000 base pair deletion within the unc119b gene, removing the bulk of the coding region, including all conserved residues. In contrast to the morpholino phenotypes, embryos homozygous for the deletion don’t show any obvious phenotypes. This effect has been described frequently with zebrafish and is thought to be due to the presence of additional paralogues. Given the conservation of the unc119 genes across evolution and the presence of three other paralogues in the zebrafish genome, this may be occurring here as well. Therefore, to accurately model human diseases in zebrafish, the creation of compound mutants may be necessary."

22 Influence of parental allocation relative to nestling competitive behaviour on food consumption in Ferruginous Hawks (Buteo regalis)

Megan Szojka, Janet Ng, Erin Bayne

Competitive behavior between Ferruginous Hawk (Buteo regalis) siblings and its effect on relative food consumption has yet to be explored. Ferruginous Hawks raise altricial nestlings in their breeding grounds of southern Alberta and Saskatchewan, where they are considered endangered. My objective is to evaluate the effect of sibling competition, hatching order, and brood size on food consumption by nestlings, relative to direct feeding by the parents. Preliminary research suggests that first-hatched nestlings are fed more food than their siblings during a feeding event, and receive more food overall than their siblings. It is not clear whether parents allocate food differentially amongst their young, or if competitive behavior between siblings has a greater influence on their relative food consumption. I hypothesize that the first hatched nestling will gain the most food consumption in the brood, as a result of competitive behavior, rather than direct parental allocation.

23 Respirometry and Swim Performance Alterations in Zebrafish (Danio rerio) acutely exposed to Hydraulic Fracturing Flowback and Produced Water.

Erik Folkerts, Greg Goss

"Horizontal hydraulic fracturing is an emerging practice in North America to extract oil and gas reserves. To date, little toxicological information is supplied for both the technologies and chemicals used during the fracturing process, and for the surface returned by-product fracturing flowback and produced water (FPW). Although salinity dominates much of the toxicity observed, other inorganic (e.g. metals, radionuclides, etc) and organic (e.g. polycyclic aromatic hydrocarbons, benzenes, etc.) molecules found in FPW can induce toxicological responses in exposed organisms.

The current study uses zebrafish (Danio rerio) as a model organism to determine if acute exposures to FPW induce developmental changes and alter organismal respiratory characteristics. To determine if exposure to dilute FPW resulted in changes in oxygen consumption, zebrafish embryos were acutely exposed to 2.5% and 5% FPW and oxygen consumption was measured. Transcriptional analyses of specific hypoxia, stress inducible, and cardiovascular genes were performed and paired with embryo metabolic rates to determine if FPW exposure affected immediate embryo oxygen consumption, and if FPW-induced respirometry changes persisted after exposure was terminated. Finally, juvenile zebrafish swim performance experiments were performed to determine if acute embryonic FPW exposure affected later stage fish fitness.

Our results aim to validate respirometry as a potential toxicological biomarker for identifying biological zones of impact when FPW spills occur, and provide regulating agencies information to help shape future FPW spill/leak remediation protocols."

24 Assessing the Relative Utility of Mesostigmatid Mites as Bioindicators of Disturbance in Forested Areas of Alberta, Canada

Matthew Meehan, Dr. Heather Proctor

Indicator taxa are often used to estimate how anthropogenic forces affect ecosystem health through their presence/absence and abundance within the environment. Many different groups can be used as bioindicators, including soil invertebrates. Soil mites have been used as bioindicators from disturbances due to land use change, agricultural management and soil contamination. Mesostigmatid mites (Arachnida: Parasitiformes: Mesostigmata) are the main soil predators in temperate forests and agricultural systems due to their abundance, which can reach up to 10,000 individuals/m2. As predators, they may face greater extinction risk due to needing more area to support their dietary needs, and by needing prey populations to be established before they can colonize a new area. In my study, I am testing whether Mesostigmata are effective bioindicators for four common disturbance types in boreal Alberta: forest fires (10 sites), forest harvest (8 sites), well pads (6 sites) and linear features (6 sites). I will be comparing Mesostigmata assemblages from these sites to assemblages within adjacent undisturbed boreal forest sites. The samples for this study were collected by the Alberta Biodiversity Monitoring Institute (ABMI), and were sampled from 2010 – 2015. I have so far identified adult Mesostigmata >300 µm in body length from10 fire disturbed sites, 8 harvest sites, 3 well pad sites and 4 linear features sites and their accompanied undisturbed sites. Thus far, 19 families and 37 genera have been recorded, including a new species in the genus Microgynium. Preliminary analysis has shown that only fire disturbance has lead to significant alteration of assemblage composition. Coarser scale analysis comparing all undisturbed and disturbed sites to one another was shown to have non-significant effects on assemblage composition.

25 Plant chemical lures for the attraction of parasitoid Apanteles polychrosidis to increase parasitism of Caloptilia fraxinella on green ash in Edmonto

McPike, S., Evenden, M.

Apanteles polychrosidis (Hymenoptera:Braconidae) is a native parasitoid wasp that has shifted hosts to exploit the ash leaf-cone roller, Caloptilia fraxinella (Lepidoptera: Gracillaridae), an introduced nuisance pest of ash (Fraxinus) in Edmonton, Alberta. As the primary parasitoid of ash leaf-cone roller (ALCR), A. polychrosidis has the potential to be an effective biological control agent. To enhance parasitoid attraction and retention on green ash trees, Fraxinus pennsylvanica, infested by ALCR, we tested synthetic copies of semiochemicals, methyl salicylate (MS), and two green leaf volatiles (GLVs),(Z) 3-hexenyl acetate and (Z) 3-hexanol, used by the parasitoid in host location. Green ash trees with either one MS bubble cap lure, one bubble cap lure of both of the GLVs, or both sets of lures, were measured for wasp attraction to yellow sticky cards, and parasitism rate (measured by presence of parasitoid pupa in ash leaf-cones), and compared to control trees with no lures. While wasp attraction was significantly increased in trees with both MS and the GLV lures, this did not increase parasitism rate of ACLR compared to the other lure treatments, or the control trees. In a 4-way olfactometer laboratory experiment, using the same volatile chemicals, we attempted to demonstrate the attraction of mated female wasp to the volatile lures. With attraction being measured by 'seconds spent' in the quadrant of the treatments over a 15 minute trial, there was no difference in time spent by mated female wasps at any of the volatile treatments compared to the solvent control.

26 Evolutionary trends in the pelvic musculature and locomotion of theropod dinosaurs

Matthew Rhodes, Philip Currie, Gregory Funston

Hindlimb musculature is reconstructed based on extant phylogenetic bracketing and direct observation of muscle attachment sites for caenagnathids, dromaeosaurids, ornithomimids, troodontids, and tyrannosaurids from Alberta. The results indicate disparate locomotory styles in theropod dinosaurs from Alberta. Trends in the evolution of theropod pelvic musculature explain how this diversity of contemporary theropod taxa coexisted, and shed light on the stepwise transitions towards bird-like muscular configuration. Caenagnathids probably engaged in omnivory to reduce competition, and dromaeosaurids were specialized predators similar to extant raptorial birds. Ornithomimids and troodontids appear built for speed and capable of rapid cursorial locomotion. Tyrannosaurids possess powerful hips that served for attachment of strong muscles for supporting and moving their enormous body mass. The progressive change from tail-driven to knee-driven locomotion is tracked by the morphology of the pelvis and the rearrangement of pelvic musculature. The loss of the supraacetabular crest at the base of Pennaraptora probably coincides with the transition to knee-driven locomotion and bird-like posture.

27 Too many podia, too little coordination? Sea stars on surface tension

Christine Simard, A. Richard Palmer

To move effectively in one direction, sea stars must somehow coordinate the motion of hundreds of podia arrayed along arms that point in multiple different directions. Podia coordination has previously been attributed to either central nervous control or to some form of local proprioceptive system. However, considerable debate still exists over how coordination is achieved. To test whether sea stars coordinate their tube feet via central nervous control or via local proprioceptive cues, we tracked the movements of multiple individual podia in juvenile sea stars that attached one or more rays to the surface tension of seawater. Sea stars were filmed walking upside-down on the surface tension and on an adjacent floating glass cover slip to compare podia movements simultaneously on both surfaces. We found that podia do attach to the surface tension and do attempt to propel the sea star, but these attached movements were much less coordinated than when podia attached to a glass surface. We also tracked the recovery strokes of individual podia and their neighbours on these two substrata to test how coordinated recovery motions were. Our results suggest that local proprioceptive cues are important for effective echinoderm locomotion but we cannot entirely rule out the contribution of central nervous coordination.

28 Boreal songbird community response to understory protection harvesting

Connor Charchuk, Erin Bayne

Traditional harvesting practices have followed the natural disturbance regime, which in Canada is the practice of logging to mimic forest fires. Young aspen is the first to regenerate in these clearcuts, and outcompete conifers resulting in pure deciduous stands. For both economical and ecological objectives, mixedwoods are often desirable. Alberta-Pacific Ltd. (Al-Pac) has implemented a novel type of harvesting, termed understory protection, to facilitate the regeneration of mixedwoods following harvest. Understory protection is implemented in areas with high understory spruce densities, and involves harvesting of mature aspen by reaching in with a feller buncher to avoid damaging the understory. The economic benefits of UP harvesting are well understood, but the ecological implications are uncertain. The purpose of my research is to survey bird communities in understory protection harvested areas to compare them against communities in traditionally harvested cutblocks. Furthermore, I use unharvested patches of forest as a positive control. Using autonomous recording units (ARUs) to acoustically survey bird communities, comparisons of richness, diversity, and overall community composition can be made. In 2015 we surveyed 47 sites (consisting of one of each treatment type), and 45 sites in 2016. I will be discussing analyses that I have conducted to compare the bird communities across these three treatment types, and how the bird communities respond as these harvested areas recover. My hypothesis was that the understory protection communities would more closely resemble the unharvested communities, and would recover more rapidly than traditional harvest blocks.

29 Do transmission lines affect Ferruginous hawk home range size and nest success?

Nick Parayko

Human disturbance and land conversion are driving factors behind the decline of native prairie grasslands across North America, which now occupy under one third of their historic range. In Alberta, mixed grasslands have undergone extensive modification, primarily from agricultural and industrial developments. Transmission lines are continuously developed and upgraded as urban populations increase and can run through critical habitat for several Species at Risk. Ferruginous Hawk (Buteo regalis; FEHA) populations in Canada have been declining since the 1980’s and in 2006 they were listed as provincially Endangered in Alberta under the Alberta Wildlife Act. Recent declines in FEHA are attributed to anthropogenic disturbance including habitat loss (industrial development, conversion for agriculture, fragmentation, and farming) and the loss of nesting structures as remnant trees are removed from the landscape. Since 2012, we have captured and transmittered 24 FEHA in southern Alberta using GSM and PTT transmitters including 11 males nesting on transmission towers. With precise and frequent GPS fixes, we are able to determine FEHA home ranges using minimum convex polygon and kernel density methods. I plan to use this data to assess how home range size and nesting success change in the presence of transmission lines. I am also interested in how non-breeding territorial males utilize transmission line towers and if their home range use differs from breeding males. My results have the potential to assist in key management decisions at both the federal and provincial level regarding transmission line placement, construction, and mitigation measures and will help to improve our understanding of how FEHA alter movement patterns in the presence of energy infrastructure.

30 Cross-platform compatibility of de novo ddRAD SNPs in a non-model butterfly genus

Erin Campbell

Genome-wide methods are being rapidly adopted for discerning population structure and phylogenetic divergences of non-model organisms in conservation and biodiversity studies. However, the reproducibility and compatibility of such de novo genotypes generated from different methodologies has not yet been tested directly. We sequenced the same 24 specimens from the butterfly genus Speyeria on two different high-throughput sequencing platforms and with different library preparation methods prior to sequencing. Wethen took a de novo approach to bioinformatic locus assembly and SNP discovery for subsequent phylogenomic analyses. We found a high rate of locus recovery despite differences in initial library preparation and sequencing platforms, as well as overall high levels of data congruence after data processing and filtering, albeit with some observed topological discordance. These results support the use and long-term viability of de novo SNP genotyping applications.

31 Morphology and gene expression associated with dispersal capability in mountain pine beetle (Dendroctonus ponderosae)

Shegelski V, Evenden M, Sperling FAH

In recent years, mountain pine beetles (MPB), Dendrochtonus ponderosae, have become major forest pests in Western Canada, causing immense economic losses in forestry. Dispersal by MPB is poorly understood and more thorough knowledge of its dispersal capabilities would improve predictive modelling, allowing more efficient allocation of management resources. Flight morphology and propensity are two key elements that often have an effect on flight performance; this study aims to identify morphological characters and genes associated with dispersal capability in MPB. Beetles were flown on flight mills to collect flight data, and measurements were made on the body and wings before RNA was extracted for RNA-seq and differential gene analysis. Dimensions of the wings, body and flight muscles were compared to flight performance. Multiple regression showed that some of the variation in flight performance could be explained with wing and body morphology based models; previous studies have demonstrated that body size often has relationship with dispersal capability, but this study shows that the inclusion of wing morphology can drastically improve the predictive power of this relationship. Regardless, only approximately 25% of the dispersal capability is explained by the morphology of the beetle. Quantile regression shows that morphology has a greater influence on strong dispersers while it seems to have little or no effect on weak dispersers. This suggests that behavioral traits - in particular flight propensity - are also likely to be important factors affecting dispersal capability. RNA-seq data has been collected and is being analyzed for differential gene expression associated with flight propensity.

32 A matter of size: do invasive Tetropium fuscum (Coleoptera: Cerambycidae) use visual cues to assess the diameter of potential host trees?

Tyler D. Nelson, Jon D. Sweeney, Neil Kirk Hillier

The brown spruce longhorn beetle, Tetropium fuscum (Fabr.) (Coleoptera: Cerambycidae), is an invasive phloeophagous beetle that is established in Atlantic Canada. Primarily, it colonizes stressed spruce trees (Picea spp.) that are greater than 10 cm in diameter at breast height (DBH), though it is unknown whether host diameter influences host selection and/or larval performance. We hypothesized that T. fuscum uses visual assessment of tree diameter when selecting a host, which we tested by counting the number of adult beetles that landed on sticky bands affixed to 29 Picea rubens ranging from 12.2–37.5 cm DBH. All trees were baited with host volatiles and aggregation pheromone to ensure that olfactory cues were equal on all potential hosts. Linear regression showed significant positive relationships between tree diameter and the mean number of T. fuscum per sticky band, and between tree diameter and phloem thickness. We conclude that there is a positive relationship between host diameter and T. fuscum landing rate, which could benefit the offspring of the beetle as larger diameter trees provide more phloem for larvae to consume. However, there was no significant relationship between tree diameter and T. fuscum per square meter of sticky band. These findings suggest that T. fuscum does not actively select hosts with larger diameter. The positive relationship between alighting and host diameter could be due to larger diameter trees serving as an obstacle to airborne beetles as such trees have a greater chance of impeding beetle flight.

33 Mechanisms of oleic acid acquisition in the intestine of the Pacific hagfish (Eptatretus stoutii)

Alyssa M Weinrauch, Greg G Goss

Hagfish are early-diverging craniates that occupy the basal position in vertebrate phylogeny and are thus useful candidates for the study of evolutionary physiology. These scavengers have been shown to persist upwards of 11 months without feeding and rely on lipid based fuel sources over these extended periods. My previous research revealed distinct intracellular lipid micelles within a variety of tissues, to a degree not recorded in any other vertebrate. With this study we sought to quantify lipid uptake strategies that would permit this necessary acquisition for extended periods of food deprivation. Utilizing intestinal flux studies with 3H-oleic acid (OA) we demonstrate the presence of a saturable transporter (Km= 58.9, Vmax= 94.4) in the hagfish hindgut. Additionally, following feeding OA transport was increased. Bioinformatics analysis revealed putative fatty acid transport protein (FATP) sequences in the hagfish transcriptome, which we will examine in future molecular studies. Furthermore, research in mammalian models has revealed an insulin-dependent translocation of FATP to apical membranes. To elucidate whether hagfish utilized similar mechanisms we injected insulin into fish 24 h prior to gut flux studies. While metabolic analysis demonstrates an effect of insulin, there was no effect on OA transport. This report represents the first examination of lipid uptake in a basal member of the vertebrate lineage and provides insight into early vertebrate evolution of lipid acquisition strategies.

34 Transcriptomic mechanisms of salinity tolerance in the Pacific spiny dogfish, Sqaulus suckleyi

Dylan Cole, Greg Goss

Environmental salinity strongly influences the distribution of aquatic organism. Euryhalinity is the ability of an organism to tolerate and adapt to a range of salinity, allowing for exploitation of new habitats. Within elasmobranchs (sharks and rays), fully euryhaline species (such as the bull shark) are rare, while increasing numbers of marginally euryhaline species are becoming recognized. The Pacific spiny dogfish, Squalus suckleyi, is a marine elasmobranch that is marginally euryhaline and can temporarily inhabit brackish water, however, prolonged exposure in the lab has shown to induce negative effects on overall health. Little is known regarding the physiology of euryhaline elasmobranchs, and less about the underlying genomic and transcriptomic mechanisms. This study aims to examine the transcriptional changes of the S. suckleyi kidney, a major osmoregulatory organ, after exposure to 21 ppt for 0, 12, and 48 hrs. It is hypothesized that large suites of genes involved in stress response, iono- and osmo-regulation, and general metabolism will be differentially expressed in response to the salinity challenge. Plasma osmolytes (Na+, Cl-, urea) and total osmolality decreased over the 48hr exposure period, consistent with previous studies. Transcriptomic data has finished sequencing and will be analyzed to identify differentially expressed transcripts.

35 Investigating transcriptional changes in neurulating Cecr2 mutant mice

Alaina N. Terpstra, Farshad H. Niri, Heather E. McDermid

Early mammalian development cannot progress without targeted temporal and spatial expression of genes. Changing the accessibility of DNA to transcriptional machinery is one critical way gene expression is controlled. This process, known as chromatin remodelling, is vital for formation of the embryonic neural tube and for spermatogenesis. Disrupted neural tube formation results in profound defects. Mutations in mouse Cecr2 can lead to either the lethal neural tube defect exencephaly, analogous to human anencephaly, or a non-lethal subfertility phenotype. Cecr2 is highly expressed in the embryo, testicular germ-line stem cells (spermatogonia), embryonic stem (ES) cells, and an established primary neural stem cell culture. The CECR2 protein forms a chromatin remodelling complex and has tissue-specific bin¬ding partners. In both ES cells and testis CECR2 binds SNF2H, a protein that drives remodeling. CECR2 binds LUZP1, a protein required for neurulation, in ES cells but not in the testes. Chromatin immunoprecipitation sequencing has identified overlapping genomic binding sites for

CECR2, SNF2H, and/or LUZP1 within the promoter region of several genes. To investigate differential gene expression in the absence of CECR2, I collected RNA from neurulating embryo heads and just recently submitted it for sequencing. Together, these results can be used as a powerful comparative approach to determine if CECR2 acts as a direct transcriptional regulator and to identify further downstream transcriptional differences. In the meantime, I am using qRT-PCR to identify transcriptional changes in genes with similar phenotypes to our Cecr2 mice. Further analysis of genes differentially expressed will include spatial and temporal assays on neurulating embryos. Genes that appear to be involved in CECR2 phenotypes, such as those that localize to neurulating tissues, will be further characterized to help us understand the role of CECR2.

36 Does a new Cecr2 mutation provide a mouse model for the human disorder cat eye syndrome?

Renee Dicipulo, Kacie A. Norton, Heather E. McDermid

Chromatin remodellers can dynamically modify gene accessibility to transcription factors through changes to chromatin architecture. Cecr2 encodes a protein that is part of a chromatin remodelling complex, that when mutated, results in the lethal brain defect exencephaly in mice. Recently, analysis of a new Cecr2 mutant made by replacing exon 4 in Cecr2 with the LacZ gene showed that this mutation was more severe than previously studied Cecr2 mutations. I have shown that the mutants have exencephaly as expected. However, unexpectedly they they also show new abnormal phenotype: the eye defect coloboma (fissue of the iris and retina) and post-axial polydactyly. Polydactyly has not been previously seen in other Cecr2 mutants. In the new mutant coloboma was observed in pigmented C57Bl/6 mice, making the defect easy to observe. However, as two previous Cecr2 mutations were characterized in albino Balb/c mice with colourless irises, there is a possibility that we have missed coloboma in previous studies. With this in mind, I will revisit these mutations to look for coloboma as well as other defects seen in the Exon 4 mutants. The presence of colobomas suggests that there may be a connection to a human syndrome associated with a duplication of a region of chromosome 22 that contains the gene Cecr2. “Cat eye syndrome” also features coloboma. In genomic disorders, there are a few instances where overlapping phenotypes are seen between reciprocal deletion and duplications of a gene. To test the possible role of CECR2 in cat eye syndrome, I will sequence the CECR2 gene in a select group of patients with cat eye syndrome-like symptoms but no chromosomal duplication, looking for deleterious mutations. This investigation endeavours to advance our understanding of Cecr2’s involvement in development, and uncover if CECR2 is involved in cat eye syndrome.

37 Examining teleost immunoregulatory receptor-mediated phagocytic responses

Dustin Lillico, Myron Zwozdesky, Chenjie Fei, Joshua Pemberton, and James Stafford

Specialized innate immune cells called phagocytes express a diverse array of immunoregulatory proteins that recognize extracellular targets and initiate an engulfment process called phagocytosis. Receptor-mediated phagocytosis uses intracellular signaling cascades to activate filamentous (F)-actin-dependent polymerization events that controls unique plasma membrane remodelling during the active capture and engulfment of targets. Most research examining the cellular mechanisms of phagocytosis, a vital component of innate immunity, have been performed in mammalian models. However, little is known about the receptor-types and associated mechanisms controlling this evolutionarily conserved innate defense strategy in other vertebrates, including fish. My research is focused on examining teleost leukocyte immune-type receptors (IpLITRs) with the goal of understanding how various LITR-types promote intracellular signaling to activate the phagocytic process. My work has established that distinct phagocytic modes exist within the IpLITR family, which are facilitated by specific sub-types of receptors with unique intracellular signaling mechanisms. Importantly, recent results also suggest that specific IpLITR-sub types when expressed in innate immune cells confer the generation and utilization of filopodia for target acquisition while others do not. Filopodia are exploratory F-actin dependent extracellular membrane structures which have both non-immune and immune-related functions. In the context of phagocytosis these structures are important for surveying the surrounding extracellular milieu for potentially harmful targets and generating initial phagocyte-target interactions leading to procuring targets to the cellular surface for internalized and destruction. One major unanswered question in filopodia research is whether or not these protrusive structures are receptor-dependent events or if they are constituently formed by cells. This data suggests for the first time that the formation of filopodia in some instances may be the result of the activation of specific receptors types and offers new insights in to their regulation.

38 Defining Roles for SVP-like Genes in the Activity-Dormancy Transition of White Spruce Terminal Buds

Gregoris A.S., Hall J.C., Scarpella E., Cooke J.E.K.

"Perennial plants survive winter by developing buds to protect shoot apical meristems, terminating meristematic cell proliferation to enter the dormant state, and resuming cell divisions the following spring. Although our understanding of the molecular mechanisms controlling the transition from active growth to dormancy at the shoot apex of perennial species is limited, recent advances suggest that there are marked similarities between regulatory networks controlling this activity-dormancy transition and the regulatory networks controlling the transition from vegetative growth to flowering. The MADS-box transcription factor SHORT VEGETATIVE PHASE (SVP) inhibits flowering in Arabidopsis thaliana, and SVP-like genes have been implicated in regulating bud formation and/or dormancy acquisition in perennial species such as peach and kiwi. To date, no research has examined the role of SVP-like genes in conifers, which diverged from angiosperms approximately 300 million years ago. Our goal is to determine the role of SVP-like genes in bud formation and dormancy acquisition in the conifer white spruce.

Phylogenetic reconstruction shows that seven white spruce SVP-like (PgSVP-like) genes are closely related to angiosperm SVP genes. Distinct expression profiles of these PgSVP-like genes suggest diverse roles in bud formation and/or dormancy acquisition. In situ hybridizations for two PgSVP-like genes showed that they are expressed in most tissues within the bud. Some PgSVP-like genes restored a wildtype flowering phenotype in Arabidopsis svp mutants, suggesting conserved functional features. Transcriptional fusions of two PgSVP-like promoters in Arabidopsis revealed transcriptional activation is localized to the shoot apical meristem for one promoter but absent in the other, demonstrating probable divergence of function. Yeast one-hybrid analysis of these two promoters revealed that one PgSVP-like promoter may be regulated by transcription factors involved in bud initiation, the other is controlled by proteins likely involved in bud development. This supports the model that these genes play distinct roles in bud formation. "

39 Monitoring Seasonal Changes in Photosynthetic Activity in Deciduous and Evergreen Boreal Trees using Remotely Sensed Indices

Kyle R. Springer, John A. Gamon

Trees species in the boreal forest cycle between periods of active growth and dormancy, altering their photosynthetic processes in response to changing environmental conditions. For deciduous species, these changes are readily visible, while evergreen species have more subtle changes in their foliage during seasonal transitions that define growing season length. In this study, we used remotely sensed optical indices, sensitive to changes in foliage, to observe seasonal changes in photosynthetic activity, or photosynthetic phenology of boreal tree species. We evaluated the efficacy of the Normalized Difference Vegetation Index (NDVI), the Photochemical Reflectance Index (PRI), and the Chlorophyll-Carotenoid Index (CCI) in tracking seasonal transitions and compared these optical indices to metrics of photosynthetic activity (photosynthetic rate and chlorophyll fluorescence) to determine if the remotely detected changes correspond to changes in plant physiology and how these patterns vary across functional types. NDVI tracked photosynthetic metrics well in deciduous species but not in evergreens. PRI illustrated seasonal changes strongly in evergreens but less prominently in deciduous species. CCI closely tracked changes in photosynthetic metrics for both functional types. We report strong annual correlations between photosynthetic rate and all three indices in deciduous species, but only with PRI and CCI for evergreen. Similar correlations were found for chlorophyll fluorescence as an indicator of photosynthetic activity. Our study indicates that CCI is capable of accurately tracking seasonal transitions and photosynthetic phenology in both deciduous and evergreen species. Our results demonstrate the efficacy of these optical indices in tracking photosynthetic phenology at proximal scales, providing an important foundation for using similar techniques at larger spatial and temporal scales to monitor, and potentially quantify, photosynthetic activity across ecosystems. With CCI now becoming available from satellite sensors, it offers new opportunities for assessing photosynthetic phenology, particularly for evergreen species which have been difficult to assess with previous methods.

40 Lethal Periods of Various Fourth Chromosome Mutations In Drosophila Melanogaster

Graeham Turner and John Locke

Chromosome 4 in Drosophila melanogaster is an essential but under-researched component of an otherwise extraordinarily well characterized and powerful genetic model system. This chromosome contains many essential genes that are in regulatory and developmental pathways whose importance is constantly showcased in current research. However many of these essential loci are woefully under characterized in that they lack mutations, which would allow for further chacterization. To provide such mutations, we have conducted a genetic screen for EMS-induced mutations and recovered 284 lines with recessive lethal mutations on chromosome 4. This collection was crossed to defined deletions and mapped to various regions along the chromosome. Additionally, these mutations were crossed inter se to identify complementation groups. I am examining nine such groups, containing 14 lines, and am in the process of confirming the chromosomal location. Next I will be determining the lethal phase of each of the lines. This will be useful in matching these complementation groups to their respective genes. Finally we will be using DNA sequencing to narrow the lethal effect down to single point mutations and identifying the genes affected. This will allow us to generate stocks for further investigation of those loci, including their action in specific developmental mechanisms.

41 The roll of MEMI in regulating C. elegans oocyte meiosis

Jens Herzog

Successful completion of meiosis is critical for gametes to inherit a haploid complement of chromosomes. In many species, oocyte meiosis relies on receipt of external signals. C. elegans, oocytes exit a meiosis I (MI) arrest after receiving a diffusible signal from the sperm. The oocyte then begins meiosis II (MII) after sperm entry. We found that the MEMI proteins represent a good candidate for an oocyte-specific factor that “senses” sperm entry triggering meiosis II. The MEMI gene has three copies; memi-1/2/3 which function redundantly. They have 87% DNA similarity and in strains missing any one or two copies of memi, embryos remain viable. When all copies of memi are deleted, embryos are not viable. In strains carrying the hypermorphic mutation memi-1(sb41), oocytes complete MI and enter MII, but they fail to exit MII properly, instead dividing to form a non-viable mass of polyploid cells. Western blots show that MEMI(+) proteins are degraded before mitosis, but that MEMI-1(sb41) protein persists into mitosis, thus memi-1(sb41) affects protein degradation. These results indicate that MEMI activity is strictly controlled; it is required after sperm entry for MII, but it must be rapidly removed prior to the start of mitosis. The memi-1(sb41) mutation perturbs post MII events, but, because of the functional redundancy with memi-2/3, it is still unclear whether the sb41 mutation interferes with meiotic functions. By crossing memi-1(sb41) into a memi-2/3 deletion background, I found that lowering total MEMI activity in the oocytes increased embryonic viability, confirming a hypermorphic nature. Worms that have only MEMI-1(+) survive, but if they have only MEMI-1(sb41), they do not survive, suggesting that MEMI-1(sb41) is not a simple hypermorph. This dichotomy in the genetic behaviour of memi-1(sb41) has prompted a re-examination of current models on the molecular regulation of MEMI proteins and the control of the meiosis-to-mitosis transition.

42 Spatiotemporal distributions for non-ophidian ophidiomorphs and implications for the origins of snakes

Campbell, M., and Caldwell, M.

"Living snakes (Serpentes) comprise one of the most diverse (spatially, ecologically, taxonomically) groups of extant reptiles. There are over 3,400 species found in almost every continent and ecosystem. However, the origins of this geographic, ecological, and evolutionary diversity have been the subject of considerable recent and historical debate. Some the major questions surrounding their Mesozoic origins are: whether they arose on Gondwana or Laurasia, whether they come from the land or sea, and what kind of ecological niche (fossorial, terrestrial, aquatic, arboreal) they initially occupied. Recent studies have used fossil snakes to try to answer these questions, but highly-modified morphologies, and the preservational, geographic, and taphonomic biases of these fossils obscure definite answers.

Some recent phylogenetic analyses place snakes within the Ophidiomorpha: a group including dolichosaurids, pontosaurs, adriosaurs and ophidians (living and fossil snakes). The non-ophidians—‘dolichosaurs'—are small-bodied aquatic forms basal to snakes that lived in shallow seaways, rivers, and reef environments. Dolichosaur biodiversity is historically difficult to study as most described species are monotypic and known only from single specimens, imparting very little information on morphological or spatial variation. Fortunately, recent finds have dramatically altered traditional views of their stratigraphic distribution and global range. Though biogeographic patterns and processes remain obscured by preservation bias, some general patterns have become clear. Our results present a clearer picture of the global evolutionary history of this group including probable Tethyan origins, several major radiations, and multiple freshwater incursions. Understanding the occurrence of this group through space and time is critical to the study of their evolution and adaption, including evolutionary drivers and their aquatic radiation. Importantly, the evolutionary history of this group also provides another lens through which to assess questions concerning the elusive origin of snakes. "

43 Geodia barretti oscule contraction behaviour and morphology: implications for sensory system evolution

Evgeni Matveev

Multicellularity necessitates the evolution of a coordinated, whole-body response to stimuli. While most animals have neurons for signal propagation and muscles for physical response, sponges have neither. Interestingly, new phylogenetic evidence suggests that ctenophores (which have both muscles and neurons) may have predated sponges as the first multicellular animal. This begs the question; did neurons evolve twice (separately in Cnidaria and Ctenophora), or did they evolve in a common ancestor of all Metazoa but were subsequently lost in sponges? To answer this question an intricate knowledge of the sponge sensory system is crucial. In this study, I attempted to characterize the response to stimulation of the North Atlantic sponge Geodia barretti. By conducting a video analysis of the contraction behaviour of the sponge's exhalent pores I could calculate the speed and extent of signal spread. The pores contracted in a localized and graded fashion relative to the point of stimulus and the signal speed was calculated to be ~44 µm s-1, suggesting that the signal was propagated via a messenger molecule (rather than an action potential). The pores were also sectioned and stained to determine what allows them to be more contractile than the rest of the body. The pores had contractile sphincters made primarily of layered collagen, with elongate cells interspersed throughout. An actin label showed up in strands along the collagen layers, potentially in the cells. Based on the amount of collagen relative to cells it is suggested that the collagen itself has contractile properties, akin to the mutable collagenous tissue in echinoderms.

44 Neural genes and sponge sensory systems

Jasmine Mah & Sally Leys

Sponges are nerveless animals with neural genes. Even in the absence of nerves, sponges have the ability to sense changes in flow and respond with a choreographed behavior. Initiation of this behavior can be linked to primary cilia in the osculum, the excurrent vent of the sponge. Thus, while the genetic mechanisms remain unknown the osculum may function as a sensory and coordinating hub. A recent hypothesis suggests that ctenophores, rather than sponges, are the most basal phylum. As ctenophores possess a full nervous system while sponges lack one, this challenges previous views about a linear progression of nervous system evolution. Thus, do these neural genes suggest the presence of a 'proto-nervous system' or nervous system loss? Bilaterian gene function is not necessarily conserved in non-bilaterian animals. Do these neural genes correlate to a sensory function? I performed a meta-analysis of sponge gene expression studies which showed that while sensory structures do express 'neural' genes, so do non-sensory cell types. I also performed an RNAseq experiment investigating the development of the osculum in Spongilla lacustris and the osculum itself in another sponge, Aphrocallistes vastus. We found that, with few exceptions, neural effector and neurodevelopmental genes were not differentially expressed during oscular development or in the osculum itself. In addition, a recent hypothesis suggested that Wnt and a battery of conserved transcription factors may be involved in patterning sensory regions. But, while Wnt pathway proteins are expressed, Aphrocallistes lacks the complement of conserved transcription factors and thus this mechanism likely does not underlie the osculum. While some genes characteristic of neural tissues are differentially expressed in the osculum, the overall picture from these studies is one of a unique sponge-specific sensory system.

45 New Species Records for Sponges of the Eastern Canadian Arctic and Subarctic

Curtis Dinn

The diversity of deep-water sponges (Phylum Porifera) in the Canadian Arctic has historically been overlooked, in part because of difficulties associated with sampling deep hard bottom environments. As part of the ArcticNET HiBio (Hidden biodiversity and vulnerability of hard-bottom environments in the Canadian Arctic) project, regions of reported coral and sponge bycatch in Baffin Bay and the North Labrador Sea were targeted for extensive sampling using remotely operated vehicle (ROV), box core, and Agassiz trawl operations aboard the CCGS Amundsen. Expeditions in July 2016 and October 2015, retrieved 114 sponge specimens from depths ranging between 72-1148 m and encompassing latitudes 60°18N to 68°15N. Morphology-based identifications indicate the presence of at least 23 species from 17 different sponge families, some of which may be new species records for the region according to the World Porifera Database. Sponges hitherto known only from the Northeast Atlantic and higher Arctic (Axinella arctica, Janulum spinispiculum, Mycale lingua, Polymastia uberrima, and Tetilla siberica) are reported for the first time from Baffin Bay. This work spans three bioregions, which were thought to contain a combined 16 known sponge taxa. Current results notably increase our present knowledge of sponge species richness on the Northeast Canadian shelf. Sponges are also being quantified from ROV video to give an indication of species richness and biodiversity in sampled sites and to facilitate species identification for specimens collected in ROV surveyed sites. This work will compare sponge distributions in relation to habitat and substrate type. Comprehensive taxonomy and ROV video annotation will provide insight into largely unknown sponge distributions and species richness from multiple sites in the eastern Canadian Arctic.

46 Role of Sip1 with Merlin in the Hippo Pathway

Linda Mbajiorgu

Neurofibromatosis Type 2 (NF2) is an autosomal dominant cancer that is identified through the exhibition of large tumours of the nervous system that develop during adolescence. NF2 tumours are either unresponsive or poorly responsive to chemotherapy and radiation. Most patients are treated by repeated surgeries. Similar to other cancers, the presence of other gene mutations are presumably important for the initiation or progression of NF2. The NF2 gene encodes the tumour suppressor protein Merlin. We propose that mutations in Merlin or Merlin-binding proteins interfere with Merlin functions as a tumour suppressor, leading to the formation and/or progression of NF2 tumours. Another important pathway in development that controls animal size is the Hippo signalling pathway. Merlin has been shown to regulate aspects of this pathways but the mechanism is unclear. Previously Merlin has been shown to be required for the localization of Warts to the plasma membrane in certain conditions. This project will analyze the potential role of the Merlin interacting protein, Sip1, in regulation of specific members of the Hippo pathway. This work will involve transfection and expression of Merlin, Warts and Sip1 in Drosophila cell culture (S2R+, neuronal cell lines) combined with co-immunoprecipitation and Western blot assays in addition to antibody immunofluorescence staining and confocal microscopy.

47 Habitat Suitability Modelling for Passage Activity: A Cautionary Tale Using Mink

April Robin Martinig

While many studies have evaluated wildlife passage effectiveness, few have explored how accurately passage activity can be modelled. I created a habitat suitability index (HSI) model for American mink (Neovison vison) using Geographic Information Systems with 17 wildlife passages located in Quebec as validation for the model. Two questions were addressed: (1) How well can HSI modelling for mink determine passage activity? and (2) how sensitive are the models to different parameterizations? To test this, I assessed how well the HSI scores generated aligned with passage activity. A generalized linear model was used to test how well the model explained the variability in passage activity (counts). Uncertainty analysis revealed that the HSI model was sensitive to extreme changes in factor weights and scale. The predictive power of all models greatly improved after including aspects related to passage construction, with the pseudo-R2 increasing between 64 – 73 %. These findings suggest that these HSI models are a poor predicator of passage use for mink, while wildlife passage characteristics are highly predictive. Transportation agencies would benefit from the ability to make informed planning decisions, however greater care is required to determine passage suitability. The proper implementation of these tools requires knowledge of not only habitat preferences, but also how movement is influenced by the wildlife passages themselves.

48 Density-Dependent Space Use Violates Assumptions of Camera Models

Kate Broadley

Cameras are an increasingly popular tool for wildlife management. Estimating relative abundance in unmarked species is often done using hit rate indices, where a change in hit rate is assumed to reflect a similar change in density. Hit rate indices assume that detectability remains constant, such that density is the only variable to cause changes in hit rate. This assumption is violated if movement rate and home range size vary with density, but camera trap studies often do not address their assumptions. Therefore, quantifying the relationships between movement rate, home range size, and density is important to determine the suitability of cameras for monitoring unmarked species. I used a systematic review and meta-analysis to determine relationships between movement, home range size, and density across terrestrial mammalian taxa. I also used telemetry data for species with different life histories (wolves, white-tailed deer, and moose) to determine how movement rate and home range size were related within populations. Overall, movement rate and home range size are significantly negatively correlated with density. Effect sizes were significantly heterogeneous, meaning the strength of the relationship varies between taxa and systems. Within populations, individual movement rate generally increases with home range size. Assumptions of constant detectability are violated across a broad range of taxa. When individuals cannot be identified with cameras, researchers and managers should consider monitoring for presence-absence rather than fine-scale changes in abundance, as those estimates are likely to be confounded.

49 Investigating Physiological Function of Cellular Prion Protein Through RNA-Sequencing Analysis

Niall Pollock, Patricia L.A. Leighton & W. Ted Allison

Aims: Cellular prion protein (PrPC) has been implicated in the pathology of Alzheimer’s Disease, the leading cause of dementia, through interactions with amyloid-β oligomers. The normal physiological functions of PrPC are still unknown. We have engineered mutants for two PrPC homologues, combined and separate, in zebrafish. Samples for zebrafish mutants for both PrP1 and PrP2 and AB wild type fish underwent RNA-sequencing to investigate levels of gene expression between fish with and without prion protein.

Methods:Wild-type AB and mutant prp1-/-; prp2-/- zebrafish were grown to three days’ post fertilisation. Triplicate samples for both wild-type and mutant fish underwent PE100-125 and HiDeq2500 sequencing. Genes of interest were identified based on log2 fold change. Once identified gene expression will be validated using qPCR. Localisation of genes of interest will be assessed using in situ hybridisation of zebrafish larvae.

Results: RNA sequencing analysis shows a change in expression of 364 genes with a log2 fold change of 1 or greater. There was an increase in expression of 256 genes and a decrease in expression of 108. Certain genes of interest were identified suggesting there may be a disruption of circadian rhythm in our mutants. One of the biggest changes was in growth hormone releasing hormone, which showed a decrease of almost 12.5x in mutants compared to wild-type. The opsins opn1sw1 and opn1sw2, both showed a double increase in expression in mutants compared to wild-type. Pd36h, also associated with circadian rhythm, showed a 9x increase in expression in mutants.

Conclusions: The data generated through RNA-sequencing suggests PrP may be involved in the development and maintenance of circadian rhythm. Behavioural changes will be pursued using Ethovision to identify changes to zebrafish circadian rhythm under controlled conditions. Rescue experiments will be undertaken through injection of PrP1 and or PrP2 mRNA into single cell zebrafish embryos.

50 Mek and RasGRP1 roles in DUSP2/5 induction during thymocyte selection

Julia May, Nancy Hu, Troy Baldwin

Extracellular signal-regulated kinase (ERK) is a mitogen-activated protein kinase (MAPK) whose activation profile differs during positive or negative selection signals in developing thymocytes. ERK is activated by phosphorylation on tyrosine or threonine residues, and can be inactivated by dephosphorylation by dual specificity phosphatases (DUSPs). Previously, it was shown that DUSP2/5 expression was greater in thymocytes undergoing negative selection compared to positive selection. In other systems, DUSP5 expression has been shown to be positively regulated by ERK signaling. We hypothesize that DUSP2/5 expression is positively regulated by ERK signaling in a RasGRP1-dependent manner. If DUSP2/5 expression is regulated by MAPK signaling, this supports the existence of a negative feedback loop in the regulation of ERK activity. We focus on investigating the differential expression of DUSP2/5 between negatively and positively selected thymocytes, and examine the impact of blocks in the MAPK signaling pathway on DUSP2/5 expression using the Mek inhibitor, UO126. We show by qPCR that DUSP2/5 are positively regulated during negative selection in vitro in a RasGRP1 and Erk-dependent manner. If DUSP2/5 are involved in a negative feedback loop that regulates ERK activity, this could explain the characteristic ERK activation profile during negative selection.

51 Characterizing Short Wavelength Cone Telodendria in Zebrafish

Nicole C. L. Noel and W. Ted Allison

Photoreceptor cells are photo-sensitive neurons within the eye that convert light information into electrochemical signals that can be transmitted to the brain for interpretation, ultimately allowing for vision. In order to do this, photoreceptors must maintain appropriate connections within the retina and receive feedback from neighbouring photoreceptor cells. Understanding how developing photoreceptors integrate into downstream neurons has been an important area of research regarding vision restoration; despite this, connections between neighbouring photoreceptors have been largely overlooked. Telodendria are fine processes that emit from the photoreceptor pedicle and connect to other photoreceptors. While telodendric structures are conserved across diverse vertebrate groups, the exact function of these structures is unknown. We exploited the genetic tractability of the zebrafish to visualize telodendria via fluorescent protein expression in specific photoreceptor types. We characterized UV and blue cone telodendria in wild-type adult retina, larval retina, and mutant larval retina. In the adult retina, blue cones have twice as many telodendric connections than UV cones. Additionally, blue cone telodendria branch twice as often as UV cone telodendria. We also observed that the pedicles of UV and blue cones are unexpectedly coupled, compared to the UV and blue cone cell bodies, which are evenly spaced. In the larval retina, UV cone telodendria are 1.3 times more numerous than blue cone telodendria. In tbx2b-/- mutant larval retina, wherein UV cones are reduced in number and rods are abnormally abundant, UV cone telodendria were reduced by half, per cell body. No evidence has been observed that telodendria extend in length or increase in number in the absence of typical cellular neighbours. In summary, we describe the development and morphology of zebrafish cone connectivity in the outer plexiform layer.

52 Assessing ecosystem dynamics in the Beaufort Sea using stable isotopes in polar bears (Ursus maritimus) and ringed seals (Pusa hispida)

Nicole Boucher, Andrew Derocher, Evan Richardson

The amount of sea ice is declining in the Arctic, which likely will result in changes of abundance and distribution of both polar bears (Ursus maritimus) and their main prey ringed seals (Pusa hispida). Changes in availability and accessibility of prey will result in changes in the diets of both species. I am proposing a study focusing on how the diets of both polar bears and ringed seals in the Beaufort Sea may have changed due to climate change using samples collected in April and May from 1985–1987, 1992–1994, 2000, and 2003–2011. I will use nitrogen and carbon stable isotopes in serially sectioned polar bear claws and hair, and ringed seal claws, to quantify diet. The objectives of this study are to 1) determine if seasonal and annual variation in polar bear and ringed seals diets has changed over time from 1985 to 2011, 2) determine whether stable isotope values in sectioned hair and claw samples are associated with polar bear space-use strategies from satellite telemetry data, 3) examine relationships between isotopic values in both species relative to sea ice breakup dates, rate of sea ice breakup and climate indices, and 4) determine if changes to ringed seal diets are associated with changes in polar bear stable isotopes.

53 Protein Quality Control in Sarcomere Assembly and Turnover

Casey Carlisle, Kendal Prill, Dave Pilgrim

In order to maintain homeostasis, defective proteins must be identified, removed, and replaced. In skeletal muscle, failures in this process can lead to a variety of incurable muscle diseases, many of which are fatal. While aspects of sarcomere protein turnover have been studied widely, the process and its components are still incompletely understood, which hinders the development of effective treatments for these diseases. Using the zebrafish model system, our goal is to elucidate the components and mechanism behind protein quality control in muscle assembly and maintenance. Until recently, it was believed that response factors known as myosin chaperones corrected myosin damage regardless of when it occurred. However, ours and other data suggests a restricted role in sarcomere assembly for at least one myosin chaperone, unc45b (Etard et al., 2015). Here we present qPCR and ISH data which extends this finding to other myosin chaperones, hsp90a1, and smyd1b. Therefore, we support a model by which these myosin chaperones only correct myosin damage during sarcomere assembly. To address sarcomere damage post-assembly, we examined the involvement of the Ubiquitin Proteasome System by examining the expression of various E3 enzymes via qPCR. Our data indicates a division of function where specific E3 enzymes respond to sarcomere damage exclusively during assembly, while others respond to sarcomere damage throughout the life of the sarcomere. An understanding of the specific role of the proteins involved in protein quality control provides the initial steps into identifying potential targets for therapeutic treatment.

54 Defining the Novel Algaecide Biosynthetic Pathway of a Marine Bacterium

Yue Xu, Anna R. Bramucci, Elizabeth Ryan, Rex Malstrom, and Rebecca J. Case

The cryptic biosynthetic pathway of roseobacticides, a novel class of antibiotics, remains unsolved. These antibiotics are only produced by the marine bacterium Phaeobacter inhibens in the presence of phenylpropanoid compounds, which are thought to be breakdown products of cell wall lignin released by senescent Emiliania huxleyi algae. Roseobacticides trigger programmed cell death in aged E. huxleyi, a defense mechanism to protect the E. huxleyi population from further pathogen invasion. As the enzymes involved in roseobacticide production are likely targets for E. huxleyi caspase-like proteases released during programmed cell death, we identified them by the presence of specific amino acid motifs recognized by these caspases. Fifty-eight mutants of genes coding for such enzymes were selected from a transposon mutant library of P. inhibens and submitted to a bioassay screen for deficiency in roseobacticides production. Three mutants with an 18% reduction and five mutants that have a more than 30% increase in the production of roseobacticides were found. These mutants correspond to genes likely involved in synthesis and host delivery of roseobacticides, yielding insights in the biology of this compound, but also a possibility for new drug discovery in pharmaceutical industries.

55 Distribution, diversity, and function of glass sponge (Porifera, Hexactinellida) reefs in Hecate Strait, British Columbia

Lauren Law, Dr. Sally Leys

Trawling and oil/gas exploration threaten to destroy ancient glass sponge reefs found exclusively in the deep sea of Hecate Strait, British Columbia. As filter feeders, sponges recycle nutrients to the water column and also serve as important habitat forming structures that increase biodiversity. The valuable ecosystem functions the reefs perform has prompted interest in establishing them as a marine protected area (MPA), but our ability to monitor and protect the reefs is hindered by a lack of baseline biological data. Given these knowledge gaps, I analyzed imagery from remotely operated vehicle surveys carried out in 2015 to generate high-resolution maps of live sponge cover and quantify biodiversity data. Reef boundaries were originally mapped prior to 2010 with multibeam bathymetry and backscatter, but preliminary results show discrepancies between our 2015 ROV surveys and these past mapping records. The reefs are distributed in smaller patches and sometimes extend beyond the boundaries implied from multibeam mapping. Biodiversity data show squat lobsters and fish to be highly associated with live reef, whereas shrimp and flatfishes correspond to dead reef. Numerous non-reef forming sponge species were also discovered living on dead glass sponge, including a potential new species of a thin encrusting sponge called Desmacella. In measuring reef distribution, diversity, and function, we aim to develop MPA management strategies effective at protecting Canada's unique sponge reef habitats.

56 Impacts of industrial noise on owl occupancy and seasonal use in northern Alberta

Julia Shonfield & Erin Bayne

Noisy environments can pose problems for animals that use acoustic signals. Owls use vocal communication to attract mates and defend territories. They also rely on acoustic cues to locate their prey. Industrial noise has been shown to negatively affect owl hunting success and reduce foraging efficiency by affecting their ability to detect prey. It remains unknown if this results in reduced habitat quality for owls in forested areas in close proximity to chronic industrial noise. To determine if owls avoid forested areas surrounding industrial noise sources in northeastern Alberta and at what scale, we conducted passive acoustic surveys for owls in the spring using autonomous recording units deployed at sites with two levels of industrial noise and sites with no noise. Detections of owls were extracted from the recordings using automated species identification and analyzed using occupancy models. Barred owls, great horned owls, and boreal owls were equally likely to occupy noisy sites compared to sites with no noise, indicating that occupancy at a home range scale was unaffected by the presence of noise sources on the landscape. However, at a local scale, barred owls showed a decline in seasonal use around stations with higher noise levels, but this was not the case for boreal owls and great horned owls. This research contributes to growing evidence that anthropogenic noise sources have impacts on wildlife and can degrade otherwise suitable habitat, though some species seem to be more sensitive than others.

57 Can song rate tell us about reproductive status in the Olive-sided Flycatcher?

Emily Upham-Mills

Male songbirds sing primarily to attract a mate and defend a breeding territory. Biologists make use of bird song to identify species and monitor population sizes and distributions across the landscape. But what if bird song can tell us more that just if a bird is present? Large amounts of time and effort are normally required to assess reproductive status for songbirds, making a simpler index attractive. A count of the number of songs produced over a given time, or "song rate", is a possible measurement. I tested this idea by measuring song rate in the Olive-sided Flycatcher, a species at risk songbird, on 26 breeding territories in northern Alberta and the Northwest Territories. Male birds were monitored throughout the breeding season to assess their reproductive status and acoustic recording units (ARUs) were deployed to see if song rate could also be detected remotely. The presentation will cover initial results of the study as well as possible implications for songbird monitoring on a larger scale.

58 Turning on the bacterial thermostat: Identifying and characterizing a cyanobacterial RNA thermometer

Logan A. Brand and George W. Owttrim.

"RNA helicases are ubiquitous among the three domains of life, wherein they fulfill roles essential to all aspects of RNA metabolism. In Synechocystis sp. PCC 6803, cold stress induced by a shift from 30°C to 20°C results in the rapid, transient accumulation of transcripts of its sole RNA helicase, crhR. Although the transcriptional mechanisms responsible for this accumulation have yet to be elucidated, preliminary data suggest that regulation occurs through a temperature-sensitive riboswitch, or RNA thermometer, present in the 5' UTR of the crhR transcript.

Term-seq, a technique which identifies the basepair at which transcription is prematurely halted by an activated riboswitch, will be used to verify the presence of this regulatory mechanism. The precise conditions affecting the activity of this riboswitch will be characterized in vivo via observing its effect on the expression of a constitutively-expressed His-tagged CrhR reporter, and allow mutagenesis of the RNA secondary structure to confirm riboswitch activity. In future work, term-seq will be applied to identify temperature-responsive riboswitches in the entire Synechocystis transcriptome. Riboswitches identified in this manner may have biotechnological applications, regulating gene expression for the production of ecologically sustainable biofuels and other biotechnological applications."

59 Phenotypically distinct subfertility in male and female mice with mutations in *Cecr2*

Kacie A. Norton, Chelsey B. Weatherill, Ross C. Humphreys, Vivian V. Nguyen, Kenji Rowel Q. Lim, and Heather E. McDermid

Mammalian reproduction is dependent on a myriad of genes, all of which must be carefully regulated spatially and temporally to ensure successful fertilization and embryonic development. This process partially depends on chromatin remodellers, which are able to affect gene transcription and other processes through modulation of chromatin structure. Mutations in chromatin remodelling gene *Cecr2* result in subfertility in both sexes.

Mutant males are most subfertile immediately after sexual maturity (42-60 days) and within 2 months litter sizes improve from 11.7% to 58.3% the size of litters from normal males. Histological analysis has revealed severe defects in the seminiferous tubules of young adults, including tubules that are lacking several cell types and are not completing spermatogenesis. These defects become less common and less severe with age, as do corresponding decreases in sperm number and motility. I have shown that the fertilization frequency of mutant males follows the same pattern, indicating that subfertility is due to fewer oocytes being fertilized. Intriguingly, transcription of *Cecr2* in the testis appears to occur from both canonical exon 1 and a novel upstream exon, the function of which is still unknown.

Female mice with mutations in *Cecr2* have both smaller and less frequent litters, yet their ovarian histology is normal. The number of oocytes fertilized and the percent of embryos surviving to blastocyst stage in culture is also normal, and there is no increase in late embryonic death. Recent data visualizing implantation sites at embryonic day 5.5 through injection of blue dye shows that mutant females have fewer successful implantations.

Overall, investigating the transcription of *Cecr2* and the cause of subfertility in *Cecr2* mutants will advance our understanding of the role that chromatin remodelling plays in reproduction. While mutations in *Cecr2* cause subfertility in both sexes, the mechanism by which this occurs appears to be different.

60 The fate of infection plasticity when subjected to directional selection in a facultative parasitic mite

Emily Durkin

Phenotypic plasticity is the ability of one genotype to produce a range of phenotypes in response to the environment. For many organisms, phenotypic plasticity is an important contributor to fitness and is subject to evolution. Plasticity can be lost as a result of directional selection and genetic fixation, i.e. genetic assimilation. The facultative parasitic mite, Macrocheles muscaedomesticae, shows variation in infection due to phenotypic plasticity. Their infectious response often correlates with environmental conditions. I tested the fate of infection plasticity when infection was subjected to directional selection in M. muscaedomesticae. Following genetic assimilation, I hypothesized a loss of plasticity in infection due to artificial selection for increased host-attachment. I generated two replicate lines of mites, each consisted of a control and a high host-attachment treatment. For ten and eleven generations, only mites that attached to a host within 60 minutes seeded subsequent generations of the “high-attachment” population. Mites in the control treatment were subjected to the same methods and conditions as the “high-attaching” mites except for selection; control mites seeded regardless of their attachment status. “High-attaching” mites showed significantly increased levels of host attachment after 5 and 7 generations of selection compared to controls. Selected mites continued to show significantly greater host attachment even after selection was relaxed for 10 generations, suggesting genetic assimilation occurred. Mites from the last round of selection produced offspring that were raised in the presence of no, low or high amounts of food. Under these three conditions, I predicted selected lines to show a loss in phenotypic (attachment) plasticity compared to control lines. Interestingly, plasticity was maintained in both selected and control lines, even as host attachment remained high in the selected mites. It is possible that plasticity is highly conserved, not linked with infectivity traits or not costly to maintain in M. muscaedomesticae.

61 Assessing recommended monitoring tools for orange wheat blossom midge in the Peace River region

Jorgensen, A., Otani, J., Evenden, M.L.

Orange wheat blossom midge, Sitodiplosis mosellana Géhin (Diptera: Cecidomyiidae), is an invasive pest of wheat (Triticum spp.). Pheromone trap monitoring and in-field scouting target the short-lived adult stage. The objective of this study was to assess different monitoring tools in order to develop recommendations for growers in the Peace River region. Different traps and lures have been commercially available for monitoring wheat midge. Green and orange delta traps were baited with either a pheromone lure: flex lure (Scotts™), a rubber septa lure (Scotts™ or Great Lakes IPM™) or no lure. Eight commercial fields of susceptible wheat in the Peace River region were monitored. Traps were spaced 50 m apart at the field edge. Treatments were randomized along a North-South transect at each site. Traps were monitored weekly from 20 June 2016 to 18 August 2016 during wheat midge flight. In-field visual monitoring was conducted at three commercial cereal crop fields (wheat and barley) over 4 nights during peak flight. Counts were done on calm nights when the temperature was above 10 °C. The number of midge on 10 heads was counted hourly in 5 locations in each field. The number of midge captured in pheromone-baited traps did not differ with trap colour. Significantly more wheat midge were captured in pheromone-baited traps compared to un-baited traps, but there were no differences in the number of midge attracted by the different lures. In-field counts of midge peaked between 9:00 pm and midnight, but continued until after dawn. This study provides growers and managers with information for implementation of monitoring and scouting.

62 In situ arrests of Glass sponge (Porifera, Hexactinellida) feeding currents

Nathan Grant

Glass sponges (Class Hexactinellida) are sessile, suspension feeding animals capable of forming deep-water reefs akin to shallow-water coral reefs. By continuously pumping water through a system of fine canals, sponges feed and respire, and in doing so, perform important ecosystem services by recycling nutrients. Glass sponges have developed the unique ability to “arrest” their pumping in response to stimuli that might impair their aquiferous system. Arrest responses have been induced experimentally in response to suspended sediments, which can clog and damage a sponge’s pumping system. Suspended sediments trigger action potentials which travel through syncytial tissues leading to the temporary cessation of pumping. Trawling near glass sponge reefs suspends sediment but does that cause feeding arrests and compromise sponge health? Arrest responses have been observed in laboratory settings but have not been recorded from sponges in situ. In 2015, flowmeters and optical backscatter sensors were placed on Fraser Reef, a kilometer-long sponge reef in the Strait of Georgia, British Columbia. Analysis shows that reef-building glass sponges do arrest their feeding currents in situ. Natural arrest responses appear to be correlated with increased suspended sediment concentrations at the reef. If trawling-induced suspended sediments harm sponges, protective boundaries around reefs are needed.

63 The evolutionary history of vertebrate vision through the eyes of Eptatretus stoutii (pacific hagfish)

Emily Dong and W. Ted Allison

"Conserved across all branches of the vertebrate tree is the camera style eye, an evolutionary novelty key in the successful radiation of this group. Despite the deep conservation of function, development, and structure, the evolutionary history of the vertebrate eye remains inadequately understood. Hagfish, a jawless fish, are living members of an ancient lineage that can offer a unique glimpse into the evolutionary history of this key vertebrate characteristic thereby providing us with a greater understanding of its ancestral origins.

Small, obscured by epidermis and frequently reported as "pineal-like", the hagfish eye is unlike that of jawed vertebrates. It appears to be primitive, lacking retinal interneurons, a lens, and shows limited opsin expression. However, preliminary data indicates that the hagfish retina may be more similar to jawed vertebrates than previously suggested. Particular regions of the retina show distinct nuclear layering highly similar to that of jawed vertebrates, and photoreceptors appear markedly more developed than those of the pineal. Additionally, the historically understudied juveniles are suggested to display a more organized retina than that of the adult.

The question of whether or not these characteristics represent the state of the common vertebrate ancestor remains unanswered. Using RNA-seq, in situ hybridization and immunohistochemistry, we aim to characterize cell types and explore the organizational structure of the pacific hagfish (Eptatretus stoutii) eye. In so doing we hope to capture conserved retinal characters that may provide us with insight into the ancestral state that allowed for the evolution of the vertebrate eye."

64 UV cone photoreceptors and putative molecular mechanisms of zebrafish magnetoreception

Spencer Balay and W. Ted Allison

"The Earth's magnetic field acts as a navigational cue for a diverse range of organisms. This sensory information is used in animal behaviours such as homing, predator avoidance, prey capture and long distance migrations. Historically, the literature has been dominated by behavioural evidence for magnetoreception, with little progress made toward identifying a mechanism for this phenomenon. Light-sensitive proteins called Cryptochromes are putative molecular candidates due to their biophysical properties: they can respond to magnetic fields, are activated by blue and ultra-violet (UV) light and have been shown to be associated with retinal UV-cone photoreceptors in birds and the homologous blue cone photoreceptors in some mammals. Although UV-cones have been suggested to be crucial for Cryptochrome expression, and thus the signal cascade of magnetic information, their role in magnetoreception has yet to be tested.

To address the role of UV-cones in Cryptochrome expression, fluorescent in situ hybridization (FISH) and quantitative-PCR (qPCR) were used. Preliminary work in zebrafish showed that UV-cones express Cryptochrome 4 (cry4). When UV-cones were ablated from the retina, cry4 expression decreased. Interestingly, when blue cones were ablated, cry4 expression was unchanged, suggesting a cell-specific interaction between UV-cones and cry4. This serves as the first experimental evidence that cone photoreceptors impact Cryptochrome expression, and further supports their role in magnetoreception.

Zebrafish represent an excellent vertebrate model for uncovering the proximate mechanisms of visually mediated behaviors, but have been under used in magnetoreception studies. Our lab has engineered a zebrafish line where UV and Blue cones can be selectively ablated from the retina, without disrupting other retinal cells. This provides a unique opportunity to study the role of cone photoreceptors in Cryptochrome expression and the mechanisms governing magnetoreception. Additionally, novel behavioral paradigms are currently being designed to test the role of UV-cones in zebrafish magnetoreceptive behavior."

Poster Presentations:

65 Effects of weather covariates on the maximum detection distance of Common Nighthawk

Lan Truong, Elly Knight, Daniel Yip, Erin Bayne

Current technology in bioacoustics is being used to monitor acoustic species and help collect population and behavioural data. There are assumptions in the current method for acoustic distance detection that are not being addressed. These include the assumption that detection remains consistent in various environmental conditions, such as weather. This study investigated the relationship between independent weather covariates—wind speed, temperature, and humidity—and the maximum detection distance of the Common nighthawk. Previous work has shown mixed results for the effects of weather covariates on the detection distances of the Common nighthawk. We observed calling data of the Common nighthawk, a distinct and highly vocal, yet largely understudied species, in order to gain more information on its physical presence in Northern Alberta. Common nighthawk calls were collected over 11 days in early July 2016 with autonomous recording units (ARUs). These were used in conjunction with audio recognizer software, SongScope, to determine maximum detection distances, while linear regression models were used to determine correlations between each weather covariate and the distance. We found that none of these weather covariates had significant relationships with the maximum detection distances of Common Nighthawk. Validating these relationships work to increase the accuracy of population estimates, by first improving the accuracy of ARUs to create reliable automated estimations of detection distances of birds.

66 Alpine plant distributions under a changing climate regime as mediated by soil factors in the Rocky Mountains.

Cole Brachmann

"Plant species distribution patterns are influenced by regional climate, local variation in edaphic conditions, and biotic interactions with neighbors. Rapid environmental changes can result in distribution shifts for alpine species. The properties of soils along these environmental gradients may speed up or slow down the rate of change of plant community development in these alpine sites.

My research questions are: how are alpine plant distributions being affected by warming temperatures, and how are soil properties along an elevational gradient impacting the changes in species distribution?

My research was conducted at the site of the 2016 Alpine Club of Canada General Mountaineering Camp in Bachelor Pass in the Northern Selkirks, British Columbia. I characterized plant and soil communities along several elevational gradients, including glacier foreland. Species abundance and cover was determined, soil samples were collected, and several soil surface properties were measured in situ. These data will be used to interpret the processes that influence the elevational distribution of plants in Bachelor Pass, as well as how the soil chemistry varies along an elevational gradient. A transplant experiment was also conducted to determine the ability of late successional plant species to grow in recently deglaciated areas. The early successional sites were dominated by broad-leaved willow-herb (Epilobium latifolia), and located adjacent to the rapidly melting terminus of the Easy Glacier. I transplanted ten individuals of four species from later successional sites within Bachelor Pass: leather-leaved saxifrage (Leptarrhena pyrolifolia), lesser blackscale sedge (Carex atrosquama), alpine bluegrass (Poa alpina), and alpine sagewort (Artemisia norvegica). Inorganic nitrogen fertilizer was added to half of the transplants to determine if nutrient limitation is restricting establishment of late successional species in recently-deglaciated soils. The plants were monitored during the summer, and will be remeasured the summer of 2017, to determine growth rate and survival."

67 Neuropathology of chronic wasting disease transmitted between white-tailed deer and transgenic elk mice

Vidhi Desai

Chronic Wasting Disease, a prion disease of moose, deer, and elk, is a neurodegenerative and infectious disorder that occurs when an animal is infected with the abnormally folded form of a prion protein. CWD is prevalent in North America and is readily transmissible within and between cervid species. Therefore, it is important to understand the effects of CWD when it is transmitted between different species. There are three different prion protein polymorphisms associated with CWD in white-tailed deer that lead to multiple genotypes including wt/wt, wt/H95, wt/S96, H95/S96. This study was conducted to determine how the prion protein polymorphisms in white-tailed deer affect the aggregation and severity of CWD in the brains of transgenic elk mice (TgElk). Lesion profile analysis and deposition of disease-associated protein (PrPd) were used to characterize the severity of CWD. It was hypothesized that sequence differences between the host and the infectious agent would lead to more severe neuropathology of CWD. CWD infected brain homogenates from white-tailed deer expressing differing prion protein were transmitted into transgenic elk mice. Based on the vacuolation and prion protein aggregation seen in TgElk brains, it was concluded that the different prion protein polymorphisms in white-tailed deer do not differentially affect the aggregation and severity of CWD. In addition to this, it was concluded that there is a difference in the neuropathology of CWD when it is transmitted from deer into TgElk compared to Elk CWD. The results of this study lead to a more comprehensive understanding of CWD and can contribute to future studies defining the CWD isolates generated when deer CWD is transmitted into elk.

68 Yukon Ice Patches: Role of Ice-entombed Bryophytes in Alpine Environments

Miller, B., and La Farge, C.

Yukon alpine glacial ice patches are rapidly disappearing, exhuming 8000 year old (BP) plant populations that have been preserved in pristine condition. A major component of the ice patch flora are bryophytes, which are critical to alpine ecosystems. However, relative to diverse archaeological work, they remained uninvestigated. Bryophytes have the incredible ability to generate a new organism from any viable cell (totipotency), allowing them to persist through extreme conditions. The regeneration of formerly ice-entombed vegetation would indicate that ice patches function as reservoirs of genetic diversity in alpine ecosystems. We have compiled a rich data set on previously ice-entombed plant assemblages from the Granger Ice Patch (SW of Whitehorse) and the Gladstone Ice Patch (NE of Kluane Lake). Sampling within the vicinity of each ice margin has documented the extant diversity, and the plant succession with ice margin retreat. Preliminary results of in vitro cultured subfossil bryophyte tissue have demonstrated a phenomenal regeneration capacity of previously ice-buried populations, and viability of ancient vegetative propagules. Next generation sequencing (double digest restriction site associated DNA sequencing) was conducted to determine the genetic variation between regenerated subfossil plants and modern populations of the dominant taxa, Polytrichum hyperboreum. Our study explores the cyclical role of bryophytes from exhumed assemblages that contribute to establishment, revegetation, and maintenance of alpine ecosystems.

69 Zebrafish muscle mutants still heart and steif demonstrate incomplete heart morphogenesis revealing novel roles for these proteins in cardiac development

Kendal Prill and Dave Pilgrim

"Heart development is a complex process involving hundreds of genes and proteins that interact in the formation of the heart. Defects, or mutations, to these factors can result in heart defects and disease. Some of the genes and proteins involved have been identified and their function understood, but there are several factors whose identity and role in heart development remains unknown. Understanding the process of cardiac muscle development, and the genes/proteins involved, can lead to developing therapies or treatments for heart disease. To understand early heart formation, we have chosen to use a simple system that provides advantages that are not possible in mammals. Heart disorders are fatal during development, but zebrafish offer the ability to study these disorders in a living system. The cardiac developmental pathway, and its factors, is shared across species, making advancements in zebrafish applicable to others.

We can observe heart development defects in the affected fish still heart and steif. Both of these mutants have completely nonfunctional hearts that fail to beat and undergo cardiac morphogenesis. Analysis of their function in skeletal muscle suggests that these two genes code for myosin chaperones, however these two zebrafish mutants demonstrate defects that are not associated with the absence of myosin from the developing cardiac sarcomere. We hypothesize that these proteins are responsible for folding transcription factors critical for heart morphogenesis. We demonstrate a change in the transcriptional network of still heart and steif when compared to wild type and drug-paralyzed hearts. Furthermore, mutant hearts fail to incorporate cardiac cells from the Second Heart Field during looping, reducing the size of the heart and the ability to develop full functional hearts. Comparisons of the still heart and steif cardiac phenotype to other cardiac mutants suggest potential client proteins for these chaperones in the heart morphogenesis pathway."

70 Mountain goats (Oreamnos americanus) susceptibility to Chronic Wasting Disease: A pilot study.

Diana Johnstone

Mountain goats (Oreamnos americanus) are potentially at risk of being exposed to chronic wasting disease (CWD) via infected ungulates. However, little is known if mountain goats are susceptible to CWD and whether a species barrier exists between mountain goats and any infected ungulate species such as white-tailed deer (Odocoileus virginianus). To identify if a species barrier exists between mountain goats and white-tailed deer as well as to identify potential susceptibility to CWD we sequenced extracted mountain goat PrnP genomic regions and compared the sequences to a white-tailed deer reference at specific polymorphic sites. We discovered an apparent mismatch between the species which could indicate the presence of a species barrier but meant we could not explicitly identify if mountain goats are susceptible to CWD. Finally, to visualize the relationship between mountain goat PrnP genomic regions and other species PrnP gene regions, we obtained the optimal bootstrap cladogram which identified domestic sheep(Ovis aries) as a more closely related species to test mountain goat susceptibility to other prion infections. More research on this topic is needed in order to determine mountain goat's susceptibility to CWD and in general more research is needed to determine other at risk species suceptibility.

71 Effect of THC Exposure During Gastrulation on Zebrafish

Ruhul Amin, Kazi Tanveer Ahmed and Declan Ali

Marijuana is the world’s third most popular recreational drug, right after alcohol and tobacco. Approximately sixty-six plant-derived cannabinoid compounds and over four hundred other chemicals are constituents of this drug. THC (Δ9-tetrahydrocannabinol) is the major psycho-active component of marijuana while CBN (cannabinol) is a non-psycho-active metabolite of THC. In this study, we sought to investigate the effect of THC and CBN on development using zebrafish as a model organism. To study the effects of THC and CBN has on development- we have chosen an important stage of development, gastrulation, to expose the embryo at various concentrations of these drugs. Our preliminary findings showed that CBN exposure during gastrulation exhibited more detrimental effects towards the development of zebrafish compare to THC. Survivability of embryos was seriously reduced with CBN but not with THC exposure. CBN also delayed the hatching of zebrafish embryo. Embryos exposed to THC exhibited reduced body length, whereas CBN exposure showed severe malformation along body, i.e. axial malformation and bent body. We observed reduced heart rate both in THC and CBN exposed embryo. In terms of neuronal development, THC exposure altered the morphology of Mauthner cell, an important interneuron associated with escape response. Locomotion experiment showed that angle of escape response (C-bend) is significantly reduced by THC exposure. Exposure to THC and CBN also alters muscle fibre morphology and motor neuron branching. Overall, exposure to cannabinoids during gastrulation cause severe developmental defects in zebrafish.

72 Songbird response to regenerating seismic lines and the influence of scale

Jocelyn Gregoire, Dr. Erin Bayne

The cumulative effects of energy sector growth in Northern Alberta have raised concerns about their impacts on the biodiversity of boreal forest ecosystems. Disturbances associated with SAGD make up a large component of this industry and pose a challenge to environmental management. Three issues exist in understanding how to mitigate the effects of such disturbances on forest birds: 1) whether the metric of successful mitigation is behavioural or numerical; 2) if traditional methods (i.e. point counts) are sufficiently precise to measure the desired response; and 3) what observational scale is appropriate to assess population effects. My thesis aims to further understand these fundamental issues. The first objective is to assess how seismic lines affect the use of disturbed and edge habitat by the Canada Warbler and species of a similar habitat association. To conduct this study, I use grids of autonomous recording units (ARUs) to locate the position of songbirds around seismic lines representing a gradient of vegetation recovery states. The grids will cover the seismic line and forested edge on either side. In this way, I will be able to identify if a bird crosses the feature or not. The second objective of this study is to determine how observational scale affects our interpretation of song bird response to linear features. I will use data collected from the ongoing ‘Big Grid’ project conducted by the Bayne Lab. Each site consists of a 10x10 grid of ARUs spaced 600m apart. These grids are established on a minimum of 10 sites that represent three levels of SAGD disturbance (undisturbed, developing and active). Overall, this research has implications for future reclamation practices and industry standards. It will help to define what is recovered from an ecological perspective and provide industry with an accurate means of evaluating ecological impacts.

73 Transcriptional regulation of RNA helicase expression in cyanobacteria

Hae-Won Son, Dr. George Owttrim

RNA helicases are found in essentially every organism and play roles vital in all aspects of RNA metabolism. The post-transcriptional regulation of the crhR RNA helicase at low temperatures (20°C) has been well characterized in cyanobacteria strain Synechocystis sp. PCC 6803. My objective is to investigate the mechanism by which temperature regulates crhR transcription. I will construct a series of plasmids combining wild type or a constitutive promoter alone or including the crhR 5’ UTR to drive expression of the crhR ORF translationally fused to the yellow fluorescent protein, acting as a fluorescent reporter of crhR expression. Fluorescent microscopy will be conducted on wild type and crhR mutant strains transformed with these constructs and grown at 30°C and 20°C. Temperature-dependent changes in the level of fluorescence will indicate the relative contribution of promoter activity vs the 5’UTR to crhR expression in response to low temperature. This work will provide unique insights into the mechanism by which temperature regulates cyanobacterial gene expression, particularly in response to expanding blooms of cyanobacteria which compromises water quality on a worldwide scale.

74 Inactivation of Conditional Proteolysis: Effect on Cyanobacteria Synechocystis sp. PCC 6803 Fitness

Arinjay Sarma

Proteolysis fulfills a dynamic role in prokaryotic and eukaryotic organisms. One example involves restoring homeostasis following abiotic stress. Expression of the RNA helicase CrhR fluctuates through two established pathways: a CrhR independent induction pathway at 20°C and CrhR dependent conditional proteolysis at 30°C. A 50 amino acid C terminal motif within CrhR has been found to be necessary for its proteolysis at 30°C. This study examined the effect of disruption to this proteolytic domain, measuring cellular fitness in conjunction with temperature stress. Mutants examined included targeted deletions to the front and back halves of this motif as well as a complete deletion. Initial results indicate all three deletions severely inhibit wild type growth at 20°C, and similarly do not complement growth of cells lacking genomic CrhR. This suggests disruption of CrhR proteolysis creates a dominant negative mutation, potentially as a consequence of CrhR dimerization. Much of the mechanism of CrhR proteolysis is still unknown. However, unraveling the physiological effects created by alteration of CrhR proteolysis on fitness will provide insight for further structure function analysis of the proteolytic mechanism.

75 THE ROLE AND THE LOCALIZATION OF ERM PROTEINS IN ZEBRAFISH EYE DEVELOPMENT

Sera Sajeev, Dr. Jennifer Hocking

Photoreceptors are the specialized light-sensing cells of the eye, while the retinal pigmented epithelium (RPE) is the supportive layer adjacent to the photoreceptors, and the two cell types are the ones most commonly affected in blindness-causing diseases. Ezrin, Moesin, and Radixin (ERM) are a family of proteins with important functions in cell morphology, cell-cell adhesion, cell-matrix adhesion, and intracellular signaling. Previous work found that the ezrin b (ezrb) gene is specifically expressed in photoreceptors, while moesin b (msnb) is localized to the RPE. In order to examine the roles of ERM proteins in photoreceptor and RPE development and maintenance, we created zebrafish mutant for ezrb and msnb through CRISPR-Cas9 mutagenesis. Founder fish carrying a seven-base pair deletion in ezrb and a one-base pair insertion in msnb were identified and bred to raise an F1 generation. Various genotyping methods were developed to test for the specific mutations. In a second part of the project, localization of active, phosphorylated ERM (P-ERM) protein was analyzed in sectioned retinas of zebrafish embryos. An optimal protocol for antibody staining was developed and used in association with various secondary antibodies and transgenics to examine the subcellular localization of P-ERM in the outer retina. Very strong staining was observed in the apical villi of the RPE, and weaker staining in specific photoreceptor domains.

76 Localization and regulation of GnRH receptors in pituitary gonadotrophs and somatotrophs of Carassius auratus

George J. Kinley, and John P. Chang

All vertebrate species have multiple isoforms of gonadotropin-releasing hormone (GnRH), which is a central regulator of reproduction and growth. GnRH acts primarily by release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from gonadotrophs, and in some teleosts also by growth hormone (GH) from somatotrophs. Most vertebrate species also have multiple GnRH receptor (GnRHR) types, which bind multiple GnRH isoforms and may activate distinct signaling responses depending on which isoform binds. Additionally, GnRHRs exhibit regulatory behavior common to the G-protein coupled receptor (GPCR) family. Distribution across different cell types, regulation of expression, and selective signaling of GnRHR subtypes is poorly understood. Goldfish make an excellent model system, as they have two isoforms of both ligand (cGnRH2 and sGnRH3) and receptor (GfA and GfB), and GnRH-induced LH and GH release has been well characterized. To investigate, our lab has generated antibodies against the ligand-binding domain of GfA and GfB. We show by immunoblotting differences between GfA and GfB protein content in response to different GnRH isoforms, and that receptor regulation also varies with reproductive cycle. Fluorescence microscopy evidence suggests that GfA is enriched on gonadotrophs, and GfB on somatotrophs, which could imply that GfA signaling favours LH and FSH release, and GfB signaling favours GH release. To quantify receptor localization across gonadotrophs and somatotrophs, we will co-stain receptor and LH and GH antibody and analyze by flow cytometry. We will also selectively immuno-block the ligand-binding domain of each receptor, and examine LH and GH release in response to sGnRH3 and cGnRH2 by radioimmunoassay, as well as intracellular messenger activation by western blot. This will allow determination of ligand and receptor-specific differences in signaling responses. These studies will provide insight into GnRHR signaling and regulation, and will be valuable in understanding GPCR behavior and neuroendocrine regulation of growth and reproduction.

77 The impact of crude oil exposure on the prey capture and male aggression behavior in sheepshead-minnow (Cyprinodon veriegatus veriegatus)

D. Philibert, D. Lyons, K. Tierney

From April to July 2010, the Deepwater Horizon oil spill released 3.19 million barrels of crude oil into the Gulf of Mexico that then spread to the surrounding tributaries and wetland habitats. In an attempt to reduce the effects of an oil slick on coastal habitat, dispersant was applied both at the wellhead and at the slick surface to break oil into smaller droplets to expedite microbial degradation. Though the effects of crude oil on survival and cardiac development of early life stages of marine fishes has been established, few studies have looked at the impact of developmental exposure on ecologically relevant behavioral endpoints that relate to both fish feeding and reproduction. Male aggression in the defense of breeding territory, and feeding behavior, play critical roles in both the growth and reproductive success of marine fishes. In our study, sheepshead minnow (Cyprinodon veriegatus veriegatus) embryos were exposed from 1-10 dpf to water accommodated fractions (WAFs) of crude oil, weathered crude oil, crude oil and dispersants, weathered oil and dispersants as well as dispersants alone. Prey capture efficiency was quantified at 60 dpf using brine shrimp nauplii. At 8-11 months the male fish were assessed for aggression-based behavior, using strikes against a mirror. Exposure to the various WAFs had no direct impact on the latency to prey capture, but caused a shift in the bold and shy behavioral phenotypes normally found. Ecologically relevant endpoints such as feeding behavior and male-type aggression behavior on marine model species, such as the sheepshead minnow, will allow for more accurate predictions of the impact of crude oil release on fish populations.

78 The ancestral role of nrl in zebrafish photoreceptor development.

Keon Collett, A. Phil Oel, and W. Ted Allison

"Rods and cones, while functionally similar, are differentiated by context – rods mediate vision in dim light, while cones mediate color-sensitive vision in bright light. Like most vertebrates, zebrafish have an abundance of cones. In contrast, mammalian retinas are rod-dominant: containing significantly larger numbers of rods, with fewer cones. While it appears that rods arose after cones in vertebrate evolution, two things remain unaddressed: whether rods evolved from cones, and the mechanisms that could account for rod differentiation into cone photoreceptors.

Allison Lab and colleagues hypothesize that pro-rod transcription factor nrl (neural retina leucine zipper) co-evolved with rods as a means of controlling rod differentiation and that as early mammals evolved, a second round of rod production provided nrl with gain-of-function and/or gain-of-location contributing to the evolutionary hijacking of cone progenitor cells to the rod fate.

Using zebrafish to represent the classical vertebrate retina, and as a model easily accessible to genetic manipulation, we investigate the function of nrl through a series of ectopic over expression, knockdown, and knock-out experiments. We show the over-expression of nrl in post-mitotic UV cones induces rod characteristics, seen as labeling with rod-specific antibody 4C12, while maintaining expression of UV cone opsin. Knockdown of nrl by morpholino reduces rod abundance, showing similarity to the knockout of NRL experiments in mice.

We continue work establishing knockout mutants via CRISPR/Cas9 mutagenesis and linage tracing lines using Cre/Lox technologies to access the ancestral roles of nrl. In addition, we seek to further compare the functions of zebrafish nrl to mammalian mouse NRL, and Nrl-like Maf genes of the lamprey and chicken, representing jawless vertebrates and birds respectively. Through a series of mRNA injection experiments we will establish ectopic overexpression of nrl orthologs in wild type zebrafish embryos and similarly attempt to rescue mutant phenotypes in nrl knockouts."

79 Comparing Automated Recognition Techniques for Barred Owl Detection

Sarah Heemskerk, Julia Shonfield, Erin Bayne

Most owl species are difficult for human observers to detect visually due to their cryptic colouration and occurrence at relatively low population densities. Owls are more effectively detected by their calls, and are often monitored using acoustic survey methods. Autonomous recording units (ARUs) are being increasingly utilized in bird acoustic surveys due to their lack of observer bias and ability to revisit the data. The objective of my project is to compare two different recognizer templates for automated detection of Barred owl territorial calls to determine which provides the greatest level of accuracy and the lowest processing time. I used ARUs to conduct owl acoustic surveys throughout Northeastern Alberta and this data was scanned and processed using two different recognizer templates. One template used annotations of the entire two-phrased hoot, and the second template used the terminal two notes of the call. The results will be compared to understand if they yield different conclusions in terms of Barred owl habitat use, as well as accuracy and time required for data processing. With Barred owls expanding their range in many parts of North America it is important to understand the most accurate method for their detection to aid management plans.

80 ASSESSING RELATIVE ABUNDANCE AND RESOURCE SELECTION FUNCTION FOR PREDICTING PREDATION RISK IN A MULTI-SPECIES PREDATOR COMMUNITY

Spilker, E., K. MacAulay, E. Merrill, R. Steenweg, M. Hebblewhite, and J. Whittington

Understanding how large carnivores interact to distribute themselves in a heterogeneous landscape is an important first step toward quantifying how they collectively pose risk to their shared prey. Resource selection models and abundance estimates are two common approaches used as measure of predation risk, yet most studies employ only one. In this study, we assess whether camera-derived estimates of relative abundance improve a multi-species resource selection function (RSF) of predation risk on the eastern slopes of the Rocky Mountains in southwest Alberta by comparing the 2 approaches or their integration to predict location of elk kill sites. We modelled a multi-species RSF based on carnivore scats collected using detection dogs along 1057 km of transects distributed throughout 48 5x5-km grid cells and estimated relative abundance of each carnivore species within the same grid cells based on remote camera data located. Using a model selection approach, we found using both carnivore RSF and relative abundance best predicted areas where elk are likely to be killed for most but not all carnivores. Multi-species predation risk quantified from this study can be used to assess forage-predation risk trade-offs of different migratory segments of the elk population in this region.

81 Do spatial differences in predator diets reflect predation risk for Ya Ha Tinda elk?

Kara MacAulay, Eric Spilker and Evelyn Merrill

There is evidence that prey perceive the risk of predation, as they navigate a “landscape of fear” and alter their behaviour in response. Previous approaches to mapping spatial risk use predator and prey distributions to estimate potential encounters. Locations of prey kill sites represent actual mortality in space, but obtaining kill site information can be cost intensive and require time to accumulate adequate sample sizes. I address an alternative to these approaches for mapping predation risk based on mortality by relating features within a buffer to contents of predator scats in Ya Ha Tinda (YHT), a multi-predator community along the eastern slopes of the Rocky Mountains. Scats collected from five carnivores across a 1200-km2 study area will be analyzed for presence of elk. The objectives of this study are to: (1) compare the diets of large carnivores, (2) determine whether % elk in scat be predicted by predator distribution, elk distribution and/or landscape features, and (3) validate risk predictions with known elk kill sites. My project is part of a 14-year study investigating the changes in migratory behaviour of the YHT elk, and will provide baseline data on predation in the Panther-Dormer region in Banff National Park before the bison reintroduction.

82 Structure/function: The effects of RNA helicase activity on localization, regulation, and cell physiology

Chase S. Stevens, Denise S. Whitford, and George W. Owttrim

RNA helicases are central components of RNA metabolism in all three domains of life. Helicases are characterized by nine conserved amino acid domains and function as ATP-dependent motors, rearranging RNA secondary structure and altering gene expression. The model cyanobacterium Synechocystis sp. PCC 6803 encodes a single DEAD box helicase, CrhR, which localizes to the thylakoid membrane and whose expression is autoregulated at low temperature. The objective is to investigate the requirement of CrhR RNA helicase activity for these processes. Initially, I will determine the physiological effects of inactivating the ATP binding and hydrolysis motifs required for helicase activity. The effects of mutations in these motifs on CrhR autoregulation will be determined in both wild type and crhR mutant strains. The analysis will be extended to determine if RNA helicase activity is required for CrhR localization to the thylakoid membrane. Sucrose gradient ultracentrifugation of the CrhR mutants deficient in ATP metabolism will identify any localization defects. These projects aim to characterize which of the RNA helicase biochemical activities are required for autoregulation of expression and cellular localization of CrhR, and how disruption of these activities affects cellular physiology.

83 Differential Transcription in Subfertile Mouse Testes

Jennifer E. Just, Farshad H. Niri, Alaina N. Terpstra, Heather E. McDermid

Transcription regulated by chromatin remodelling is crucial for spermatogenesis and can lead to varying degrees of infertility if disrupted. One way chromatin remodellers can regulate transcription is by shifting nucleosomes to make promoter regions either more or less accessible to transcription machinery. In the McDermid lab, we study the gene Cecr2, which is part of the chromatin remodeling complex called CECR2- containing remodelling factor (CERF). A severe reduction of Cecr2 through mutation can result in the lethal neural tube defect exencephaly, equivalent to human anencephaly, causing perinatal death. However, only ~50-75% of these Cecr2 mutants show exencephaly, while mutant mice with normal neurulation survive to adulthood and show subfertility. Subfertility in males manifests as a decrease in litter size and a decrease in sperm count and motility. In the testis, SNF2H is a known member of the CERF complex. The overlapping chromatin binding sites of CECR2 and SNF2H were previously identified in testis through chromatin immunoprecipitation sequencing (ChIP-Seq).Using the ChIP-Seq identified binding sites in the promoter region of genes, we are looking for candidate genes that may be contributing to the subfertility in Cecr2 mutant mice. Candidate genes were selected based on the presence of known phenotypes seen in Cecr2 mutant mice: subfertility and excencephaly. Currently, I have extracted RNA from biologically matched wildtype and mutant testis samples and I have designed PCR primers for select candidate genes. I will perform qRT-PCR on these tissues to look for any transcriptional differences between wildtype and mutant testes, as would be expected if the candidate gene CERF binding sites identified through ChIP-Seq are functional. This will allow genes involved in spermatogenesis and subfertility to be further characterized to help us understand the role of Cecr2 in reproduction.

84 Transcriptomic analyses of responses of Pinus contorta to Grosmannia clavigera under contrasting levels of nitrogen availability

Louisa Normington, Chandra McAllister, Rhiannon Peery, Adriana Arango-Velez, Janice Cooke

The mountain pine beetle (Dendroctonus ponderosae, MPB), is a bark beetle that has been devastating North American forests since 1999. Lodgepole pine, Pinus contorta ssp. latifolia, has a range that overlaps extensively with that of MPB, and it is one of the main hosts of MPB. In recent years, MPB has spread from British Columbia across northern Alberta, killing more than one million hectares of lodgepole pine and lodgepole pine hybridized with jack pine (Pinus banksiana) in in Alberta to date. The microbial community hosted by the MPB seems to affect the ability of the beetle to overcome tree defenses and to successfully colonize the tree. This complex microbial community includes several species of pathogenic blue-stain fungi, including Grosmannia clavigera. Lodgepole pine have evolved both constitutive and inducible defense measures to repel or eliminate this pathogen chemically as well as physically. G. clavigera detoxifies defensive chemicals in tree resin which serves to pitch out or kill the MPB and provides a nutrient source for the beetles and their offspring. Lodgepole pines grown in nitrogen-enhanced soil show increased growth, though the effects of fertilization on fungal pathogen induced responses remains unclear. In this project, RNA-Seq transcriptomic analysis is being used to identify genes that are differentially expressed in Pinus contorta in response to Grosmannia clavigera in trees subjected to low and high amounts of soil nitrogen availability. The detection of differentially expressed defense-associated transcripts will be used to assess how nitrogen fertilization impacts the defense response. The identification of transcription factors is also a necessary step to understand the regulatory networks associated with pathogen defense processes in lodgepole pine, and data analysis will include correlation network analysis between transcription factors and differentially expressed defense related genes.

85 Bacteriophage DLP1 receptor identification for adherence to pathogenic Pseudomonas aeruginosa and Stenotrophomonas maltophilia strains

Jaclyn G. McCutcheon, Danielle L. Peters and Jonathan J. Dennis

The opportunistic bacterial pathogen Pseudomonas aeruginosa is a major cause of nosocomial infections, particularly in immunocompromised individuals, and is the second most prevalent pathogen found in the lungs of cystic fibrosis patients. The inability to control P. aeruginosa infections with antibiotics due to its intrinsic and adaptive multi-drug resistance and range of virulence factors is cause for concern. A possible alternative treatment is phage therapy, the clinical application of bacteriophages to selectively kill target bacteria. While bacteriophages typically have a very narrow host range, a novel phage DLP1 previously isolated in our lab is capable of infecting across taxonomic orders, lysing strains of both P. aeruginosa and Stenotrophomonas maltophilia, another highly antibiotic resistant bacterial pathogen. Characterization of the primary receptor for DLP1 and the mechanism of attachment for both host species may explain this unique ability. My preliminary screening of a 2,242 member P. aeruginosa PA01 mutant library suggests that DLP1 uses the type IV pilus, a virulence factor on the surface of the bacterium, as its primary receptor for adherence. Results show that mutants with transposon insertions in genes encoding pilus structural and regulatory components are resistant to DLP1 infection and complementation of disrupted pilin subunit genes restores DLP1 adherence and lysing ability. Visualization using electron microscopy also suggests that DLP1 interacts with pilus structures on the surface of PA01, however further analysis is needed. Future research will examine mutagenesis of pilin associated genes in the main S. maltophilia host strain, D1585, with the prediction that this will knockout DLP1's ability to adhere and lyse the cells. This research will further characterize DLP1 as a candidate for phage therapy in the treatment of highly antibiotic resistant bacteria from multiple genera.

86 Ecological Relevance of Ucrit: The Link Between Sustained Swimming Performance and Prey Capture and Predator Avoidance Behaviour in Juvenile Rainbow Trout (Onchorhynchus mykiss)

Kyle Dehaan, Keith Tierney

Swimming performance in fish is a key aspect of their fitness and relates to activities such as predator avoidance, prey-capture, migration, spawning, maintaining position in currents, and establishment of dominance hierarchies. There are three classifications of swimming in fish: sustained swimming, prolonged swimming, burst swimming. Ucrit is used as a measure of maximum sustained swimming prior to fatigue in fish, and has allowed researchers to compare the swimming speeds of different species. While this has been useful for comparisons, the link between Ucrit and ecologically relevant behaviours has not been well documented. By using intraperitoneal injections of carbamazepine (CBZ), chlorpyrifos (CHP), tributyltin (TBT), and pentachlorophenol (PCP), I intend to test if a decrease in Ucrit is associated with the prey capture ability and predator avoidance behaviour of juvenile rainbow trout (Onchorhynchus mykiss).

87 Comparing the toxicity of crude oil in fresh and saltwater systems: linking chemistry and gene expression

D. Lyons, D. Philibert, K. Tierney

Crude oil is highly toxic to fish during early life stages. As an exceptionally complex mixture, oil induces numerous abnormalities in developing fish including cardiac and morphological deformities. This study compares the molecular responses of saltwater and freshwater fish species (sheepshead minnow and zebrafish) to crude oil exposure. We exposed sheepshead minnow and zebrafish embryos to water accommodated fractions (WAFs) of crude oil, weathered crude oil, crude oil plus dispersant, and dispersant alone. We measured gene expression levels in both sheepshead minnow and zebrafish embryos after exposure, targeting genes involved in neurological and cardiac development as well as biotransformation. In our exposures, source oil WAFs had a higher concentration of polycyclic aromatic hydrocarbons (PAHs) than weathered oil WAFs. The combination of dispersant and oil vastly increased the amount of PAHs in the WAFs when compared to oil alone. This increase in PAHs occurred to a greater extent in saltwater than freshwater, indicating that oil and dispersant interact differently in these two aquatic environments. Due to the increase in PAHs, the oil plus dispersant WAF was more toxic to developing fish embryos than the other exposures. Though biotransformation gene expression levels were upregulated in both species in response to oil exposure, they were induced to different extents with zebrafish having a much higher expression level. The results of this study enable us to compare and contrast the consequences of oil spills in both freshwater and saltwater as well as demonstrate the implications of using a chemical dispersant in these environments. We were also able to help establish whether zebrafish, a common model species, are valid models for studying marine oil spills.

88 AGBE regulates iron homeostasis via IRP1A in Drosophila larval prothoracic gland

Nhan Huynh, Kirst King-Jones

Iron homeostasis and heme synthesis is strictly regulated at tissues where iron is being constantly required. In Drosophila, ecdysone is the primary steroid hormone involve in metabolism, molting and growth regulation. Its synthesis relies on cytochrome P450 enzymes, those require heme as a cofactor for normal functions. These enzymes are highly expressed in larval prothoracic gland of Drosophila, the novel tissue for ecdysteroidogenesis. As a result, iron metabolism and heme production in this tissue has to be strictly regulated. Our previous screening has identified a list of 34 candidates might participate in this regulation. At the center of this proposal is our study on the roles of AGBE in relationship with IRP1A. Preliminary data suggest potential roles of these genes in iron homeostasis and metabolism for which we aim to find out through this proposal.

89 Endogenous and exogenous factors influencing Dendroctonous ponderosae dispersal

Kelsey Jones and Maya Evenden

The mountain pine beetle (Dendroctonus ponderosae) is a well-known forest pest insect in western North America. Unlike many other bark beetle species, the mountain pine beetle will attack and kill healthy trees within the genus Pinus (pine species). In the most recent outbreak that started in the early 2000s, they have killed over 18 million hectares of pine in British Columbia, and have begun to expand their range towards North-Central Alberta. The mountain pine beetle has begun attacking the naive pine trees of Alberta and threatens expansion into the boreal forests. Although the mountain pine beetle has been of great interest in the last few decades, much is still unknown regarding the beetle's dispersal capabilities. My research assesses the endogenous and exogenous factors that influence dispersal of the mountain pine beetle at the leading edge of infestation. I will assess how host and non-host tree chemical cues influence mountain pine beetle behaviour during the pre-flight and dispersal phases. I will further test if flight is required for response to semiochemical cues from host trees and conspecific beetles. This research will fill gaps in our knowledge about the flight and orientation behaviour of the mountain pine beetle during its obligatory dispersal phase to find a new host. My research, paired with other research being conducted at the University of Alberta can help further quantify the mountain pine beetle spread risk into the boreal forest.

90 The first oviraptorosaur (Dinosauria: Theropoda) bonebed: evidence of gregarious behaviour in a maniraptoran theropod

Gregory F. Funston, Philip J. Currie, David A. Eberth, Michael J. Ryan, Tsogtbaatar Chinzorig, Demchig Badamgarav & Nicholas R. Longrich

A monodominant bonebed of Avimimus from the Nemegt Formation of Mongolia is the first oviraptorosaur bonebed described and the only recorded maniraptoran bonebed from the Late Cretaceous. Cranial elements recovered from the bonebed provide insights on the anatomy of the facial region, which was formerly unknown in Avimimus. Both adult and subadult material was recovered from the bonebed, but small juveniles are underrepresented. The taphonomic and sedimentological evidence suggests that the Avimimus bonebed represents a perimortem gregarious assemblage. The near absence of juveniles in the bonebed may be evidence of a transient age-segregated herd or 'flock', but the behaviour responsible for this assemblage is unclear. Regardless, the Avimimus bonebed is the first evidence of gregarious behaviour in oviraptorosaurs, and highlights a potential trend of increasing gregariousness in dinosaurs towards the end of the Mesozoic.

91 Investigating the function of redundant cone-rod homeobox-like genes and their role in photoreceptor development and maintenance using an in vivo zebrafish model

R Rosca, WT Allison

Severe vision loss affects life for thousands of Canadians. Our lab, in part, aims to study vision loss-related diseases that are linked to inherited genetic mutations using in vivo zebrafish modeling. Zebrafish are ideal candidates for their heavily cone-populated retinas, similar to the human fovea, and regenerative capabilities. One gene we aim to study, cone-rod homeobox (crx), is normally involved in photoreceptor differentiation and growth. These specialized cells are necessary for nighttime vision (rods) or colour vision (cones). crx mutations result in photoreceptor degeneration in humans, causing diseases like retinitis pigmentosa. Orthodenticle homeobox 2 (otx2) and otx5 are genes proposed to have similar functions as crx, partly due to the high level of amino acid conservation within zebrafish and between zebrafish and human lineages. These genes are being investigated alongside zebrafish crx to better understand photoreceptor development and maintenance. Development will be determined by characterizing photoreceptor abundances resulting from mutations using fluorescent tools. In the future, we hope to compare phenotypes from different mutations to elucidate overlapping functions between these genes (functional redundancy). Photoreceptor maintenance will also be explored using optical coherence tomography (OCT), a technique used to image human retinas for diagnostic purposes. This tool will be utilized to monitor in vivo crx-mutant zebrafish retinas, alongside quantified otx2 and otx5 mRNA expression. Data collected from OCT work will demonstrate whether gene functional redundancy exists, further contributing to the photoreceptor development and/or maintenance gene regulatory network(s). Ultimately, in order to establish clinical interventions of degenerative blinding disorders, understanding the mechanisms involved through photoreceptor development and maintenance is needed.

92 Spinules as a Source of Synaptic Plasticity Following Cone Photoreceptor Ablation

Quinton D. Schmidt, W. Ted Allison

Vertebrate photoreceptors receive feedback and integrate signals from adjacent photoreceptors via the horizontal cells. In teleost fish this process is modified via spinules, labile membranous extremities of horizontal cells that project into photoreceptor pedicles. Spinules are known to respond to light conditions by fluctuating their abundance and thereby modifying the interaction of horizontal cells with their respective associations. Thus spinules are an intriguing hypothetical source of plasticity. Here, spinules in zebrafish were quantified in relation to a recently observed and unexpectedly rapid recovery of vision following pharmacogenetic ablation of certain cone photoreceptor subtypes. Upon ablation of UV cones ~25% of the remaining cone pedicles were observed to experience an abundance of spinules (4-fold increase in spinules when UV cones ablated vs. wildtype; $p<0.022$; $n=47$ or 20 pedicles, respectively, from multiple fish), whereas ablation of blue cones led to ~50% of remaining pedicles with an abundance of spinules (5-fold increase, $p<0.001$; $n=79$ or 49 pedicles, respectively). Spinules were 57% more abundant within the remaining cone pedicles following blue cone ablation relative to UV cone ablation ($p=0.0085$). This pattern of increased spinule abundance is coincident with significantly greater recovery of visual function after blue cone ablation that was observed previously. Though a causal link between spinules and recovery of vision remains to be formally demonstrated, this data uniquely supports a role for spinules in retinal plasticity. The data also tentatively suggest a morphological source of plasticity that may enable the unexpectedly rapid recovery of vision following ablation of blue cones in zebrafish, though other hypothetical mechanisms of plasticity remain untested.

93 Exploring SOD1 and TDP43 association in ALS as modeled in Danio rerio

Natalie Snyder, Michèle DuVal, Ted Allison

Amyotrophic Lateral Sclerosis (ALS) is a neuromuscular degeneration commonly characterized by protein misfolding, aggregation, and toxicity of superoxide dismutase encoded by SOD1, with Tar DNA Binding Protein (TDP43) thought to play a role in influencing misfolding of the SOD1. The mechanism in which these two proteins interact is unknown, although an association between the tryptophan (Trp) residues between the two proteins is hypothesized to play a key role in this interaction. Therefore we will be comparing various treatment groups to observe whether swapping residues from Trp will reduce SOD1 toxicity. Building upon many experimental breakthroughs in ALS completed in cell culture, this study aims to produce a zebrafish model for ALS by injecting SOD1 and TDP43 mRNA into embryos, and measuring effects on zebrafish motor neurons, as well as motor output via behavioural assays. By quantifying abnormal branching in motor neurons (axonopathy) and comparing motor abilities of wild-type to injected treatment groups using touch-evoked escape response, it will be possible to observe deficiencies as well as abnormalities in a zebrafish model. Preliminary data aims to display increases in axonopathy in zebrafish injected with wild-type SOD1 and TDP43. These results are expected to illustrate the hypothesized enhancement of axonopathy when both proteins are present in comparison to their presence individually, with results pending on the effect wild-type SOD1 has on motor abilities in this model. These results aim to support and build upon cell culture studies, in order to display whether results including axonopathy and the direct effect on muscle output allow for an accessible animal model of ALS, such as zebrafish.

94 Exploring genetic background effects on regeneration and revertant fibre expansion in Duchenne muscular dystrophy mouse models

Kenji Rowel Q. Lim, Merryl Rodrigues, Yusuke Echigoya, Rika Maruyama, So-ichiro Fukada, Toshifumi Yokota

Duchenne muscular dystrophy (DMD) is a fatal X-linked recessive disorder characterized by progressive muscle weakness. DMD is caused by out-of-frame mutations in the DMD gene coding for dystrophin. Surprisingly, numerous studies have observed sporadic dystrophin-positive fibres in DMD patients and animal models. Called revertant fibres (RFs), these are thought to expand due to satellite cell (i.e. muscle stem cell) activity during muscle regeneration. Among other factors, genetic background is considered highly influential in RF formation. This study aims to establish the effect of genetic background on muscle regeneration and RF expansion. Two DMD mouse models with the same mutation type (mdx) but different strains, C57BL/6 (mdx-B6) or DBA/2 (mdx-DBA) were examined. We found that mdx-DBA muscle had significantly decreased centrally nucleated fibre counts, and little to no developmental myosin heavy chain-positive fibres, compared to mdx-B6 at 2-18 months of age; they represent cumulative and current regeneration, respectively. Also, mdx-DBA muscle had fewer RFs and RF clusters than mdx-B6, a difference becoming more significant with age. Overall, we show that genetic background significantly affects regeneration and, thus, RF expansion. Our study further suggests that assessment of DMD therapeutics be done using mdx-DBA, as fewer RFs are present to confound efficacy results.

95 Posterior lateral line phenotype of zebrafish with prion protein mutations

Jordyn Ko, Niall Pollock, W. Ted Allison

Cellular prion protein (PrPC) has been implicated to be involved in the development of various neurodegenerative diseases, for instance, Alzheimer's disease in humans. Little is known about the normal function of PrPC because knockout mice do not present major phenotypic differences from wild-type mice. In this experiment, zebrafish were used as test organisms because they are widely studied and their genetics are comparable to humans'. We sought to observe a phenotypic difference in the development of the posterior lateral line (PLL) of wild-type zebrafish compared to prp1ua5003/ua5003; prp2ua5001/ua5001 compound homozygous mutant zebrafish. Fish expressing GFP in the PLL (under the claudin-b promoter) were bred to three days post fertilization then fixed in 4% PFA. Images of mutant and wild-type fish were taken using fluorescent microscopy, and the width of the PLL was measured in 3 locations between the first and fourth neuromasts using Image J. The wild-type fish population (n=20) had a mean PLL width of 5.45 ± 1.50 pixels. The mutant fish population (n=12) had a mean PLL width of 10.38 ± 3.90. The majority of mutant fish were heterozygous for one prion protein and homozygous for the mutation in the other protein. The width of the PLL in mutant zebrafish appears to be greater than that of the wild-type fish, suggesting that the loss of function of these prion proteins results in a decreased amount of cell-to-cell adhesion in the developing PLL. More fish are currently being genotyped to raise the sample size to the point where a difference can be confirmed. Rescue experiments will then be performed to see if the mutant phenotype can revert back to the wild-type phenotype when prp1 and prp2 mRNA is injected into fertilized zebrafish embryos that are suspected to be mutant zebrafish.

96 Alzheimer's Modeling in Zebrafish

Hadeel Alyenbaawi, and W. Ted Allison

Background: Alzheimer's disease (AD) is one of the most prevalent forms of dementia affecting aging populations. It is characterized by two neuropathological hallmarks: senile plaques composed of A1-42 peptides and/or oligomers, which originate from APP processing, and neurofibrillary tangles (NFTs) that consist of abnormally phosphorylated microtubule-assembly tau protein. Due to the important role of APP in AD pathogenesis, various animal models expressing APP with familial AD mutations have been generated. Despite the valuable information these models provided, the cause and mechanisms leading to neurodegeneration are unknown. Thus, there is a need for new models that could provide complementary advantages in terms of investigating the molecular mechanism of the disease as well as screening for potential therapeutics. Zebrafish are transparent, amendable to genetic manipulation and have intact CNS, which make them ideal to model AD. In this study, our main aim is to work towards creating an AD model in zebrafish using the CRISPR/Cas9 system. Methods & Results: CRISPR was used to target the appb gene, one of the zebrafish's paralogues of human APP, and was injected into one-cell embryos of appa ua5005/ua5005 knockout fish, along with a template for homology-directed repair to humanize the zebrafish gene. In order to validate and enrich for successful CRISPR, we co-injected the gRNA target tyrosinase gene, which is associated with the albino phenotype. Preliminary evidence suggests germline transmission of mutant appb has been achieved. Additionally, we designed CRISPR targeting mapta and maptb, the zebrafish paralogues of human MAPT, to gain a deeper understanding of the physiological functions of MAPT. Our data shows that most of the CRISPR we designed are working efficiently. Conclusion: Engineering of the humanized appb and mapt knockout model will not only shed light on the molecular mechanisms of AD, it will also provide valuable models for drug screening.

97 Phylogenies of mosasauroid reptiles inferred under multiple phylogenetic methods

Oksana Vernygora, Tiago Simões, Ilaria Paparella, Paulina Jimenez-Huidobro, Michael W. Caldwell

Mosasauroids is a clade of secondarily aquatic reptiles. However, it is still uncertain whether mosasauroids acquired their aquatic adaptations only once in their evolutionary history, or multiple times. The most recent phylogenetic analyses have given greater support for multiple origins of fully aquatic mosasauroids. However, mosasauroid relationships have only been tested so far using traditional (unweighted) maximum parsimony (TMP) search methods. Maximum likelihood (ML) and Bayesian estimates provide complementary approaches to this problem, which are able to test (unlike TMP) for the effects of branch lengths and varying evolutionary rates. Here we present the first analysis of mosasauroid reptiles using multiple phylogenetic methods, including ML, Bayesian inference, and implied weighting parsimony (IWMP), in addition to TMP using contingent and multistate coding schemes. Contingent TMP recovers a monophyletic Mosasaurinae, with halisaurines and Dallasaurus at their stem, as well as a monophyletic Russellosaurinae. Multistate TMP finds halisaurines in a politomy with Mosasaurinae and Russellosaurinae. IWMP recovers a very similar overall topology to both TMP analyses, but with halisaurines falling as the most basal member of Russellosaurinae, instead. Differently from studies with dinosaurs, in which likelihood based methods have more similar results to IWMP, ML obtained more similar result to contingent TMP: halisaurines and Dallasaurus at the stem of Mosasaurinae. Finally, the Bayesian estimate deviates the most from all other results: halisaurines are the sister taxon to all other mosasauroids; Russellosaurinae is not monophyletic; and Clidastes forms a monophyletic group with Globidens. The overall conclusion is the uncertainty in the position of halisaurines, and Dallasaurus as a mosasaurid, instead of nesting with other not fully aquatic mosasauroids (aigialosaurids). This provides greater support for either a multiple independent invasion of the aquatic realm by mosasaurs, or that Dallasaurus may represent a lineage that lost aquatically adapted features during the early evolution of mosasaurids.

98 Omics-based Bioactives and Drug Discovery in Bacillus

Albert Remus R. Rosana, Mohamed Seghir Daas, Jeella Zarah Acedo, Farida Nateche, Salima Kebbouche-Gana John Vederas and Rebecca J. Case

Several strains of Bacillus spp. were isolated from across Algeria, the largest African country. The strains were bioprospected from several economically-relevant environments such as oil-mining sites, salty lakes, coal mines, deserts and oil-contaminated water basins. Hundreds of isolates were extensively screened for extracellular enzyme and lipid-emulsifying properties and pathogen-inhibition activities. Potent strains were further subjected to both genomics and proteomics-metabolomics based-pipelines for gene-cluster and bioactive peptide discovery, respectively. Whole genome shotgun sequencing revealed new strains of Bacillus spp. namely: the insecticidal B. thuringensis; the biosurfactant-producing B. amyloliquefaciens; the bacteriocin-producing B. paralicheniformis; the food borne-pathogen B. cereus and the soil-borne pathogen B. anthacis. Cluster gene analyses revealed an extensive repertoire of secondary metabolite families encoded in the genome of these new Bacillus strains such as bacteriocins, terpenes, lassopeptides, lipopeptides, homoserine lactones, and siderophores. These bioinformatically predicted gene inventories were coupled with metabolomics platforms to confirm a number of biotechnologically relevant molecules. Nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography-mass spectrometry (LC-MS) identified several potent bioactives such as fengycins, lantipeptides and microcins with strong bactericidal activity against common food borne-pathogens such as E. coli, Salmonella and S. aureus. Here, we will present the direct biotechnological application of genomics and targeted metabolomics in microbial bioactive predictions and drug discovery.

99 Characterization of GnRH (Gonadotropin Releasing Hormone) Signaling in Immune Cell Types

Enezi Khalid, Greg Goss, James Stafford, and John Chang

GnRH (gonadotropin releasing hormone) is a highly conserved hypothalamic decapeptide classically involved in the hypothalamic-pituitary-gonadal axis, regulating the release of gonadotropins, as well as growth hormone, from the pituitary into systemic circulation, whereby these hormones are involved in a variety of biological processes including, but not limited to, growth, reproduction, and sexual maturity. It’s neuroendocrine actions have been well described in a variety of vertebrate and invertebrate species. In addition to these classical roles, GnRH has also been implicated in directly playing a part in immune function in extra-pituitary sites, possibly in an auto/para -crine fashion. In both mammals and fishes, as shown in several in vitro as well as in vivo studies, GnRH is capable of modulating the processes of phagocytosis, cytokine production, chemotaxis, and degranulation. However, relatively little is known about the intracellular signaling mechanisms by which GnRH brings about its actions in immune contexts. Using rodent basophilic and macrophage cell lines as well as various goldfish primary immune cells, my project aims to elucidate GnRH signalling in immune cell types, identify the specific GnRH-receptor isoforms involved, and examine the conservation in GnRH signaling between immune and endocrine contexts.

100 AVIAN INFLUENZA IMMUNE EVASION – SPECIES-SPECIFIC PROTEIN-PROTEIN INTERACTIONS IN HUMAN AND DUCK HOSTS.

Danyel Evseev, Robert G. Webster and Katharine E. Magor

"The ability of influenza viruses to block innate immune responses during the early stages of infection is crucial to its success in a host. The influenza non-structural protein 1 (NS1) is the main viral antagonist to innate immunity within host cells. In humans NS1 blocks the RIG-I signaling pathway for viral detection, by interacting with an essential co-activator – tripartite motif-containing protein 25 (TRIM25). I am investigating whether NS1 interacts with duck TRIM25, because ducks are the reservoir hosts of influenza and mount robust innate immune responses to highly-pathogenic flu strains that kill chickens and humans. I compare the ability of NS1 proteins from several influenza strains to interact with the divergent human and duck TRIM25, and show that different NS1 proteins interact with the human and the duck orthologue, despite similar subcellular distribution patterns. Notably, NS1 proteins from a fatal human influenza isolate and from a closely-related avian isolate have different binding affinities for human TRIM25. Work is ongoing to test interactions with other RIG-I pathway components, to determine by mutagenesis the critical amino acid residues in NS1 that determine these interactions, and to perform in vitro infections with recombinant viruses. Knowing the sequence features of NS1 that contribute to human virulence is important for global surveillance and disease control. Comparing this to the function of NS1 in ducks will expand our understanding of the changes that occur when influenza jumps the species barrier.

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101 Investigating the role of the melanoma antigen (MAGE) protein MAGEL2 in circadian rhythm

Mercedes Zoeteman, Dr. Rachel Wevrick and Dr. Heather McDermid

Background: Prader-Willi Syndrome is a complex genetic disorder characterized by neonatal hypotonia, endocrine dysfunction, intellectual disability, hyperphagia, and deficits in sleep and circadian rhythm. Circadian rhythm is a process that is regulated within the hypothalamus, and functions as an endogenous 24-hour cycle that regulates physiology and behaviour. On a molecular level, circadian rhythm is tightly regulated by a complex transcription-translation feedback loop and post-translational modifications (PTM's) such as ubiquitination. Previous research has indicated that MAGEL2 is highly expressed in the suprachiasmatic nucleus of the hypothalamus in a circadian rhythm expression profile. Additionally, mice lacking Magel2 have circadian rhythm deficits in the absence of external light cues, suggesting Magel2 has a role in circadian rhythm. MAGEL2 is a member of the MAGE family of proteins, which interact with RING E3 ligases involved in ubiquitination, however the specific function of MAGEL2 in circadian rhythm has yet to be elucidated. Methods: Cellular-based assays such as the BioID system and abundance assays were used to determine if MAGEL2 interacts with or modifies the abundance of proteins involved in circadian rhythm. Results: My analysis suggests that MAGEL2 interacts with the E3 ubiquitin ligase RBX1 via the MAGE Homology Domain. MAGEL2 proximally interacts with the cryptochrome proteins (CRY1 and CRY2) involved in circadian rhythm. Furthermore, the presence of MAGEL2 destabilizes both RBX1 and CRY1. Conclusion: This data suggests a link between MAGEL2 and circadian rhythm, however further studies are needed to determine how the inactivation of MAGEL2 results in circadian rhythm dysfunction.