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Otterbein University Department of Biology & Earth Science, "2018 Senior Research Symposium" (2018). *Biology and Earth Science Student Scholarship*. 2.
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Department of Biology and Earth Science

Senior Research Symposium

April 11, 2018

Podium Presentations – Riley Auditorium

- 8:10** **Opening Remarks, Dr. Halard Lescinsky**
- 8:15** **John Michael Wright:** Evaluating *Streptomyces scabies* SCAB27101 Deletion Mutant Life Cycle Alterations
- 8:30** **Taryn J. Chudo:** Sibling Rivalry: A Comparison of Giant Panda (*Ailuropoda melanoleuca*) Twin Behavior
- 8:45** **Samantha M. Bonifas:** Developmental Growth Plasticity in American Toads (*Anaxyrus americanus*)

9:00 – 9:25 Refreshment Break – Riley Lobby

Podium Presentations – Riley Auditorium

- 9:25** **Madison T. Flasco:** Characterization of Two Cyclic di-GMP Phosphodiesterases Hypothesized to Regulate Virulence in *Streptomyces scabies*
- 9:40** **Abigail E. Smith:** Prediction Factors for Release of Wildlife at a Midwestern Rehabilitation Facility
- 9:55** **Caroline N. Fulmer:** Differential Expression Analysis of the *Streptomyces coelicolor* Conservon
- 10:10** **Amanda M. Drake:** Effects of Seasonality and Plant Maturity on Levels of Toxicity to Equine Erythrocytes in 45 species of *Acer*

10:25 – 10:50 Refreshment Break – Riley Lobby

Podium Presentations – Riley Auditorium

- 10:50** **Rachel T. Nguyen:** Identifying RmdA Protein Interactions in *Streptomyces* Using a Bacterial Two-Hybrid System
- 11:05** **Delaney H. Galbraith:** Variation in the Vocal Repertoire of The Cape Parrot (*Poicephalus robustus*) in a Behavioral Context
- 11:20** **Sean G. Kirk:** The Identification and Characterization of a Putative Chromosome Segregation Gene in *Streptomyces coelicolor*
- 11:35** **Closing Remarks, Dr. Halard Lescinsky,
Group Photograph**

2018 ABSTRACTS OF SENIOR RESEARCH PROJECTS (In Alphabetical Order)

Samantha M. Bonifas

Developmental Growth Plasticity in American Toads (*Anaxyrus americanus*)

Growth rate plasticity is the ability of organisms to adjust growth in response to environmental change. The environmental matching hypothesis predicts that individuals perform best in environments similar to those experienced early in development. Alternatively, compensatory growth predicts that organisms reared in resource-poor environments will have restricted growth followed by rapid growth when resources become available. Last, the silver spoon hypothesis predicts that individuals raised in high quality environments early in development maintain an advantage, even when later exposed to poor quality environments. We tested these hypotheses in American Toads, *Anaxyrus americanus*, by manipulating early environmental conditions of developing tadpoles. Tadpoles were initially reared at high (N = 14) or low density (N = 14) (20 or 5 tadpoles per 12-liter tank). Each tank had equal food levels creating different per capita food resources. Once tadpoles reached a standard mass, we transferred three tadpoles from each tank to a new tank that was either resource-rich or -poor. We measured one-week growth rates, intake, gut area, and toadlet size at metamorphosis. Low-density tadpoles gained more mass than high-density tadpoles in both resource-rich and -poor treatments (analysis of variance). High-density tadpoles ate less food even under ad libitum conditions. Low density tadpoles metamorphosed into heavier toadlets than the high density tadpoles in both resource levels. Also, tadpoles from the resource-rich treatment metamorphosed into heavier and longer toadlets. The results support the silver spoon hypothesis.

Taryn J. Chudo

Sibling Rivalry: A Comparison of Giant Panda (*Ailuropoda melanoleuca*) Twin Behavior

Expanded knowledge on an animal's individual behavior would be useful in reintroduction programs for endangered species around the globe. Currently, reintroduction program criteria focus primarily on physical characteristics such as age, sex, and overall health for the selection of candidates. However, behavioral deficiencies have been found in captive populations, producing adverse effects on reintroduction program success. The giant panda (*Ailuropoda melanoleuca*) is a species that would benefit from understanding the impact of behavior on reintroduction success. This study focused on the relationship of behavior with kinship, birth weight and age. No significant correlations were found between birth weight and activity, inactivity, social, or solitary levels. No significant correlations were observed between cub age at the start of the study and activity, inactivity, social, or solitary levels. No significance was found within

or between twins and singletons. Although this study did not find any correlations between physical and behavioral characteristics, it should be explored more to see definitively see if any physical correlations exist.

Amanda M. Drake

Effects of Seasonality and Plant Maturity on Levels of Toxicity to Equine Erythrocytes in 45 species of *Acer*

Maple leaf toxicosis is a condition that affects equines after they have ingested wilted leaf tissue from certain species of the genus *Acer*. The objective of this research is to: 1) screen 45 *Acer* species originating from North America, Asia, and Europe for toxicity, 2) determine the severity of toxicity among common species of *Acer* in North America, and 3) assess the effect of seasonality and plant maturity on toxicity. Leaves were collected in 2015-2017, dried, and ground. The toxin(s) were extracted in buffer and incubated with equine erythrocytes. Toxicity, as measured by percentage hemolysis (%H) and methemoglobin (%M), was quantified based on the absorbencies of erythrocyte/extract suspensions at 560, 576, and 630 nm. Among the 45 *Acer* species tested, there was a continuum of responses ranging from low to high %H and %M. However, the response for %H and %M were not correlated among the 45 species ($P=0.12$). For species common in North America, %H and %M were correlated ($y = 0.95x - 2.9$ $R^2 = 0.83$, $P<0.0001$), and certain species exhibited higher toxic levels than those caused by *A. rubrum*. In addition, certain species responded no differently than the control. Also, there were no differences in toxicity among samples collected in different months or collected from different stages of maturity. *Acer* species not previously cited as potential threats to equines were toxic in our study. Lack of correlation between %H and %M suggests that there are multiple oxidative-stress inducing compounds in members of the *Acer* genus. In addition, the toxic compounds are produced consistently throughout the growing season of young and mature plants.

Madison T. Flasco

Characterization of Two Cyclic di-GMP Phosphodiesterases Hypothesized to Regulate Virulence in *Streptomyces scabies*

Common scab is a devastating disease that affects the marketability of potatoes. The disease results in raised and pitted lesions seen on the surface of the potato and is caused by the soil pathogen, *Streptomyces scabies*. The *S. scabies* genome has two cyclic-di-GMP phosphodiesterases, RmdA and RmdB, both regulators of morphology and development. These proteins break down the ubiquitous second messenger molecule cyclic di-GMP, a molecule in charge of cell cycle progression and virulence. Both proteins contain diguanylate cyclase domains and phosphodiesterase domains, GGDEF and EAL, respectively, while RmdA contains an additional PAS_4 domain. Despite similar domain configurations, the two appear to have little homology. Amino

acid sequence alignments indicate there is only 35.47% identity between them. The two also differ structurally, unlike RmdA, RmdB has six transmembrane regions. BLAST searches reveal both RmdA and RmdB are highly conserved amongst streptomycetes and those species outside of the genus. Nothing is known regarding the influence of phosphodiesterases on the virulence of *S. scabies*. *Arabidopsis thaliana* seedlings were inoculated with the both the wild-type and $\Delta rmdB$ pathogenic strains of *S. scabies*. When compared to the wild-type, the mutant strain showed less severe infection. Seedlings inoculated with *rmdB* removed had longer roots and more cotyledon development than the wild-type. Preliminarily, the differences seen provide evidence of phosphodiesterase involvement in virulence. Understanding how these proteins affect the virulence of *S. scabies* can allow for improved treatment and prevention of the disease.

Caroline N. Fulmer

Differential Expression Analysis of the *Streptomyces coelicolor* Conservon

Streptomyces coelicolor is a gram-positive Actinobacterium. It is a filamentous soil organism that performs complex multicellular development. Its physiological and morphological differentiation produces active secondary metabolite antibiotics that have pharmaceutical applications. Studies of *S.coelicolor* show that cyclic di-GMP proteins regulate physiological and morphological development. *S.coelicolor*, has 13 copies of an operon that are collectively named “conservon” because they are highly conserved. The operons are named cvn1-13 and genes in each operon are denoted *cvnA-D*. RNA-Seq data of *S.coelicolor* showed differential expression between wild type and a cyclic di-GMP phosphodiesterase double mutant strain for many of the operons. Previous studies of *S.coelicolor* have shown varied effects of deleting these operons. Kenji Ueda et al., 2011 made null mutants lacking all genes in the operon for all 13 operons which resulted in wild-type phenotypes for all operons except cvn1. However, deleting *cvnA9*, *cvnD9*, and *cvnA10* exhibited abnormal phenotypes. This provides evidence that deleting the entire *cvn7* operon may not exhibit a phenotypic change, but deleting one gene of the operon could. A bioinformatics analysis shows that gene SCO6796 (*cvnC7*) is of interest because according to strepdb it contains conserved protein domains from the *arsR* family which contain a DNA-binding helix-turn-helix motif implying involvement in gene expression. Gene SCO6798 is interesting because it is an unnamed gene at the end of the *cvn7* operon. It contains a conserved GAF domain which could imply the ability to sense cyclic nucleotides. A concurrent analysis of a transposon mutant of SCO5292, *cvnD5* presents a phenotype with a difference in pigment production, but appears to sporulate like wild type *S.coelicolor* at 6 days growth. Distinct mutant phenotypes of each of these genes could shed light on the function of each gene and the complete conservon in *S.coelicolor*.

Delaney H. Galbraith

Variation in the Vocal Repertoire of The Cape Parrot (*Poicephalus robustus*) in a Behavioral Context

Vocal learning assists in the acquisition of a vocal repertoire through social interactions and is found in a plethora of socially complex taxa including parrots, whales, elephants, and bats. Psittaciformes (parrots) represent an interesting case study, the taxa having the necessary cognitive social complexity and composited native habitats to give rise to complex communication. Multiple species of parrots have been shown to have specific vocalizations for a given behavioral context. Here we aim to evaluate the vocal repertoire of wild Cape parrot (*Poicephalus robustus*) populations of the Eastern Cape province, South Africa. Data collection of the Cape parrots' behavior and vocalizations were conducted using continuous sampling of focal group. A total of 73.97 minutes of recording revealed vocalizations that were associated with five behavioral contexts; calls were evaluated on ten acoustic parameters. The Cape parrot's repertoire was revealed to have a high diversity of call types, with several being behaviorally specific. Contextual flexibility was also observed as several call types were utilized throughout behavioral contexts. The Cape parrot is a vulnerable species recently separated from other *Poicephalus* species; this study helps to extend the knowledge of the variation in vocal repertoire for this genus. The Cape parrot's vocal repertoire shows to be significantly larger than previous studies of *Poicephalus*, being three times that of findings for the grey-headed parrot (*Poicephalus suahelicus*). Further exploration into the vocal repertoire of other *Poicephalus* species could be contrasted with this work to provide insight into taxonomic distinctiveness and shared vocal origins of this genus.

Sean G. Kirk

The Identification and Characterization of a Putative Chromosome Segregation Gene in *Streptomyces coelicolor*

Streptomyces coelicolor is a soil bacterium that is a model for bacterial development. It is a filamentous, sporulating bacterium known to produce many medically utilized antibiotics. The goal of this research was to examine several developmental mutants and characterize novel genes of interest. Previously generated random transposon insertion mutants were analyzed using visual and microscopic phenotyping. Mutants of interest were further pursued and each transposon disruption site was identified by Inverse PCR and DNA sequencing. One of the novel genes is suspected to be involved in DNA segregation and codes for a putative membrane protein. Staining with propidium iodide was conducted to visualize DNA under confocal microscopy. Preliminary screening shows a significant, twenty-two percent anucleation rate in this novel mutant as compared to the wild-type parental strain. This is the highest known rate of annucleation in *Streptomyces* segregation mutants. A modified CRISPR-Cas9 system is being utilized to construct a deletion of the putative chromosome segregation gene.

Upon isolation of the deletion mutant, genetic complementation will be completed to confirm that the phenotype is caused by the gene of interest.

Rachel T. Nguyen

Identifying RmdA Protein Interactions in *Streptomyces* Using a Bacterial Two-Hybrid System

Streptomyces is a genus of the phylum actinobacteria most commonly found as soil bacteria and is used as a major source of antibiotics. RmdA and RmdB are phosphodiesterases that break down the ubiquitous second messenger cyclic-di-GMP which determines colony morphology and development of *Streptomyces*. The objective of this research is to determine whether RmdA will have interactions with itself and/or RmdB using the Bacterial Adenylate Cyclase Two-Hybrid (*BACTH*) System. Each gene was cloned and fused into one of two *BACTH* vectors containing the DNA sequence corresponding to a single fragment of the catalytic domain for the adenylate cyclase gene from the bacterium, *Bordetella pertussis*. The adenylate cyclase gene naturally possesses both fragments (T18 and T25). One of the genes was fused with the *BACTH* vectors pKT25 and pKNT25 and the other gene was fused with the other complimentary vectors pUT18C and pUT18. They were then cotransformed into the *BACTH* indicator strain, BTH101. The transformants were then plated on indicator plates, LB-X-Gal and MacConkey-maltose and incubated to qualitatively show their possible interactions. If the proteins interact, they will produce beta-galactosidase on LB-X-Gal or ferment maltose on MacConkey-maltose which will be seen as blue or red colonies respectively. It is hypothesized that RmdA will have interactions with itself through homodimerization as seen in other phosphodiesterases and will possibly interact with RmdB because inactivation of either phosphodiesterase gene results in a similar phenotype. The cotransformation of two plasmids is currently being done to determine whether or not RmdA interacts with itself.

Abigail E. Smith

Prediction Factors for Release of Wildlife at a Midwestern Rehabilitation Facility

Wildlife rehabilitation is a large and internationally known field with thousands of rehabilitators around the world. Resources, such as time and money, are extremely limited in rehabilitation facilities and not all animals are candidates for release due to injuries or illnesses that make them unlikely to survive in the wild. Understanding what factors predict release would be useful in focusing limited resources, but there has been little investigation previously. The purpose of this study is to determine what factors affect the survival of mammals and birds at an American Midwest wildlife rehabilitation center. It was found that animals admitted with trauma and fracture or injuries to the skull, hindlimb/pelvis, spine/tail, or nervous system had low rates of release. Weight was also a factor of release for young mammals; animals that were below average weight for

their life stage were less likely to be released. This data may be of use in improving triage techniques in the American Midwest and abroad.

John Michael Wright

Evaluating *Streptomyces scabies* SCAB27101 Deletion Mutant Life Cycle Alterations

The bacterial genus *Streptomyces* is responsible for the production of most of the world's antibiotics currently available on the market. This research was conducted to further characterize SCAB27101, a gene of the root-vegetable pathogen *Streptomyces scabies*. A microscopy time-course of a strain where the gene was deleted showed a delayed life cycle lagging two days behind the wild-type strain. This supports SCAB27101 being involved in *S. scabies* growth and development. Furthermore, preliminary results show that the deletion mutant may differentially express the phytotoxin thaxtomin A, the major virulence factor of this pathogen.