

DEPARTMENT OF NATURAL SCIENCES [Biology & Chemistry]

SARA ANDERSON – Biology

Rescuing a molecular motor in dilute mice

Myosin Va is a molecular motor. A mutation within a myosin Va causes the *dilute* phenotype in mice and Griscelli's syndrome in humans. Myosin Va and *dilute* melanocytes were used for this study. Transfections were done to test whether or not the phenotype of a *dilute* melanocyte could be restored by a chimeric myosin Va. The main goal of this research was to create a chimeric myosin Va, with portions of both myosin Va and Vb. This chimeric myosin would then be transfected into *dilute* melanocytes to see if normal distribution

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of the melanosomes was possible. Another goal of the research was to test an old construct of a chimeric myosin. This construct was transfected into *dilute* melanocytes. Distribution of the melanosomes within the cell was monitored via immunofluorescence.

ANISHA M. BOETEL – Biology

Using RNA Secondary Structure as an Aid to Aligning ITS2 rDNA Sequences in Phylogenetic Analysis

In order to determine whether using RNA secondary structure as an aid to DNA sequence alignment makes highly variable ITS2 rDNA sequences suitable for phylogenetic analyses at familial and ordinal taxonomic levels, I tested the hypothesis that the freshwater sponge, *Potamolepis* sp., is more closely related to marine sponges belonging to the order Hadromerida than to other freshwater sponges, belonging to the order Haplosclerida. Secondary structural models of ITS2 rRNA were generated for seven hadromerid species, 10 haplosclerid species and *Potamolepis* sp. These structures were then combined with the primary sequence data to produce an alignment that was used for phylogenetic analysis. The results of the analysis indicate that (1) *Potamolepis* sp. should be grouped with the freshwater sponges and (2) when secondary structural information is considered, ITS2 rDNA alignments are improved and can be used for analyses at the familial and ordinal levels.

JILLIAN BYBEE – Biology

Detection of Sporadic Genetic Mutations Using an EndoV/Ligase Scanning Assay

The identification of sporadic point mutations in tumor-suppressor genes, which are responsible for regulating cell growth, has been linked to the early detection of cancer, as well as with identifying those individuals with a genetic predisposition to developing cancer. My research investigates the use of a single-step endonuclease V/ligase scanning assay in the detection of unknown point mutations in genomic DNA. Specifically, the research focuses on the K-Ras gene, which has been linked to several different types of cancer, including colorectal cancer. A universal PCR strategy using fluorescently labeled universal primers and unlabeled gene specific primers allowed for amplification of K-Ras oncogene sequences. Amplification was followed by heteroduplex generation from the universal PCR products. This procedure resulted in products with one of two mismatches (A/C or G/T) that could be used for endonuclease treatment. Endonuclease V recognizes and cleaves base pair mismatches within the gene sequence, but it also cleaves some correctly paired bases. A highly specific ligase was used to reseat these misleaves, thus increasing signal intensity. Capillary gel electrophoresis was employed to distinguish the single-stranded products of the EndoV/ligase assay based on fragment-size differences. The early detection and identification of mutations could lead to increased survival rates for individuals testing positive for those mutations. Further research should include the potential to transfer the assay to a microelectrophoretic device with possible clinical applications.

TAMERA CALDWELL – Biology

Alleviation of the negative effects of restraint stress with estrogen and tamoxifen on cognitive learning and memory retention in female rats

Selective estrogen receptor modulators (SERMs) either mimic or block the effects of estrogen (Goldstein, 1998). Tamoxifen, a SERM, blocks the effects of estrogen in breast tissue (Walling, 2005), but its effects in other tissues, specifically in the brain, are poorly understood. Tamoxifen may reproduce the effect of estradiol in the brain (Le Saux *et al.*, 2005) or it may inhibit memory (Jenkins *et al.*, 2004). This study used ovariectomized rats injected with pharmacological doses of estrogen or tamoxifen. Cognitive learning and retention were evaluated based on performance in the Morris water maze. The hypothesis was that tamoxifen would simulate the effects of estrogen in the brain, resulting in cognitive learning and retention similar to estrogen-injected rats but different from the control group. While no statistically significant results were noted in the retention protocol, a statistically significant difference between the tamoxifen and the control groups on days four and five in the learning protocol was observed. However, when comparing average learning times, the results suggested that tamoxifen does not mimic the effects of estrogen in the brain.

KRISTEN CHRISTENSEN – Biology

Biogeographical Origins of Hawaiian *Diplopterygium pinnatum* by Long Distance Wind Dispersal

This study was designed to investigate a possible biogeographical origin and mechanism of dispersal of the Hawaiian fern *Diplopterygium pinnatum*. Molecular phylogenetic analyses were conducted on *trnL-F* and *rbcl*, cpDNA regions, for seven species of the genus *Diplopterygium* and three outgroups. Maximum parsimony and maximum likelihood analyses were used for phylogenetic reconstruction. Three hypotheses were tested: that the ancestor of *D. pinnatum* originated from 1) an Indo-Pacific source and traveled to Hawaii via the jet stream, 2) an American source and traveled to Hawaii via the trade winds, or 3) an Austral source and traveled to Hawaii by a combination of a ITCZ shift, Hadley cell air movement, and the trade winds. The two methods of reconstruction, maximum parsimony and maximum likelihood, produced similar trees with similar support. The results suggest an ancestor originating from an Indo-Pacific source or an Austral source.

DANIELLE DAEHNKE - Biopsychology (MDM)

Screening for antibiotic, antifungal and antitumoral compounds in natural products extracted from freshwater sponges

The secondary metabolites of marine sponges include many bioactive products (e.g., Ara-C) that have found uses in the health field. Even though freshwater sponges share many characteristics with their marine cousins, little is known about their secondary metabolites. This study looks at the effects of the secondary metabolites extracted from three freshwater sponge species on the survival of bacteria, fungi and brine shrimp. It found that none of the extracts were toxic to the bacteria or fungi. Preliminary results did, however, show that

extracts of all three species were active against brine shrimp. This study suggests that the secondary metabolites of freshwater sponges should be tested more extensively to see if they contain useful bioactive compounds.

ANNABELLE C. KLEIST – Biology

Molecular phylogenetics and biogeography of Hawaiian members of the pteridophyte genus *Cibotium* (Dicksoniaceae)

Although ferns comprise a significant proportion of Hawaii's native vascular-plant flora, few phylogenetic studies have been performed to infer relationships among taxa and to deduce their biogeographical origins. Species from the pteridophyte genus *Cibotium* can be found among the canopy in the Island's montane regions and were the focus of this study. Four species of *Cibotium* are endemic to the Hawaiian Archipelago and ten species exist worldwide. The goal of this study was to determine whether Hawaiian *Cibotium* is monophyletic and to discover the biogeographical origins of the species. Two chloroplast DNA fragments, *ttnL-F* IGS and *rbcl*, were sequenced and maximum parsimony, maximum likelihood, and neighbor joining analyses were performed. These analyses support a South American origin for a monophyletic Hawaiian *Cibotium*. This supports the hypothesis that the ancestor of Hawaiian *Cibotium* colonized Hawaii by wind dispersal of spores via a combination of the ICTZ, Hadley Cells, and trade winds.

ALLIE MAFFIT – Biology/Chemistry

Identification of Key Binding Partners of Elongin C

Cancer is the second leading cause of death in the United States. Prostate cancer affects one in seven men in the United States. Unfortunately, not much is known as to how normal prostate cells are transformed into a cancerous state, or how these cells remain in the cancerous state. A study performed by Parkka *et al* in 2002 revealed that Elongin C is over expressed in prostate cancer cell lines. I hypothesize that the over expression of Elongin C could inappropriately stabilize or destabilize key proteins involved in cell cycle regulation leading to transformation and or proliferation of cancerous cells. Through the use of a two-hybrid screening process four proteins within the prostate cell library were found to interact with Elongin C.

BEN MATELICH – Chemistry

Synthesis of $(\text{Bi}_2\text{Te}_3)_x(\text{Sb}_2\text{Te}_3)_y$ and $(\text{TiTe}_2)_x(\text{Sb}_2\text{Te}_3)_y(\text{TiTe}_2)_x(\text{Bi}_2\text{Te}_3)_z$ Superlattices: An Investigation into Thermal Conductivity Dependence on Superlattice Period

There is disagreement concerning the effect superlattice period has on thermal conductivity. Accordingly, $\text{Bi}_2\text{Te}_3/\text{Sb}_2\text{Te}_3$ and $\text{Bi}_2\text{Te}_3/\text{TiTe}_2/\text{Sb}_2\text{Te}_3$ superlattices were synthesized to determine whether differing thicknesses of the layering structure affected thermal conductivity significantly. In the $\text{Bi}_2\text{Te}_3/\text{Sb}_2\text{Te}_3$ superlattice, the two components were found to interdiffuse slightly upon annealing rather than crystallizing ideally. Consequently, the research shifted to include a TiTe_2 barrier between Bi_2Te_3 and Sb_2Te_3 . Calibration of the new system resulted in more ideal crystalline structure following annealing. Once fully calibrated, this titanium barrier system could be subjected to thermal conductivity analysis.

BRIAN MOREAUX – Biology

Prion infection of the nasal cavity: site for agent shedding?

The mechanism of prion shedding in prion disease is currently unknown. The purpose of this study was to determine if the nasal cavity is a site of prion infection. Hamsters were intracerebrally inoculated with the HY strain of transmissible mink encephalopathy agent. Once clinically ill, the hamsters were euthanized and their skulls were removed and examined for prion infection using immunohistochemical and dual immunofluorescence staining techniques. In infected hamsters, PrP^{Sc} was shown to be present in the olfactory receptor neurons of the olfactory sensory epithelium (OSE) as well as in the nasal-associated lymphoid tissue and the vomeronasal receptor neurons in the vomeronasal sensory epithelium (VSE) of the vomeronasal organ. PrP^{Sc} infection was specific to sensory and not to non-sensory epithelium in both the OSE and the VSE.

CHRISTINE LAUREN NELSON – Biology

Biogeographical origins and phylogeny of the endemic Hawaiian species of the pteridophyte genus *Cibotium* (Dicksoniaceae): a molecular perspective based on atpB sequences

A great deal of speculation exists as to the biogeographical origins and phylogeny of endemic Hawaiian ferns; yet only a handful of studies has actually examined the evolution of these endemics and their relationships to continental species. The endemic fern genus *Cibotium* (Dicksoniaceae), consisting of four Hawaiian endemics and five non-Hawaiian species, is the focus of this study. The goals of my research were to resolve whether Hawaiian *Cibotium* is monophyletic and to test the hypothesis that the common ancestor of Hawaiian *Cibotium* originated from the Indo-Pacific region. Although the long-distance dispersal mechanism of fern spores to Hawaii was not directly investigated; a pathway can be inferred based on the geographical location of the colonizing ancestor. I sequenced the chloroplast DNA fragment *atpB* for eighteen taxa and performed phylogenetic analyses using both maximum parsimony and maximum likelihood. These analyses strongly indicate that Hawaiian *Cibotium* is monophyletic and suggest that the colonizing ancestor is of South American origin. From this we may infer that the ancestral spores arrived on Hawaii via the combined effects of Hadley cells, trade winds, and seasonal shifts in the inter-tropical convergence zone (ITCZ).

SARA O'CONNELL – Biology

Damaging Capacity of Five Metal Cations on Escherichia coli Plasmid DNA

The toxic effects of five metal cations, frequently in contact with humans, have been analyzed in this study. Anthropogenic activities, such as mining, cause elevated levels of cobalt in soil and water. Other common environmental pollutants found in mining effluent include the heavy metals: copper, iron, and manganese. Stannous chloride (SnCl_2) is widely used in nuclear medicine as a reducing agent for products radiolabeled with technetium-99m. The ability of these five heavy metals to mediate a Fenton-like reaction was observed through

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the alteration of plasmid topology. Treatment of *Escherichia coli* plasmid DNA with CuCl_2 , SnCl_2 , and FeCl_2 resulted in DNA single-strand breaks in a dose-dependent manner. Sodium benzoate, a free radical scavenger, and EDTA, a metal ion chelator, both inhibit the DNA damage. The data indicate a correlation between the formation of reactive oxygen species and DNA damage. Also, the presence of a slow-migrating band after treatment with copper, tin, and iron suggests these metal cations produce an additional plasmid topology.

ELISE PLETNIKOFF – Biology

Sexually Dimorphic Effects of Prenatally Applied Fluoxetine (Prozac) on Behavior and Cognition in Rats

The safety of prenatally applying fluoxetine (Prozac), one of the antidepressant drugs currently most commonly prescribed to pregnant women, has been inadequately studied. The object of this study was to determine gender differences in behavioral and cognitive effects in rats prenatally exposed to fluoxetine. Differences were investigated across the following four groups: females exposed to Prozac, males exposed to Prozac, females unexposed, males unexposed. Because males are commonly more acutely affected by prenatal drug exposure than females, it was hypothesized that fluoxetine would more noticeably affect behavior and cognition in prenatally exposed males than in females. Cognitive performance of rats that had been prenatally exposed to fluoxetine showed no significant differences from rats that had not been prenatally exposed to fluoxetine. Rats that had been prenatally exposed to fluoxetine were found, in three of seven behavioral tests, to respond differently to the stress of a predator's scent than rats unexposed to fluoxetine. This may indicate heightened awareness to presence of danger in rats prenatally exposed to fluoxetine. In this research, male rats prenatally exposed to fluoxetine were not found to respond to the presence of a cat towel in the same way that female rats prenatally exposed to fluoxetine respond to the presence of a cat towel. This pilot study indicates that prenatally applied fluoxetine affects some behaviors in rats differentially according to gender.

DEVEN ROBINSON - Biology

AAV Mediated Alpha CaM-Kinase II Suppression and Up Regulation: Behavioral and Western Blot Analysis

The goal of the present study was to both inhibit the α CaM-Kinase II (α CaM-K II) enzyme, and to over-express it in neurons in order to observe if the enzyme has a role to play in memory as determined by behavioral and learning tests. An adeno-associated virus (AAV) with either sense or anti-sense RNA to the α CaM-K II protein was designed for the over expression and suppression of the enzyme, respectively. Morris-water and open field mazes were used to test for the effects of the differences in protein concentration on memory and behavior. An increase in protein in the rats receiving sense RNA, as apparent in the Western Blots, correlated with significantly greater probe trial times when compared to the controls, as expected if α CaM-K II protein was involved in the establishment of memory. Concentrations of α CaM-K II protein in the hippocampi were determined 29 days after surgery using Western Blots. The concentrations of α CaM-K II protein in the rats receiving sense RNA ($n = 6$) were statistically larger ($p < 0.05$) compared to the controls ($n = 5$). There were no statistical

differences seen between the rats with the anti-sense RNA and the control rats in the behavioral and learning tests. This correlated with the lack of difference in the concentrations of the enzyme in the hippocampi of the rats receiving the anti-sense RNA and controls.

KATHRYN STYREN – Biology

A Cytogenetic Analysis of the *Simulium arcticum* complex at the Little Blackfoot River, Powell County, Montana

This two-year study of the chromosome diversity within the *Simulium arcticum* complex within the Little Blackfoot River Drainage addressed three objectives: 1) determination of the distribution and frequency of *S. arcticum* s.s., *S. brevicercum*, *S. apricarium*, and *S. arcticum* III-18 at high, intermediate, and low elevation sites; 2) investigation of the reproductive status of these taxa at the Elliston; and 3) investigation of sibling-specific criteria for *S. brevicercum* to determine the status of III-st/st males at Elliston.

Regarding the first objective, the null hypothesis would suggest that these siblings would be distributed evenly in the drainage. However, it was observed that *S. arcticum* s.s. occurred in abundance at the high and intermediate locations whereas *S. apricarium* was only found at the low elevation site. *S. brevicercum* and III-18 were abundant at the intermediate location. Based on this, the null hypothesis was rejected. Elevation seemed to influence the distribution of these siblings.

I was unable to address the second objective because the low frequency of autosomal polymorphisms at Elliston precluded determination of the reproductive status of III-18, *S. arcticum* s.s. and III-st/st males.

For the third objective, the criteria for identification of *S. brevicercum* were compared among standard males, *S. arcticum* s.s., and III-18 males to determine if the standard males were *S. brevicercum*. The criteria for identification of *S. brevicercum* were not unique to the standard males; therefore, they could either be sex-exceptional males or a divergent type of *S. brevicercum* with the characteristics being variable within the species.

ADRIENNE UDELHOVEN – Chemistry

Developing Proteomics Techniques for Glycosylated Proteins

The ability to study proteins and their role in cell function is a central area of scientific study. To learn more about proteins and glycosylation, a form of post-translational modification, a means of detecting specifically for glycosylation is necessary. The modification of an existing dye to meet this need was the goal of my research. N-benzyl-4-nitroaniline and methyl ((4-(4-nitrophenyl)amino)methyl)benzoate, two intermediate compounds in the synthesis were created and purified.