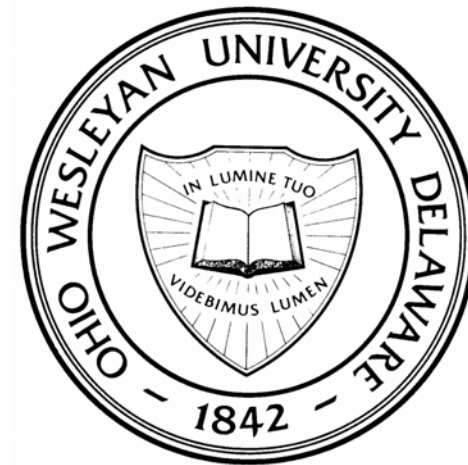


**2004**  
**SUMMER SCIENCE**  
**RESEARCH SYMPOSIUM**



**Ohio Wesleyan University**  
**Delaware, OH**

**SUMMER SCIENCE RESEARCH SYMPOSIUM**

**Atrium**

**Conrades • Wetherell Science Center  
Thursday, September 30, 2004**

**PROGRAM**

**8:00 – 10:00 Poster set-up**

**12:00 – 12:20 Opening Remarks**

Edward H. Burt Jr., Department of Zoology

President Mark Huddleston

**12:20 – 1:10 Poster Session 1**

<sup>1</sup> Vinodkumar Saranathan

<sup>2</sup> Charini Perera

<sup>3</sup> Sarah Miller

<sup>4</sup> Rebecca Shaner

<sup>5</sup> Stefanie Fluke

<sup>6</sup> Abigail Polter and Stefanie Fluke

<sup>7</sup> Jennifer Brunsdon

<sup>8</sup> Audrey Schiavo and Mikaela Ebitz

<sup>9</sup> Mikaela Ebitz

<sup>10</sup> Laura Becker, Hilary Comeras, Marian Homan, and Vivek Venugopal

<sup>11</sup> Sebastian Gorham

<sup>12</sup> Carley Shulman

<sup>13</sup> Alexandra Wise

**1:10 – 2:00 Poster Session 2**

<sup>14</sup> Elizabeth Curseen

<sup>15</sup> George Hamaoui Jr.

<sup>16</sup> Emily Carleton

<sup>17</sup> Kathryn Noonan

<sup>18</sup> Gyasi Dapaa

<sup>19</sup> Kumar Chheda

<sup>20</sup> Rushini Perera

<sup>21</sup> Myla Ashfaq

<sup>22</sup> Michael Nelson

<sup>23</sup> Umut Aypar

<sup>24</sup> Nga Nguyen

<sup>25</sup> Amanda Wibley

**ADDITIONAL EVENTS – SCIENCE CENTER DEDICATION CEREMONIES**

2:00 – 4:00 **Tours of the Science Facilities**

4:00 **Lecture: Frontiers of biotechnology**

Science Center Atrium

Introduction: Amy Downing

Lecture: Paul Schimmel

Professor of Molecular Biology and Chemistry  
The Scripps Research Institute

5:00 **Reception**

Science Center Atrium

8:00 **Lecture: Frontiers of physics**

Science Center Atrium

Introduction: Mark Huddleston

Lecture: Brian Greene

Professor of Physics and Math  
Columbia University

**ACKNOWLEDGEMENTS:**

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Associate Dean of Academic Affairs

Dr. Mark Huddleston  
President, OWU

Mary Ann Nelson  
Secretary, Department of Psychology

Staff of Building and Grounds

OWU Food Service

All faculty supervisors and student volunteers

Parents and guardians of student researchers

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**SUMMER SCIENCE RESEARCH SYMPOSIUM**

## 2004 ABSTRACTS



### Poster Session 1

12:20 – 1:10

#### <sup>1</sup> Vinodkumar Saranathan

Faculty Mentor: Dr. Edward H. Burtt, Jr.  
Department of Zoology

#### **Project Title: Variation in plumage microbial communities among Song Sparrows (*Melospiza melodia*) of Arizona, Ohio and Washington**

Populations of Song Sparrows with limited genetic contact occur in geographically and ecologically different regions of North America. We sampled three sub-species of Song Sparrows: *M. m. fallax* in the riparian corridors of southeastern Arizona, *M. m. melodia* in old field habitat in Ohio and on Kent Island, New Brunswick, Canada and *M. m. morphna* in the Olympic rainforests of western Washington (Arcese, P., M. K. Sogge, A. B. Marr, and M. A Patten. 2002. Song Sparrow. The Birds of North America, No. 704. The Birds of North America, Inc., Philadelphia, PA.). We compare the variation in plumage microflora within and among these subspecies of Song Sparrows. We found differences in the occurrence of feather-degrading microbes, especially *Bacillus licheniformis*, *Streptomyces* spp., *Fusarium* and *Acremonium* spp. among the three sub-species. We speculate on the causes of differences in the microbial communities of the plumage in these populations. We also call attention for the need to study how such differences in plumage microflora may affect the morphology, behavior or ecology of sparrows in the different populations.

#### <sup>2</sup> Charini Perera

Faculty Mentor: Prof. Robert Harmon  
Department of Physics and Astronomy

#### **Project Title: Searching for transits of exosolar planets**

The first planet orbiting another star was discovered in 1995 by Swiss astronomers Mayor and Queloz, by observing Doppler shifts in the star's spectral lines, caused by the gravitational tug of an orbiting planet. To date 123 such exosolar planets have been detected via this technique. To accurately deduce a planet's mass, the inclination angle of the orbital plane must be known. However, this information cannot be found via the Doppler Shift method. On the other hand, if a planet transits (passes in front of) the star, it can be deduced that its orbital plane is almost edge-on to us, so its mass can be found. A transit can be observed by the drop in the star's brightness it produces. Until recently, only one planet was known to transit its star, HD 209458. Due to competition at large international observatories, an organization called transitsearch.org was set up to incorporate small college observatories and advanced amateur astronomers into the search for transits. Our research was carried out using a Meade LX-200 8" Schmidt-Cassegrain telescope with an SBIG ST-8E CCD camera to capture images of stars predicted to show transits. These images were processed using Software Bisque's CCDSoft. Aperture photometry was carried out on the target star and comparison stars in the field using MIRA AP. We observed the following stars: HD 209458, HD 130322, HD 143761 ( $\rho$  CrB), HD 168443, and HD 192263. We successfully observed the end of a transit of HD 209458, the star known to transit, thus verifying our ability to detect a transit. With the exception of  $\rho$  CrB, the other stars observed showed no transits. Our observations of  $\rho$  CrB showed a possible transit, and we are awaiting confirmation or refutation of this result with more observations of this star by ourselves and other observers.

#### <sup>3</sup> Sarah Miller

Faculty Mentor: Dr. Laura Tuhela-Reuning  
Department of Botany and Microbiology

#### **Project Title: Evaluation of Techniques to Detect and Identify both Culturable and Non-Culturable Bacteria**

Culturing bacteria in microbiological media has been the primary method of collecting, isolating, identifying and studying bacteria for the last 150 years. However, as many as 99% of the bacteria on earth have not yet been described, and many are non-culturable. To detect all bacteria present *in situ*, such as on the feathers of a live bird, alternate, culture-free detection methods are necessary. Polymerase Chain Reaction (PCR) using primers that amplify portions of 16S rRNA was used to obtain products for sequencing. The sequences are unique to different bacteria, and successful identification of *Aeromonas schubertii*, *Pseudomonas fluorescens*, and *Bacillus licheniformis* was achieved. PCR of DNA obtained from soil samples was also done. Fluorescent staining was used for direct imaging of cells using epifluorescent and confocal microscopy. Fluorescent Gram stain differentiation using the dyes hexidium iodide and SYTO 9 was used to successfully differentiate *Escherichia coli* and *Staphylococcus aureus* in samples. Bacteria collected

using adhesive tape and tabs from environmental sources were successfully imaged using confocal microscopy after staining with propidium iodide and SYTO 9 despite some autofluorescence of the tape. Viability of the bacterial cells was also determined on the tape samples. Hexidium iodide and SYTO 9 were used for quantitative enumeration of bacteria, and fluorescence intensity was measured using a luminescence spectrometer. While fluorescence intensity varied linearly with bacterial count, consistent data were difficult to obtain. Detection methods using PCR and confocal imaging were most successful. This work laid the foundation for ongoing research using fluorescent *in situ* hybridization (FISH) and cloning of PCR products to further detect non-culturable bacteria.

#### <sup>4</sup> **Rebecca Shaner**

Faculty Mentor: Dr. Heather Grunkemeyer  
Department of Chemistry

#### **Project Title: Design of an Optical Sensor for the Detection of Lead in Water supplies**

We have investigated the synthesis of an optical sensor for the quantification of lead in water supplies. The sensor design was based on developing a supramolecule that acts as a fluorescent receptor for lead. The supramolecule was designed to consist of three components; a cyclodextrin molecule, a lead binding ligand, and a fluorescent probe. One important factor in the design of the optical sensor was choosing a probe whose fluorescence was environmentally sensitive. Dansyl chloride is a fluorescent molecule that has shown environmentally sensitive fluorescence. In an effort to understand the fluorescent sensitivity we investigated the fluorescent properties of dansyl chloride in a variety of solvents having different polarities, thus providing different environments. We focused on the fluorescence quantum yield and fluorescence emission spectrum of dansyl chloride. Based on these studies we feel the environmental sensitivity of dansyl chloride is due to two different excited species differing in polarity and orientation. The dominant species emitting in solution is dependent on the solvent environment.

The design of the supramolecule must also consist of a lead binding site. We chose 1,5,9,13-Tetrathiacyclohexadecane-diol for this purpose. We have successfully linked the lead ligand to our dansyl chloride probe. The TLC results confirmed the presence of two products, mono- and di-dansylated ligand which we have successfully separated. We investigated the environmental sensitivity of the complexes in various solvents finding that the complex also shows extreme environmental fluorescence sensitivity.

We also studied the lead binding capacity of the 1,5,9,13-Tetrathiacyclohexadecane-diol ligand. To investigate the binding we attempted to extract lead from an aqueous layer into the center of the ligand in an organic layer. These samples were investigated using electrospray MS.

#### <sup>5</sup> **Stefanie Fluke**

Faculty Mentor: Dr. Chris Wolverton

Department of Botany / Microbiology

#### **Project Title: Auxin Flow in Gravistimulated Lateral Roots**

Young lateral roots extend from the main root at an angle of approximately sixty degree. When the main root is rotated the lateral roots curve back to their initial angle relative to gravity. This curvature is thought to involve the plant hormone auxin. Auxin affects many aspects of development and growth in plants, and the regulation of auxin transport plays a key role in these processes. Auxin flow can be visualized using DR5:GFP, a reporter fusion consisting of green fluorescent protein driven by the auxin responsive DR5 promoter. We have introduced this reporter gene into Arabidopsis plants in order to observe the flow of auxin in gravistimulated lateral roots. Nine to eleven day old roots were scanned before and after a thirty degree rotation to assess the direction and rate of curvature. Roots were then analyzed by confocal microscopy.

Trends were seen for lateral roots that were rotated either upward or downward. Those that were rotated upward usually had an auxin gradient on their lower side, inhibiting growth on that side and causing the upper side to experience faster growth. Eventually the root would then curve downward returning the lateral back to its original position with respect to gravity. The opposite pattern was observed on those laterals on the downward side of the main root. A gradient of auxin was seen on the upper side, inhibiting that side's growth and allowing the lower side to grow faster, causing upward curvature. Upon returning to the initial angle, GFP gradients were dissipated, supporting the idea that lateral roots have a non-vertical set point angle. Implications of these results for overall root architecture will be discussed.

#### <sup>6</sup> **Abigail Polter and Stefanie Fluke**

Faculty Mentor: Dr. Chris Wolverton  
Department of Botany / Microbiology

#### **Project Title: Gravitropism in akt1-1 roots lacks a significant DEZ contribution**

Plant roots are responsive to a number of environmental stimuli, including light and gravity. Upon reorientation in the gravity field, primary roots begin differential growth within 10 – 20 min in the distal elongation zone (DEZ). Differential growth in the DEZ is composed primarily of an increased rate of cell expansion on the upper flank. The plant hormone IAA plays a role in root gravitropism, but it is thought to act by inhibiting cell expansion on the lower flank of the central elongation zone, leaving open the question of DEZ regulation. Previous work has shown that DEZ cells are stimulated to elongate by the application of an exogenous electric field, raising the possibility that ionic signaling participates in DEZ regulation. To test this, we analyzed gravitropism in roots of an Arabidopsis potassium channel mutant, akt1-1, using high-resolution image analysis. We found that roots of akt1-1 have a greatly reduced gravitropic response, taking over 70 min to initiate curvature compared to 20 min for wild-type. In wild-type roots, the DEZ contributes the majority of

curvature in the 1 h following the latent period. In akt1-1 roots, the DEZ contribution was almost completely lacking over the same period. Conversely, akt1-1 roots appear to have an intact phototropic response. Thus, the akt1-1 mutation appears to specifically disrupt the participation of the DEZ in gravitropism. We tested whether this disruption in gravitropism was due to an inability of the mutant to sense or respond to IAA, and found that mutant roots have a wild-type response to applied IAA. Future work aimed at the interaction between potassium uptake and auxin responses may shed light on the regulation of the DEZ.

## <sup>7</sup> Jennifer Brunson

Faculty Mentor: Dr. N. Kyle Smith  
Department of Psychology

### **Project Title: Accentuating the Positive: The Effects of Comedic Movies on the Attention Bias**

Previous research has suggested that there is a tendency to automatically focus more attention on negative information than positive information (Hansen & Hansen, 1988; Pratto & John, 1991). Recent findings have suggested that priming people with positive stimuli can eliminate or decrease the magnitude of the attention bias (Smith, Larsen, Chartrand, Cacioppo, Savage & Moran, 2004). The current study focused on whether or not real world experiences, such as watching a movie, could also affect the attention bias. We hypothesized that, similar to positive priming, watching a comedic movie would attenuate the attention bias. Sixty-seven participants were recruited at a local suburban movie theater. Participants' tendency to attend to positive and negative information was measured both before and after watching either a comedy or a non-comedy. This was accomplished by presenting participants with a visual search paradigm (Ohman, Lundqvist, Esteves, 2001) in which they had to detect a happy or angry oddball face in grids of nine faces. A three-way interaction between test time, oddball valence, and movie type was significant  $F(1, 65)=4.286$ ,  $p=0.042$ . Pre-movie measurement results showed that, there was no difference in the attention bias of participants before watching a movie  $F(1, 65)=0.986$ ,  $p<0.324$ ; because independent of the type of movie viewed all participants displayed an attention bias towards negative information  $F(1, 65)=16.739$ ,  $p<0.001$ . However, the type of movie seen (comedy vs. non-comedy) significantly moderated the attention bias to negative information in post-movie measurements  $F(1, 65)=5.219$ ,  $p=0.026$ . Consistent with our hypothesis, in the post-movie measurements participants who watched non-comedies still displayed the attention bias  $t(38)=4.447$ ,  $p<0.001$  whereas participants who watched comedies did not  $t(27)=0.7738$ ,  $p=0.442$ . These findings demonstrate that everyday, real world experiences, such as watching a comedy, can attenuate or eliminate the attention bias to negative information.

## <sup>8</sup> Audrey Schiavo and Mikaela Ebitz

Faculty Mentor: Laurel J. Anderson

Department of Botany and Microbiology

### **Project Title: A Population Study of *Alliaria petiolata* in Krauss Preserve in Central Ohio**

*Alliaria petiolata* (Garlic Mustard) is a highly invasive biennial, native to Europe and with no known predators in the United States. Populations of *A. petiolata* displace native wildflowers and compete with tree seedlings for resources. Permanent plots have been constructed in Krauss Preserve, a mixed deciduous mesic forest in Central Ohio, to monitor the spread of *A. petiolata* populations. Total plant counts, in both the basal rosette and mature stage, were conducted from spring 2003 through summer 2004, and bi-weekly soil moisture measurements were done in summer 2004. Unlike the results of greenhouse experiments, total plant density did not effect plant survival in the field. Also, the drier sites had significantly more new rosettes than the sites with higher soil moisture readings. Significant edge effects were present, with the greatest numbers of *A. petiolata* found near the preserve edge, though a number of new rosettes were found further inward, suggesting that the 'front' is migrating toward the preserve interior with every successive generation.

## <sup>9</sup> Mikaela Ebitz and Audrey Schiavo

Faculty Mentor: Dr. Laurel J. Anderson  
Department of Botany and Microbiology

### **Project Title: Photosynthesis as a competitive trait of *A. petiolata* in the Ohio Wesleyan Krauss Preserve**

*Alliaria petiolata* (Garlic Mustard) is a highly invasive plant species native to Europe. This biennial herb has no predators in the USA and interferes with native plant species in many deciduous forests throughout the country. To better understand what makes *A. petiolata* such a strong competitor with native plants, we measured the photosynthetic rates of its rosettes along with the rates of two other rosette species, *Geum vernum* and *Viola striata*, during summer 2004. For each leaf, photosynthesis was measured at three light levels. *G. vernum* had significantly higher photosynthetic rates than the other two species under all three light conditions. Changes in daytime temperature did not affect photosynthetic rates. Therefore while *A. petiolata* competes well with native plants, summer photosynthesis is not its key advantageous characteristic.

## <sup>10</sup> Laura Becker, Hilary Comeras, Marian Homan, and Vivek Venugopal

Faculty Mentors: Dr. Harry Bahrack, Dr. Lynda Hall, and Mindy Baker  
Department of Psychology

### **Project Title: The Effects of Cognitive Aging on Access to Names of Famous People**

The purpose of our research is to discover how cognitive aging affects access to semantic memory, particularly for names. Recall of names declines with age more rapidly than recognition of names, and the goal of this study is to quantify this *differential* decline and learn about variables that influence it. In addition to age, these variables include the total amount of relevant knowledge, the elapsed time since learning the names, and the type of memory cue used to trigger recall.

By comparing access (recall) of names learned at the same time by various age groups we will be able to separate the effect of elapsed time from the effect of age of the learner. Our final test will present 84 portraits and 84 unique, brief verbal descriptions of famous people. One half of the questions of each type will be presented in recognition format (to determine the available knowledge of the individual), the other half in recall format (to determine the portion of accessible knowledge).

Based on pilot test results, questions presented in recognition and recall format will be selected to be equally recognized; likewise, the names cued with portraits and with verbal descriptions will be equally available. This will enable us to quantify the decline of access as a function of age and type of cue.

The pilot test also allows us to estimate when participants learned the names, which we accomplish by selecting famous people who were only in the news for a short time and who are not recognized by individuals who are too young to have learned the name. Finally, the pilot test will permit us to select names of appropriate difficulty by eliminating names known by nearly all or by very few participants.

The final test will be computerized and administered to three age groups.

### <sup>11</sup> Sebastian J. Gorham

Faculty Mentor: Bart S. Martin  
Department of Geology and Geography

#### **Project Title: Xenolith-bearing Dikes of the Grande Ronde Basalt, Columbia River Basalt Group, Southeast Washington State**

Xenolith-bearing dikes in the Grande Ronde Basalt were examined at the northern end of the Chief Joseph Dike Swarm along the Snake River near Lower Granite Dam. The dikes range in width from 0.3 to ~10 m; internal chilled margins suggest that the larger dikes may represent multiple magma injections. The dikes are aphyric and contain xenoliths that may represent the granitic rock underlying this part of the Columbia Plateau. Geochemical data indicate that the dikes are correlative with the Sentinel Bluffs and possibly Umtanum Members of the Grande Ronde Basalt.

Xenoliths were found sporadically in these dikes; however, a narrow dike adjacent to a compositionally similar and larger, Sentinel Bluffs dike contained a significant concentration of xenoliths. Contacts between the xenoliths and host basalts varied from sharp to diffuse suggesting a range of lava-xenoliths and host basalts varied from sharp to diffuse indicating a range

of lava-xenolith interactions. They were rounded to angular; they displayed a wide range of textures, from crystalline to glassy and fully melted. The xenoliths contained quartz, plagioclase, pyroxene,  $\pm$  alkali feldspar; accessory apatite; sparse crystallites were present some of the glasses. The plagioclase feldspars varied from largely intact to those displaying well-developed sieve- or spongy-textures. Alkali feldspar was identified in two xenoliths. Fine-grained, euhedral, ortho- or clino-pyroxene and/or pigeonite were common in xenolith glasses adjacent to rounded quartz grains. Glasses in the xenolith ranged from colorless to brown; they were SiO<sub>2</sub>-rich and mostly peraluminous. Fe was present in most glasses; Na and K were generally more abundant than Ca. Normative feldspar compositions indicate that the glasses are largely granitic and trondhjemitic.

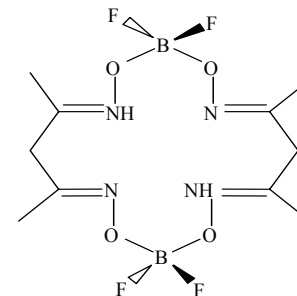
The differences in the proportions of normative quartz, albite, and orthoclase in the glasses follow a trend in the Ab-Or-Q ternary system that mimics the temperature minimum over a range of pressures, suggesting that the granitic crustal material may have melted over a range of depths. In addition, the compositions of the plagioclase and alkali feldspars in the xenoliths suggests that the granitic xenoliths may be similar to the nearby granodiorite of Granite Point, an outlier of the Idaho Batholith.

### <sup>12</sup> Carley J. Shulman

Faculty Mentor: Kim A. Lance  
Department of Chemistry

#### **Project Title: The Synthesis and Characterization of Precursors to a Manganese(III) Epoxidation Catalyst**

The synthesis of catalysts capable of enantiomeric epoxidations has drawn the interest of industry and academe alike. We wish to report the synthesis and characterization of the precursors to a manganese(III)  $\beta$ -dioxime complex that will be capable of epoxidizing hydrocarbon substrates.



Structure I

The free ligand structure (Structure I) has been formulated via a template synthesis using copper(II) and nickel(II). The free ligand was created by the demetallation. Bubbling hydrogen sulfide gas was passed through the transition-metal containing solution and filtered resulting in the precipitated copper(II)

sulfide and the desired product. Attempts to demetallate the nickel(II) complex proved to be fruitless.

We will report the complete synthesis of the copper(II), nickel(II) and free ligand (Structure I) in addition to our attempts to produce the manganese(III) complex.

<sup>13</sup> **Alexandria Wise**

Faculty Mentors: Dr. James Russell and Allison Berent- Spillson  
Department of Neurology, University of Michigan

**Project Title: Transforming Growth Factor  $\beta$ : Potential Regulator of Diabetic Neuropathy**

Peripheral neuropathy is a major complication of patients with diabetes, and is the leading cause of lower limb amputation in the United States. It is thought that the nerve loss and damage associated with diabetes are due to the effects of elevated glucose on the dorsal root ganglion (DRG) neurons in the lumbar spinal region. When exposed to high levels of glucose, DRG neurons die via a caspase-dependent programmed cell death (PCD) pathway (Russell, et al.). We hypothesize that elevated glucose associated with diabetes results in upregulation of transforming growth factor  $\beta$  (TGF- $\beta$ ), which subsequently induces undue activation of programmed cell death (PCD) pathways in DRG neurons.

The TGF- $\beta$ s are apart of a family of cytokine growth factors that aids in a variety of growth promotion processes within cells as well as functioning in cell cycle regulation throughout the body (Day et al.). There are three isoforms (TGF- $\beta$  1, 2, 3) found in mammals, which are differently regulated and expressed. All three TGF-  $\beta$ s have both pro- and anti-cell death properties, depending on context and location. However, within diabetics it has been shown that TGF- $\beta$  has an adverse affect on the kidneys and retina, causing glomerulosis and retinopathy, respectively.

Neurite growth was measured from DRG explants exposed to each of the 3 TGF- $\beta$  isoforms, and compared growth to that of control DRG explants from 15-17 week old mice pups. We found that DRG exposed to the TGF- $\beta$ s showed decreased neurite growth as compared to control, as well as showing signs of axonal degeneration. TGF-  $\beta$  1 had the least effect on decreasing neuronal outgrowth. This may be due to preferential expression of TGF-  $\beta$  1 by Schwann cells rather than DRG, thus having less interaction with axonal regulation and other neuronal processes. Future experiments include determining a TGF- $\beta$  dose curve for all three isoforms, hypothesizing a dose-dependent increase in caspase-3 activation (a component of the PCD pathway), and begin to determine a mechanism through which TGF- $\beta$  affects cell growth and survival.

**Poster Session 2**

**1:10 – 2:00**

<sup>14</sup> **Elizabeth J. Curseen**

Faculty Mentor: Dr. Ramon Carreno  
Department of Zoology

**Project Title: Parasites from Millipedes (Diplopoda) of the Kraus and Bohannan Preserves.**

An inventory of the eukaryotic parasites of millipedes (Diplopoda) was made from the Kraus and Bohannan preserves. Several species of gregarines (Protozoa: Apicomplexa) were recovered. Two species of parasitic nematodes, *Rhigonema thysanophora* (Nematoda; Rhigonematidae) Crites, 1965 and a thelastomatid species (Nematodea; Thelastomatidae) Leidy, 1849 were recovered from the hindguts of a polydesmid millipede, from the Kraus Preserve along with some protozoan parasites. A parasitic nematode also resembling a thelastomatid species was recovered from the hindgut of a different species of polydesmid millipede at Bohannan. Light and scanning electron microscopy were used to study the parasites' morphology and PCRs (polymerase chain reaction) were carried out on DNA extracted from both male and female nematodes to confirm their single-species identity. Analysis of microscopy images, measurements, and PCR products indicated that the nematodes from both species of millipedes represented undescribed species.

<sup>15</sup> **George S. Hamaoui Jr.**

Faculty Mentor: Dr. Edward H. Burt Jr.  
Department of Zoology

**Project Title: Analysis of feather-degradation by *Bacillus licheniformis* from the plumage of Botteri's Sparrows (*Aimophila botterii*) living in wet and dry habitats near Elgin, Arizona**

Burt and Ichida (2004) found that strains of *Bacillus licheniformis* in the plumage of Song Sparrows (*Melospiza melodia*) occupying humid habitats in northwestern Washington degraded feathers more rapidly and more completely than strains from the plumage of Song Sparrows in arid habitats in southeastern Arizona. In this study we compared the ability of *B. licheniformis* from the plumage of a single population of Botteri's Sparrows (*Aimophila botterii*) to degrade feathers. Some of the Botteri's Sparrows occupy low lying, damp washes while others occupy dry mesa habitat near Elgin, Arizona. The washes are subject to periodic flooding and may have standing water during the wet season when our study occurred. The mesas are dry and rainfall disappears quickly into the sand gravel mix that comprises these sparsely vegetated grasslands. However, unlike the Song Sparrow populations that



were hundreds of miles apart, the different sites occupied by Botteri's Sparrows were within site of each other. To quantify bacterial degradation rates, 2 centimeter pieces of white goose feathers were placed in test tubes containing feather media. The tubes were then inoculated with bacterial suspensions obtained from the plumage of Botteri's Sparrows. The tubes are incubated at 40°C for ten days. Each day, we rated the degradation of the goose feathers based upon a six-point scale. We found that there was no significant relationship between feather-degrading *B.licheniformis* from the wash and feather-degrading *B.licheniformis* from the mesa ( $\chi^2= 1.87$ ,  $df=1$ ,  $P=0.050$ ). Also, we found there was no significant difference in the number of days it took wash *B.licheniformis* and mesa *B.licheniformis* to degrade feathers. The difference in feather-degrading ability of strains of *B.licheniformis* found on Washington and Arizona Song sparrows is not present in the strains of *B.licheniformis* found on the Botteri's Sparrows. Thus, the relationship seems to exist only across large geographical distances.

### <sup>16</sup> Emily A. Carleton

Faculty Mentor: Dr. Danielle R. Hamill  
Department of Zoology

### **Project Title: PHENOTYPIC AND GENETIC ANALYSIS OF THE CELL DIVISION MUTANT *or576ts* IN *CAENORHABDITIS ELEGANS***

Mitosis, or cell division, is a complex process that must be carefully regulated. Problems during mitosis can result in cell death or in a mutation that can cause severe defects in the organism. During embryonic development, mitosis is crucial to the growth of the organism. *Caenorhabditis elegans* is a model system that can be used to study the effects of mutations in mitosis on developing embryos. We isolated a mutant called *or576ts*, which has a temperature-sensitive, embryonic-lethal mutation that affects chromosome segregation and cytokinesis (the cleavage of a cell into two daughter cells). As a result, embryos grown at the restrictive temperature of 25°C are unable to progress normally past the 1- or 2-cell stage. This phenotype was observed through the use of time-lapse movies and immunofluorescence microscopy. We have not yet identified the gene responsible for this mutant phenotype, but we have determined that it is near the center of chromosome II. By crossing *or576ts* mutants with worms possessing mutations at known positions, it is possible to locate the approximate position of the unknown gene relative to these cloned marker genes. This summer we generated 11 data points that indicate that *or576ts* is located at approximately 1.3 Map units, which is consistent with previous data collected in the lab. Additional data points are needed to determine more precisely the genetic map position of our gene. Once the location of the gene is approximated, the candidate gene can be sequenced to determine if there is an alteration in the DNA.

### <sup>17</sup> Kathryn Noonan

Faculty Mentor: Dr. David Markwardt  
Department of Zoology

### **Project Title: Identifying “Natural” Targets of Nonsense Mediated mRNA Decay in *Schizosaccharomyces pombe***

Nonsense mediated mRNA decay, (NMD), is a process that selectively degrades mRNA transcripts containing premature termination codons (PTCs). These transcripts are typically nonfunctional and likely deleterious to the organism. All eukaryotic organisms tested have been shown to use NMD. Natural targets of NMD are those mRNA transcripts consistently degraded by the NMD pathway in *wild-type* organisms. An important class of natural targets in higher eukaryotes is PTC-containing products of alternative splicing events. This phenomenon, called Regulated Unproductive Splicing and Translation (RUST) is thought to play an important role in the genome-wide regulation of gene expression in eukaryotic systems. Our goal is to understand the mechanism and utility of this phenomenon using the fission yeast, *Schizosaccharomyces pombe*. *S. pombe* is a useful model for this study because, unlike *S. cerevisiae*, almost half of all *pombe* genes have introns. As a result, it can be used to study alternative splicing and RUST. Although NMD is known to exist in *S. pombe*, potential gene targets have not been discovered. Our study attempted to identify potential gene targets of NMD in this organism. We began looking for targets by using homologs of targets of NMD in organisms such as *C. elegans*. We used reverse transcription polymerase chain reaction (RT-PCR) and northern blots to analyze these transcripts. To date, we have yet to find targets of NMD in *S. pombe*. However, we intend to expand our search using whole genome microarrays.

### <sup>18</sup> Gyasi Kwabena Dapaa

Faculty Mentor: Dr. R. S. Linder  
Department of Mathematics and Computer Science

### **Project Title: Sampling Distribution of the Correlation Coefficient when Data is Subjected to Censoring.**

Of interest to many researchers is the strength and direction of the relationship between one variable, Y, and another, X. Researchers typically employ the Pearson population correlation coefficient,  $\rho$ , as a measure of the strength of such dependence and estimate it with the sample correlation coefficient, r. The probability distribution of r is needed for inference about  $\rho$ .

When X and Y are independent, the exact probability distribution of r is known and is commonly used to test for dependence between X and Y. Of greater utility, R.A. Fisher found a remarkable transformation of r that has approximately a normal distribution, even for small sample sizes. Using Fisher's transformation, one may construct approximate confidence intervals for  $\rho$ , and hence measure the nature of the relationship between X and Y. Together, these results provide a rather complete collection of statistical methodologies for inference on  $\rho$  based on complete samples.

In many applications, complete samples are not collected. Suppose, for example,  $n$  monkeys are fed an arthrogenic diet. As monkeys die, we measure the age at death ( $X$ ) and the progression of disease ( $Y$ ). After  $p$  monkeys die, we end the experiment, and the remaining  $n-p$  bivariate observations have been censored by  $X$ . Specifically, we say that our data has been subjected to Type II censoring (Type I censoring refers to the case where the experiment ends at a fixed time, so that the number of observations is random). In fact, both censoring schemes occur often in practice, and they induce a dependence structure on the bivariate observations that makes the mathematical determination of sampling distributions generally difficult.

We consider the distribution of  $r$  when  $X$  and  $Y$  are bivariate normal in distribution, and data is subjected to Type II censoring. Here, the exact distribution of  $r$  obviously depends on  $\rho$ ,  $n$  and  $p$ , but is mathematically intractable. We develop an algorithm for obtaining an approximation to this distribution, which researchers may use to make inferences on  $\rho$ .

Using simulation, an empirical study of the sampling distribution of  $r$ , suitably transformed, suggests that the distribution may well be approximated by a beta distribution, whose parameters are functions of  $n$ ,  $p$  and  $\rho$ . We establish a relationship between the parameters which characterize this approximating distribution and the values  $n$ ,  $p$  and  $\rho$ . Specifically, we used a method of matching sample moments (mean and variance) of  $r$ , based on extensive simulation, to the mean and variance of a beta distribution to obtain estimates of the beta distribution parameters. These estimated parameters vary predictably as  $n$ ,  $p$  and  $\rho$  change. We establish closely fitting models for determining beta distribution parameters as functions of  $n$ ,  $p$  and  $\rho$ . Of course, the researcher would use  $n$ ,  $p$  and  $r$  to determine these parameters. These, in turn, provide the researcher with a suggested approximating distribution for inference on  $\rho$ .

We find that under certain conditions, the algorithm provides a reasonably good fit to the distribution of  $r$ . In other conditions, the fit is poor. When the fit is good, tail percentiles for the distribution of  $r$  are approximated with relative error less than 3%. In this case, a researcher has been provided with a simple recipe for correlation analysis based on data subjected to Type II censoring.

#### <sup>19</sup> Kumar Chheda

Faculty Mentor: Dr. Sean McCulloch  
Department of Mathematics and Computer Science

#### **Project Title: Analysis and Optimization of an Algorithm to Route an Integrated Circuit**

Routing the nets on an integrated circuit is a complex and a difficult problem because there are many components that must be connected to realize a net and there are a lot of nets that need to be routed. There are routing tools available for this purpose, all of which have many drawbacks. One such routing tool is called Quark and embodies an auction-based methodology where the nets can bid on the components they need to realize their entire route. Each net in Quark has a personality which defines its behavior in the

bidding process. This summer, the study was directed towards Quark's Focused personality, which is specialized for multi-terminal nets and is the most complex of all the personalities. Our work concentrated on how the Focused personality can be made stronger so as to achieve better results. In analyzing the existing personality's performance, we noticed a problem that affected the efficiency of the routing because of extreme competition between nets for resources. We then created an algorithm that attempted to make the bidding process more efficient in the Focused personality by rerouting if certain parts are heavily bid upon. We also attempted to implement the new addition into the existing framework of the Quark router.

#### <sup>20</sup> Rushini Perera

Faculty Mentor: Dr. Ramon Carreno  
Department of Zoology

#### **Project Title: Phylogenetic Analysis of some Trichostrongyloid Parasitic Nematodes as inferred from Ribosomal RNA gene sequences**

An inventory of the parasites of mammals collected from northwestern Costa Rica was made to help assemble data for ongoing research on the phylogeny of lungworms from mammals as well as to contribute to the NSF-sponsored Tree-of-Life project which deals with the phylogeny of all nematodes. Two species of nematodes were recovered, one from the coati (*Nasua narica*) and the other from the hooded skunk (*Mephitis macroura*). Both were identified as members of the superfamily Trichostrongyloidea. Our goal was to study the phylogeny of these two species by analyzing partial sequences of large sub-unit ribosomal RNA (rRNA) and small sub-unit rRNA. The DNA was extracted using the DNAzol kit, the relevant genetic loci were amplified by PCR and the DNA was directly sequenced. A BLAST search of the sequences inferred that both species matched trichostrongyloid sequences. Results also suggest that these species may be a possible outgroup to the superfamily Metastrongyloidea. These gene sequences will provide useful data for inferring the phylogeny of the lungworms.

#### <sup>21</sup> Myla Ashfaq

Faculty Mentor: Dr. George Schwartz  
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#### **Project Title: Investigation of Transport Metabolons on the Basolateral Membranes of Kidney Proximal Tubule Epithelial Cells**

Carbonic anhydrases (CA's) are enzymes that catalyze the reversible hydration of  $\text{CO}_2/\text{HCO}_3^-$ . CA's facilitate  $\text{HCO}_3^-$  transport through association with  $\text{H}^+/\text{HCO}_3^-$  transporters in the plasma membrane, and thus maintain cellular acid/base homeostasis. Carbonic anhydrase IV (CAIV) is tethered to the

plasma membrane via a glycosyl-phosphatidylinositol anchor (i.e. GPI-linked) and thus is targeted to the apical membrane of polarized epithelial cells. However, CAIV has also been found on the basolateral membrane of renal proximal tubule epithelial cells and has been reported to associate with the  $\text{Na}^+/\text{HCO}_3^-$  (NBC1) exchanger. Fractionation of apical and basolateral membranes confirmed that CAIV is expressed predominantly on the apical membrane with NHE3. However, carbonic anhydrase XII is another CA isoform that is basolaterally expressed in the kidney cortex and is therefore more likely to form the transport metabolons with NBC1. Western blotting showed the co-expression of CAXII and NBC1 in the total membranes of mouse kidney. The CAXII gene was cloned into a prokaryotic expression vector so that direct interactions between CAXII and NBC1 can be investigated *in vitro*.

## <sup>22</sup> Michael Nelson

Faculty Mentors: Dr. Susan Pfiffner, University of Tennessee, Department of Microbiology; Dr. Tom Kieft, New Mexico Tech., Department of Biology; Tom Gihring, University of Florida; Dr. T.J. Phelps, Department of Energy, Oak Ridge National Laboratory; and Dr. Laura Tuhela-Reuning, Ohio Wesleyan University, Department of Botany and Microbiology

### **Project Title: Culturing *Desulfotomaculum*-Like-Organisms (DLO) from Deep Subsurface South African Au Mines.**

Traditionally, sulfate reducing bacteria have been difficult to culture. This has especially applied to samples obtained in the deep subsurface environment, locations lying 500m or more below the surface. Projects involving the deep subsurface gold mines of South Africa have previously identified via 16sRNA sequencing a group of as yet uncultured organisms who are most similarly related to *Desulfotomaculum* spp. By developing a culture medium capable of sustaining growth of biological samples from deep subsurface gold mines in South Africa, we should be able to isolate these *Desulfotomaculum*-like-organisms in pure culture. Through the use of electron donor and acceptor pair enrichments, we have developed a set of media capable of stimulating growth of biological fissure water samples from deep subsurface gold mines. A phosphate buffered basal medium containing  $\text{Na}_2\text{SO}_4$  and either of  $\text{CO}$  or  $\text{H}_2$  produced the strongest growth. To date, FISH analysis has failed to identify *Desulfotomaculum*-like-organisms in cultured specimens, however, colony typing has not yet been conducted to determine species present in active cultures.

## <sup>23</sup> Umut Aypar

### **Project Title: In Search of New Markers for the Study of Glial Restricted Precursors**

Faculty Mentors: Lin Silver, Christoph Pröschel, Mark Sullivan, Mark Noble

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The ancestry and the development of oligodendrocytes, the myelin supplier of the central nervous system, have been widely studied with different approaches resulting in two extreme hypotheses. Glial-restricted precursor (GRP) cell hypothesis suggests that neuroepithelial stem cells give rise to GRP cells from which oligodendrocytes and also astrocytes are generated. The other extreme, motor neuron-oligodendrocyte precursor (MNOP) cell hypothesis, suggests that MNOP cell that give rise to motor neurons also generates oligodendrocytes, and proposes that astrocytes are generated by a separate lineage. The fact that both GRP and neuron-restricted precursor (NRP) cells have been isolated from developing tissues and shown to express predicted properties *in vivo* and also after transplanted into central nervous system (CNS) regions, supports the GRP hypothesis. The availability of better markers for glial restricted precursors will be helpful in further characterizing the role of GRP cells in neural development. This study takes two different approaches to search for new markers to analyze GRP cells: array based and single chain antibody based.

The microarray based approach compares RNA isolated from neuroepithelial precursor, GRP and oligodendrocyte-type-2 astrocyte progenitor (O2A) cells by hybridization to the Rat 230 Affymetrix GeneChip probe array. Candidate genes were chosen according to the following criteria: preferential expression at the GRP cell stage and known membrane protein or EST/novel protein. Differential expression was validated by relative quantitative PCR. Rapid amplification of cDNA ends was performed to obtain the complete cDNA sequence of the ESTs.

A direct approach for the isolation of cell type specific surface markers is the use of M13 filamentous phage expressed single chain antibodies (scFv). This involves selecting M13 phage expressing single chain antibodies on the envelope that bind to GRP cells ( $\text{A2B5}^+$ ,  $\text{PSA-NCAM}^+$ ) but not other neural lineage cells. While scFv selection is restricted to surface expressed markers, it has the potential to identify a wider array of epitopes, including proteins, phospholipids and glycosylated molecules.

Together these approaches will yield new tools that will enable a more detailed study of glial cell generation and progressive lineage restriction.

## <sup>24</sup> Nga Nguyen

Faculty Mentors: Chen Liu, M.D., PhD., John Elyar, and Haizhen Zhu  
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### **Project Title: Cloning and expression of Hepatitis C Virus Core protein**

Hepatitis C Virus (HCV) is a plus-sensed single-stranded RNA (ssRNA) virus. HCV core protein is assumed to bind to viral RNA at the 5' non-translated region (5'NTR) to form the nucleocapsid. Currently, there is little knowledge as to the specific interaction of HCV core protein/RNA. For

example, it is unknown as to whether core protein binds ssRNA of sense or anti-sense origin or if binding is dependent on a double-stranded RNA (dsRNA) intermediate. Ongoing research has focused on how HCV core protein binds to its RNA forming the viral virion. To purpose of this study is to investigation such interactions between core protein and HCV RNA. HCV core protein RNA was reverse transcribed into cDNA. HCV core-specific oligonucleotide primers were designed with restriction enzyme sites to subsequent ligation. Such primers were used to amplify core protein by polymerase chain reaction (PCR). Upon ligation into an appropriately restriction-digested vector, pQE32, vector was transformed into *E. coli* DH5 $\alpha$  and DNA mini-preparation was performed by the Promega Wizard Kit. Restriction enzyme digests were performed to screen clones capable of dropping the potential HCV core insert. Positive samples were then sequenced by Big Dye Terminator 3 Reaction. In order to evaluate protein expression, HCV core constructs were transformed into *E. coli* M15. HCV core protein production was induced by addition of isopropyl-beta-D-thiogalactoside (IPTG). SDS-PAGE analysis demonstrated the presence of core protein only upon IPTG-stimulation in a time dependent manner. A Qiagen Ni-NTA Kit was used to perform purification of the core protein. SDS-PAGE stained with Coomassie Blue demonstrated purification of the HCV core protein. Subsequent Western Blot analysis demonstrated negative results for HCV core protein. Since an appropriate sized product was induced by IPTG-stimulation and protein purification, immunoblotting will be repeated. Decreased antibody affinity or specificity due to PCR amplification of slightly altered core protein may have caused such results. In addition, HCV core protein was produced in bacteria, which do not glycosylate proteins. Subsequent analysis will involve using a different panel of HCV core-specific antibodies. Future studies will include producing high amounts of core protein to be coated on a Covalink plate. Biotinylated sense, anti-sense, and dsRNA samples, generated by *in vitro* transcription of DNA, will be evaluated for HCV core-specific binding. Such research could be applicable to future drug therapeutic and prophylaxis studies investigating potential compounds capable of inhibiting the interaction of the HCV core protein and RNA.

activity. Further work would involve insertion of the mutant plasmids into known *Pseudomonas aeruginosa* AlgG mutants to see if they would complement a epimerase defect (in strain FRD462), or a polymerization defect (in strain FRD1200).

<sup>25</sup> **Amanda Wibley**

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59717

**Project Title: Mutagenesis of AlgG, the *Pseudomonas aeruginosa* alginate epimerase.**

Certain strains of *Pseudomonas aeruginosa* produce high levels of alginate, an exopolysaccharide that confers a mucoid phenotype. Such strains are isolated from cystic fibrosis patients with chronic pulmonary infections. Alginate is a linear co-polymer composed of  $\beta$ -D-mannuronic acid (M) and variable amounts of its C-5-epimer,  $\alpha$ -L-guluronic acid (G). AlgG is a periplasmic C-5-epimerase that converts poly d-mannuronate to the mixed M+G sequence of alginate. Based on structural predictions of AlgG, point mutants were made to further understand the role and mechanism of AlgG