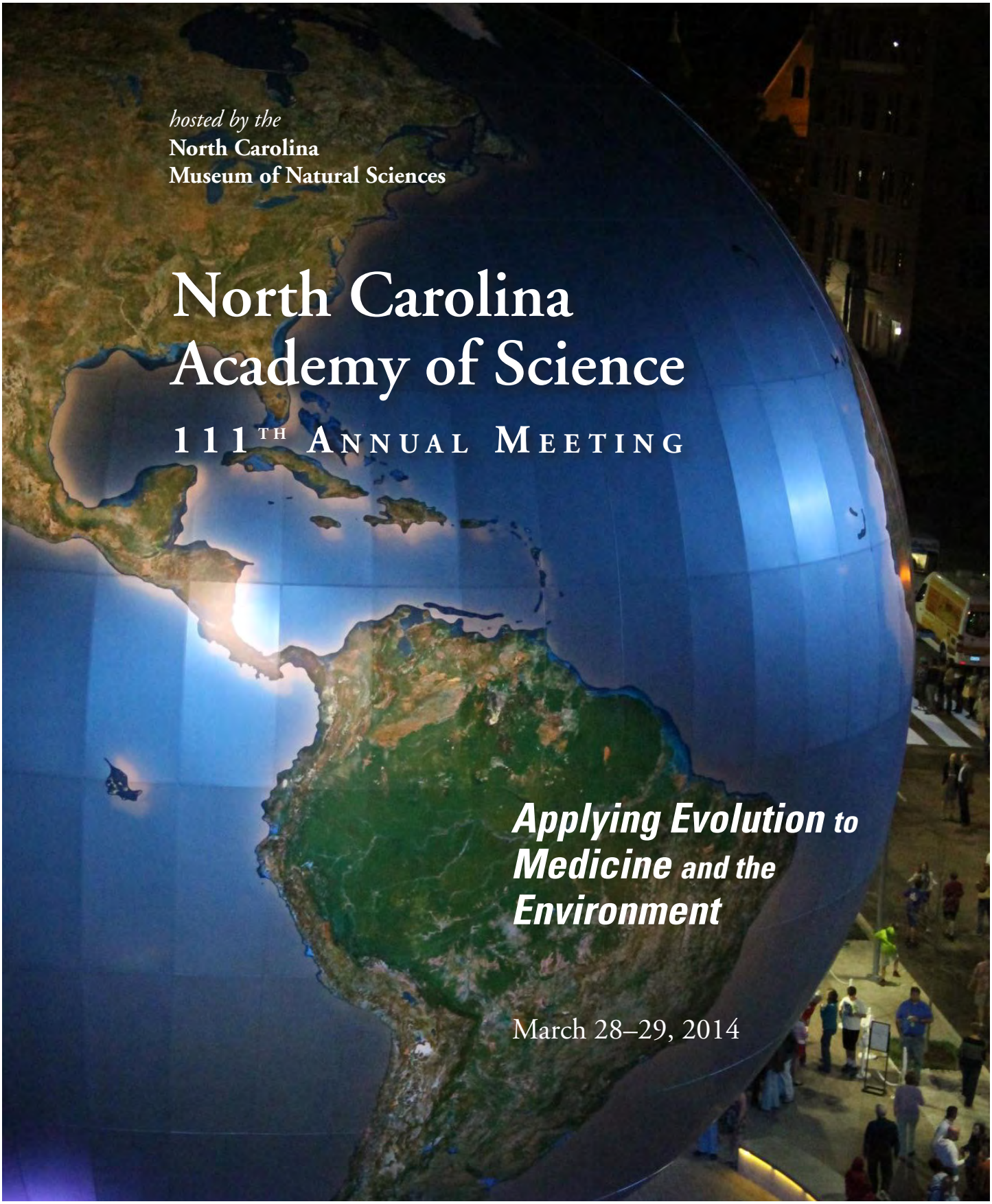

hosted by the
North Carolina
Museum of Natural Sciences

North Carolina Academy of Science

111TH ANNUAL MEETING

Applying Evolution to Medicine and the Environment

March 28–29, 2014

North Carolina Academy of Science
Since 1902

NORTH CAROLINA
Museum of Natural Sciences

WELCOME!

NCAS 2014 Local Arrangements Committee

On behalf of the 2014 Local Arrangements Committee, I would like to welcome you to the North Carolina Museum of Natural Sciences. We hope that you will enjoy all of our engaging speakers and be excited by the opportunity to interact with the public during this meeting. The local arrangements committee worked hard to organize speakers and sessions to engage you in science, so please share your excitement about the meeting and thank them for their hard work when you see them.

We are excited to build up our social media engagement so please follow us on Facebook (http://on.fb.me/1avViAn, http://www.facebook.com/naturalsciences) and Twitter (@NCAcadOfSci, @naturalsciences, @NRCjulie). When you tweet or post to Facebook about the event, please use the **#NCAS2014** hashtag.

Let me know if there is anything I can do to make your experience more enjoyable!

Julie Horvath, *Chair, Local Arrangements Committee*

2014 Local Arrangements Committee Members

Antonio Baines	John Clamp	Cindy Bogan	Bethany Cool
Sarah Council	Carla Davis	Heather Farrington	Julie Horvath
Seth Howe	Cathy Silver-Key	Julie Urban	Kari Wouk
Lisa Yow			

NCAS President's Welcome

Welcome to the 111th Annual Meeting of the North Carolina Academy of Science at the preeminent North Carolina Museum of Natural Sciences! Our theme this year is "Applying Evolution to Medicine and the Environment." This is indeed a fitting venue for our annual meeting because of our shared history and missions. The founding curator of the Museum of Natural Sciences, Herbert H. Brimley, was one of the founding members of the North Carolina Academy of Science. H.H. Brimley and his brother Clement S. Brimley, who later served as the President of NCAS, were enthusiastic and supportive members of the Academy. Both the Museum and the Academy have grown considerably since their founding but both continue in their shared mission to promote public understanding and appreciation of natural science and scientific research.

We are very excited that you could join us as we consider newly emerging scientific perspectives, showcase the talents of the next generation of researchers, promote new collaborations and renew old friendships. This year's meeting features a Friday evening plenary address by Dr. Randolph Neese entitled "Evolution and Medicine: The Great Opportunity" and a Saturday afternoon keynote address by Dr. Rob Dunn entitled "Understanding the Ecology and Evolution of Human Bodies and Homes—Lessons from Students, Solenodons and Face Mites," as well as a variety of engaging workshops, special sessions, and contributed poster and paper sessions.

The Museum's new wing, the Nature Research Center, teaches visitors how we know what we know in addition to engaging the public in the process of scientific inquiry. This offers an unprecedented opportunity for our members to interact with members of the public during an annual meeting event. We hope this stimulates you to engage in meaningful conversations with museum visitors as well as fellow members of the Academy as you share your enthusiasm for science.

I would like to thank Dr. Emlyn Koster, Director of the Museum of Natural Sciences, for inviting us to hold our 111th annual meeting in this historic monument to the natural history of the southeastern U.S. Its collections, exhibitions, and staff have educated the citizens of our state for 135 years. I would also like to commend the outstanding leadership of Dr. Julie Horvath, Director of the Genomics & Microbiology Research Laboratory in the Nature Research Center and jointly appointed in the Biology Department at North Carolina Central University (NCCU), in addition to the many others throughout the Museum and NCCU who worked tirelessly to develop such a timely and interesting program for our 111th Annual NCAS meeting.

Michael Kingston, *NCAS President, 2013-2014*

Museum Director's Welcome

It is with warm "welcome back" sentiments that the North Carolina Museum of Natural Sciences greets the 111th Annual Meeting of North Carolina Academy of Science. On behalf of the State Department of Environment and Natural Resources, as well as the Museum's entire staff and corps of volunteers, the Museum is thrilled to be the public stage for your conference theme of "Applying Evolution to Medicine and the Environment". Since the Museum's founders, the Brimley Brothers, were also a significant founding influence on the Academy, the Museum is honored to host your meeting to highlight our history and grow our partnership. In particular, I wish to commend Academy board member Julie Horvath, PhD, who heads the Museum's genomics research program, for her organizational dedication to your Meeting's success.

Your meeting takes place in both wings of the Museum. The 2000 wing, recently named the Nature Exhibition Center (NEC), focuses on "what we know" with a North Carolina lens. The 2012 Nature Research Center (NRC) wing probes "how we know what we know" with a global research lens. Ranked again this year as providing an outstanding level of community service by the federal Institute of Museum and Library Services, the North Carolina Museum of Natural Sciences is an innovative force for good about the interdependence of humanity and nature. With over one million visitors annually, we encourage you to interact with a sample of them to broaden their appreciation for science while also furthering your interest in public science communication.

We are delighted that you could all join us for this Meeting and hope you will be back soon to delve more deeply into the Museum's remarkable abundance of learning and teaching resources about the natural world. Please be an ambassador for our efforts.

Best wishes,

Emlyn Koster, PhD

Director, NC Museum of Natural Sciences

The North Carolina Museum of Natural Sciences

The North Carolina Museum of Natural Sciences, located in the heart of downtown Raleigh, is the largest institution of its kind in the Southeast. The museum's original mission, "To illustrate...the natural history of the state..." was set down in 1887 by the North Carolina General Assembly. Throughout its 135 year history, the words of the museum's first director, Herbert Hutchinson Brimley, have lighted the way: "The building of a museum is a never-ending work. A finished museum is a dead museum, and such a one must deteriorate and begin to lose usefulness from the time its growth stops."

In 2000, the Museum opened its current, seven-story, 200,000 square-foot facility. It features a 265-seat high-definition 3D theater, the most complete *Acrocanthosaurus* skeleton on display in the world, a distance learning theater that facilitates statewide outreach, and is home to enough live animals (used in programming and incorporated into permanent exhibits) to qualify it as a small zoo.

In 2005, the Museum expanded to include Prairie Ridge Ecostation, a 46-acre field station located a short drive from the Museum and used as an outdoor classroom to reconnect the public with nature. Comprised of restored prairies, ponds, other habitats and walking trails, Prairie Ridge is an environmental oasis nestled in northwest Raleigh.

In April 2012, the Museum expanded again by opening a new wing, the Nature Research Center (NRC). The NRC is an 80,000 square-foot "public laboratory" where visitors can experience science in action by visiting state-of-the-art labs and participating in real scientific research (such as sequencing DNA), observing veterinary staff perform medical procedures on Museum animals and listening to presentations by scientists on their ongoing research.

Within North Carolina, the Museum has become annually the most visited museum and one of the top overall attractions, welcoming more than 1 million visitors for the second year in a row in 2013. Also for the second consecutive year, the Museum was named a Finalist for the National Medal for Museum and Library Service, the nation's highest honor conferred on museums and libraries for service to the community.

SPONSORS & VENDORS

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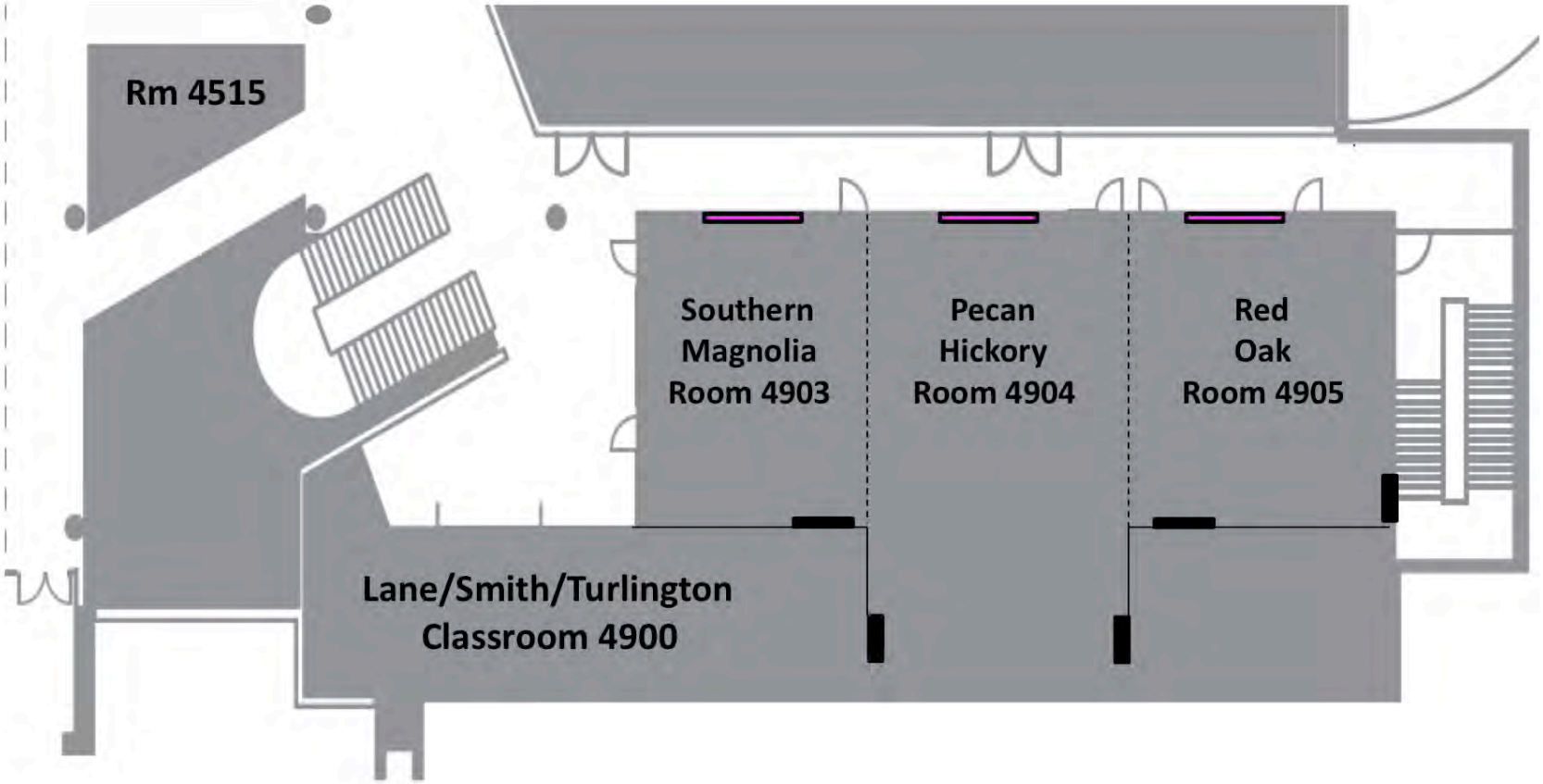
Nature Research Center at 121 W. Jones Street, Raleigh, NC
NRC Fourth Floor and Environmental Conference Center



Summary Schedule

Friday, March 28, 2014

1:00-2:00 **Finance & Strategic Planning Committee Meetings,** Nature Research Center (NRC), 4th Floor Workroom Rm 4515

2:00-5:00 **Board of Director's Meeting,** NRC 4th Floor Lane/Smith/Turlington Classroom Rm 4900

5:00-6:30 **VIP Cocktail Event,** Main Wing 5th Floor Board Room

5:15-6:00 **Registration and Vendor Setup,** NRC First Floor Lobby

5:15-6:00 **Poster Setup for Collegiate Academy (CANCAS),** NRC First Floor

5:15-6:00 **Poster Setup for Senior Academy (NCAS),** NRC Second Floor

5:30-6:00 **Poster Judges Meeting,** NRC 4th Floor Lane/Smith/Turlington Classroom Rm 4900

6:00-7:30 **Poster Session and Reception with heavy hors d`oeuvres,** NRC 1st and 2nd Floors

7:15-7:45 **Poster Judges Meeting,** NRC 4th Floor Lane/Smith/Turlington Classroom Rm 4900

7:45-9:30 **Welcome Remarks & Plenary Address**

Daily Planet Theater and NRC 4th Floor, Environmental Conference Center

Welcome Remarks

Dr. Julie Horvath, Local Arrangements Committee Chair

Dr. Emlyn Koster, Director, NC Museum of Natural Sciences

Dr. Michael Kingston, Elon University, President of NCAS

Plenary Address

Dr. Randolph Nesse, Arizona State University
"Evolution and Medicine: The Great Opportunity"

Saturday, March 29, 2014

7:30-8:15 **Judges & Session Moderators Meeting,** NRC 4th Floor Lane/Smith/Turlington Classroom Rm 4900

8:00-4:00 **Registration,** NRC 1st Floor

8:00-10:00 **Student Academy Poster Setup,** NRC 2nd Floor

8:00-10:15 **Breakfast,** NRC 4th Floor

8:00-3:00 **Practice Presentation Preview Room (with projector and computer),** NRC 4th Floor Workroom Rm 4515

8:30-1:30 **Student Academy (NCSAS) Posters Available for Viewing**

(attended from 12:45-1:45) NRC 2nd Floor Windows on Research

8:30-4:00 **Exhibits of Sponsors & Vendors,** Nature Research Center

8:30-9:45 **Oral Session I,** NRC 4th Floor, specific rooms indicated in program for each topic session

9:45-10:15 **Coffee Break,** NRC 4th Floor

10:15-11:15 **Oral Session II,** NRC 4th Floor, specific rooms indicated in program for each topic session

10:00-11:00 **Student Academy Brunch,** Main Wing (NEC) 4th Floor "Explore on Four" Room *__Meet Volunteer at Registration Desk at 9:55am__*

11:30-11:35 **Welcome Remarks, NRC Daily Planet Theater**

***Viewable in ECC, 4th Floor NRC**

Dr. Julie Horvath, Local Arrangements Committee Chair

Dr. Michael Kingston, Elon University, President of NCAS

11:35-11:45 **Dr. Jim Fuller, Award Presentation, Daily Planet Theater**

11:45-12:30 **Keynote Address, Daily Planet Theater & NRC 4th Floor ECC**

Dr. Rob Dunn, North Carolina State University

"Understanding the Ecology and Evolution of Human Bodies and Homes—Lessons from Students, Solenodons and Face Mites"

12:45-1:45 **Box Lunches,** NRC 4th Floor

Seating available in all NRC 4th Floor rooms or outside 4th or 1st Floors

1:15-2:15 **Student Academy (NCSAS and CANCAS) Poster Session Judges Meeting,** NRC 3rd Floor Instrumentation Lab

2:00-3:00 **Special Sessions,** NRC 4th Floor, specific rooms indicated below

The One Health Challenge: Respecting the Many Interfaces between People, Animals, and the Environment, Southern Magnolia Rm 4903

Physiology of Wearable Robots, Lane/Smith Classroom Rm 4900

A Science Comedian's Guide to Communicating Science, Pecan Hickory Rm 4904

Exploring the Solar System: Voyage to the Sun, Asteroid Belt, and Beyond, Red Oak Rm 4905

3:00-3:15 **Coffee Break,** NRC 4th Floor

3:15-4:15 **Oral Session III,** NRC 4th Floor, specific rooms indicated in program for each topic session

4:30-5:30 **Workshops**

Big Data Science and You, Lane/Smith/Turlington Classroom Rm 4900

Macrophotography of Tiny Arthropods, Red Oak Rm 4905

What Citizens? How Science?, Southern Magnolia Rm 4903

Graduate and Professional Panel Discussion, Pecan Hickory Rm 4904

5:00-5:30 **CANCAS Officers Award Preparation Meeting,** NRC 3rd Floor Instrumentation Lab

5:30-6:30 **NCAS Business Meeting,*** Pecan Hickory Rm 4904

5:30-6:30 **CANCAS Business Meeting,*** Southern Magnolia Rm 4903

5:30-6:30 **Free time on Floors 2-4 of Main Wing**-Please visit floors 2-4 **only** during this time as the caterers will be setting up on the 1st floor

6:30-8:30 **Banquet & Awards Ceremony, Main Wing 1st Floor**

8:30-9:00 **Board of Directors Meeting,** Southern Magnolia Rm 4903

* Everyone is welcome to participate in the business meetings. All participants are considered members of the NCAS or CANCAS because membership is included in the registration for those who were previously non-members.

Plenary Address

Dr. Randolph Nesse

Arizona State University

Presentation: "Evolution and Medicine: The Great Opportunity"

Randolph Nesse is a Professor of Life Science at Arizona State University where he directs the Center for Evolution, Medicine, & Public Health. His research ranges from the neuroendocrinology of anxiety to the evolution of altruism. At the center is his preoccupation with finding evolutionary explanations for traits that leave bodies vulnerable to diseases. In collaboration with George Williams and others, this work has given rise to the field of evolutionary medicine. His mission is to establish evolutionary biology as a basic science for medicine and public health. He is eager to make connections with others who share this goal. http://RandolphNesse.com, http://EvMedReview.com

Keynote Address

Dr. Rob Dunn

North Carolina State University

Presentation: "Understanding the Ecology and Evolution of Human Bodies and Homes -- Lessons from Students, Solenodons and Face Mites"

Rob Dunn is an evolutionary biologist and writer at North Carolina State University. His research focuses on the ecology and evolution of species that live in our backyards, homes or bodies (www.yourwildlife.org). His writing tells the stories of biological discovery and mystery (www.robrdunn.com). His writing appears in National Geographic, Scientific American, Natural History, Nature Magazine, BBC Wildlife, New Scientist and in many other magazines. His first book, Every Living Thing, told the story of the search for the limits of life. His second book, The Wild Life of Our Bodies, considered the influence of wild species (be they parasites, symbionts or predators) on our health and well-being. His newest book (to be published in the spring), "The Man Who Touched his Own Heart," considers the human heart, its history, surgeries, evolution and mysteries.

Friday Schedule Details

Poster Session and Reception with Heavy Hors d'oeuvres

Friday Evening, Nature Research Center, First Floor

6:00-7:30 pm

Entertainment provided by Jacob Dowdy
Playing a collection of instrumental jazz and pop tunes on solo electric guitar

CANCAS Poster Session & Titles

Zoology, Botany, Ecology and Environmental Science

1. *__Privette, Zachary W__., Chelsea L. Crocker, Ismael Gomez, Sean P. Graham and David A. Beamer. *The Phylogeography of the Northern Seepage Salamander (Desmognathus aeneus)*
2. *__Hernandez, Joel C__., Shelby L. Spencer, Justin J. Joyner, Sean P. Graham and David A. Beamer. *The Phylogeography of the Southern Seepage Salamander (Desmognathus aeneus)*
3. *__Akers, Nathaniel T.__, and David A. Beamer. *Does the Northern Two-Lined Salamander* (*Eurycea bislineata) occur in North Carolina?*
4. *__Crocker, Chelsea L.__, Zachary W. Privette, Ismael Gomez, Sean P. Graham* and David A. Beamer. *Independent Nuclear Markers Reveal Cryptic Diversity within Seepage Salamanders (Desmognathus aeneus)*
5. Spillman, Taylor, *__Finn Furstenwerth__, Duncan Cameron, Jay Bolin. *Isotopic Investigation of Mycoheterotrophy in the Southern Blue Thread (Burmannia capitata)*
6. *__Masecar, Susie__ and Amanda Chunco. *Effects of Varying Agricultural Practices on Mammal Diversity*
7. *__Stewart, Anthony C.,__ Martin Tsz-Ki Tsui, and Parke A Rublee. *Development of Mercury Methylating Communities in Contaminated Sediments*

Cell & Development, & Physiology

8. *__Askew, Lauren__, Claire Gordy, and Mara Duncan. *Glucose Starvation Induces Microautophagy in Yeast Cells*
9. *__Okechukwu, Charles__. *Contribution of Thyroid Hormone Levels to Estrogen Receptor Activation in Breast Cancer Cells*
10. *__Powell, Destinee__ and Dayami Lopez. *Testing of a Rat LDL Receptor Minigene in Hypothyroid Medium*
11. *__Fuller, Rachelle__, Melissa Macias, Karen Guzman. *Expression of Chondrocyte Markers in ATDC5 Cells Exposed to Osmotic Stress*
12. *__Bealer, Warren E. III__, Qingping He, and Jonathan Sexton. *High-Throughput Screening and Cell-Based Assays to Discover Small Molecules That Overcome Endogenous GTP Inhibition of L-Glutamate Dehydrogenase in Cellular Respiration*

13. *__Baba, Kayla__, Brittani Hodges*, Karen Guzman. *Expression of Oxidative Stress Markers in Zebrafish After Acetaminophen Treatment*
14. __Sumner, Chelsea,__ *Eunbyeol Goh,* Erin Byrd, Dr. Sharon Mason, Dr. Michelle Thomas. *Isolation and Characterization of Fluoroquinolone Resistant Gram-Negative Bacteria From Hog Fecal Samples*
15. *__Rahman, Meredith__, Michael C. Granatosky, and Daniel Schmitt. *Effects of habitual loading on bone material properties in the mammalian post-cranial skeleton*
16. *__Valle, Tania__ and John A. Mecham. *Comparative Muscle Sarcomere Lengths*

Microbiology, Molecular Biology and Genetics

17. __Chafin, Grace L.__, James W. Brown, Melanie J. Lee-Brown. *Mutagenesis of the FMN Riboswitch of Photorhabdus luminescens and an analysis of mutagenic effects on its pathogenicity on Caenorhabditis elegans*
18. *__Long, Christopher, H__. Romine, J. Hwang, S. Ruiz, B. A. Bahr, and U. S. Ikonne. *A Clearance Pathway for Treating Age-related Diseases: Enhancing the Lysosomal Pathway with PADK Promotes Protein Clearance in Models of Alzheimer's and Parkinson's Disease*
19. *__Kienka, Tamina__, Horia Todor, Amy Schmid. *The Effect of Carbohydrates on Cell Morphology in Halobacterium salinarum*
20. *__Llorente, Kasey M__., Jennifer K. Uno. *The effect of Valproic Acid on Notch Signaling in Intestinal Cell development in Danio rerio*
21. *__Stubbs, Caleb__ and Maria S. Santisteban. *Investigating Mutant Suppressor of Synthetic Lethality between htz1D and RPB2-2^{SL} in Saccharomyces cerevisiae*
22. *__Fowler, Conner__, Morgan Packer, Michael Wisniewski, Gregory Buhrman, and Taek You. *Engineering and Expressing an Upstream Insulin Promoter-binding Fusion Protein*
23. *__Neal, Aaron J.__, Lauren E. Chun, & Robert L. Spencer, *Basal mRNA Expression of Clock Gene Bmal in the Hippocampus and the Amygdala of Male and Female Rats*
24. *__Corona, Armando__, P.M.P. Quizon, H. Romine, W. Kelly, V. Naidoo, S. Ruiz, S. Suggs, B. Mbugua, R. Jackson, L. Elliott, C. Long, J. Ellerbe, K. Stephens, J. Showers, A. Makriyannis, and B.A. Bahr. *Drug Discovery Efforts to Enhance Neuroprotective Endocannabinoid Signaling through Dual Inhibition of FAAH and MAGL Enzyme*

Chemistry, Physics, Biotechnology and Behavioral Science

25. *__McCormick, John__. *Inhibition of Peroxynitrate-Induced DNA Damage by Caffeine*
26. *__Conte, Juliana__, Thomas Benton, Carmony Hartwig and Mark Sabo. *Investigating Peroxidation of Lipid by Heme-Artemisinin Adducts in vitro*
27. *__Kinney, Garrison__. *Synthesis of Peropyrene Derivatives*
28. *__Shields, David__, Nahibi Maldonado and Stephannie Walker. *The Informed Consent Process: The Effectiveness of Alternative Delivery Methods*
29. *__Chouinard, Roxanne__, Francesca Ceppi, Steven Greco, William Martin and Stephannie Walker. *Comparison of Physiological Reactions to Stress in College Freshman and Senior Student-Athletes*
30. *__Howard, Jason__ and D. Shukla. *Exploring charge dependence of the strong force by modeling neutron-proton scattering*

31. *__Foster, A. C.__, and W.D. Brandon. *Novel Approach to a Generalized Theory on Nanostructure Devices*
32. *__Bullock, Bianca__, Samantha Whitaker, Jeffrey E. Barrick, Scott H. Harrison. *Degeneracy and Reassignment of Instruction Sets in a Digital Evolution Experiment*

NCAS Posters

Friday Evening, Nature Research Center, Second Floor 6:00-7:30 pm

Microbiology, Ecology, Environmental Science and Botany

1. **Spurrier, Ariel,** and Parke A. Rublee. *Abundance of Select Cyanobacteria OTUs in Six North Carolina Drinking Water Supply Reservoirs*
2. **Shah Halley**, Christina Saunders*, Adam Speen, Elizabeth Brooke, Palanisamy Nallasamy, Hong Zhu, Yunbo Li, Zhenquan Jia. *Protection of HepG2 Cells from Acrolein Toxicity by CDDO-Im via Glutathione-Mediated Mechanism*
3. **Mickle, James**, Maria Rosaria Barone Lumaga, Mario Coiro, Boglarka Erdei *Cycad cuticle micromorphology: What role does ecological pressure play?*
4. **Blackburn, Joe**, Eric S. Anderson, Matthew O. Schrenk. *Serpentinizing Environments as a Potential Source of Novel Antibiotic Compound*
5. **Ayad, Amira**, D. A. Gad El-Rab, and S. A. Ibrahim. *Acid and Bile Salt Tolerance of Microencapsulated Probiotics Strains*

Immunology, Molecular Biology and Biotechnology

6. **Hughey, Joshua**, Tamina Kienka, Vijay Sivaraman. *Examination of Mediators of Interleukin-1 Receptor Antagonist (IL-1RA) duringYersinia Pestis Pulmonary Infection*
7. **Sengupta, Dipendra, Jharna Sengupta** and Scott Armel. *Analyzing DNA sequences through Graph Entropy and Chaos Game*
8. **Faulkner, Nicholas**, TW Christensen, PL Fletcher Jr. and ES Anderson. *Expression of a Novel Scorpion Venom Metalloprotease* in *Escherichia coli*

Cell Biology and Development

9. **Lehman, Emily**, and Paul Steimle. *Cellular studies of Dictyostelium myosin II heavy chain kinase D*
10. **Herring, Ashely**, Kyle Burgetta and Paul Steimle. *Studies of the signal transduction pathways regulating myosin II localization and activity in the social amoeba Dictyostelium discoideum*
11. **Alonzo-Johnsen, Martha**, Margaret Kirby, Kathleen Smith, Mary Hutson, Julie Horvath (Sponsor). *The Role of Cardiac Neural Crest in Outflow Tract Septation of Danio rerio and Trachemys scripta*
12. **Chitrakar, Rojin**, Halley Shah, Adam Speen, John McCormick, Palanisamy Nallasamy, Philna Joubert, Hong Zhu, Yunbo Li, Zhenquan Jia. *Acrolein toxicity in endothelial cells involves lipid peroxidation, protein damage, reduced cellular GSH, and augmented monocyte adhesion*

Plenary Address

Friday, Daily Planet Theater (NRC 1st Floor) & Environmental Conference Center (ECC), NRC 4th Floor

7:45-8:00 **Welcome Remarks**

Dr. Julie Horvath, Local Arrangements Committee Chair

Dr. Emlyn Koster, Director, NC Museum of Natural Sciences

Dr. Michael Kingston, Elon University, President of NCAS

8:00-9:30 **Plenary Address,**

Dr. Randolph Nesse, Arizona State University
"Evolution and Medicine: The Great Opportunity"

Saturday Schedule Details

Oral Session I

Saturday Morning, ECC, Nature Research Center 4th Floor,
specific rooms indicated below

8:30-9:45 am

Physics and Engineering

Southern Magnolia Rm 4903

8:30-8:45 *__Stewart-Nunez, Michi__. *Shining a Light on the Physics of Charge Transfer within Organic Photovoltaics*

8:45-9:00 *__Thompson, Marc__, Pinar Huri and Warren Grayson. *Printing Cell-Laden Alginate Fibers*

9:00-9:15 *__Macklin, Bria__, Sravanti Kusuma and Sharon Gerecht. *Encapsulation of Derived Bicellular Vascular Population in Type I Collagen Gel*

9:15-9:30 *__Brown, Jazmine__, George Anderson and Ellen Goldman. *Thermal Stability and Refolding Capability of Shark VNAR*

9:30-9:45 *__Smith, Marsalis__. *Development of a Calibration Scheme for In Vivo Microiontophoresis*

Microbiology and Chemistry

Pecan Hickory Rm 4904

8:30-8:45 *__Worley, Jonathan__, Melanie Lee Brown and James W. Brown. *Characterization of Pseudomona* sp. *(ATCC 55646)*

8:45-9:00 *__Lusk, Niageria__, Samantha Palace and Jon Goguen. *Developing a novel class of antibiotics: targeting essential metabolic pathways to combat antibiotic resistance*

9:00-9:15 *__Biggs, Jeannette__, Melanie Lee Brown and James W. Brown. *Multi-locus-sequence-typing of Azotobacter zettuovi: an organism without a place*

9:15-9:30 *__Dietrich, Sarah__, Steven Cartier, Langdon Martin, David Ellum, Katherine A. Borkovich, Johnathon R. Blahut, Loren E. Prudhomme, Patrick C. Schacht, Ilva E. Cabrera. *Phenotypic Analysis of Transcription Factor Mutants in Neurospora crassa*

9:30-9:45 *__Healey, Mark.__ *Solvent Effects on the Copper(I)/TEMPO-Catalyzed Aerobic Oxidation of Primary Benzylic Alcohols*

Genetics

Red Oak Rm 4905

8:30-8:45 *__Nelson, Jonathan__, Shan Yu and Karen Katula. *Epigenetic Modulation of WNT5A Expression in the Human Colorectal Cell Line HCT-116*

8:45-9:00 *__McRae, Briana__ and S. Catherine Silver Key. *Comparing the Dot chromosome of Drosophila biarmipes to Drosophila melanogaster based on similarities in the amino acid sequences of the exons*

9:00-9:15 *__Harrison, Krystal__, Sowjanya Kallakuri and Zhaoxia Sun. *Dissecting the underlying mechanisms involved in brain hemorrhage in cilia mutants*

9:15-9:30 *__Worthy, Kelsey__, Zhong Fang and Patricia C. Dos Santos. *The Role of Cysteine Desulfurase (NifS) of Bacillus subtilis in the Assembly of the [4Fe-4S] Cluster of Quinolinate Synthase (NadA)*

Ecology

Lane/Smith/Turlington Classroom Rm 4900

8:30-8:45 *__York, Joshua__, Jael Martinez, Marlon Barber, Bruce Harrison and Carmony Hartwig. *Morphological and Molecular Identification of Mosquito Diversity in the Fred Stanback Jr. Ecological Preserve at Catawba College, Salisbury, North Carolina*

8:45-9:00 *__Moore, C. Travis__ and Megan White. *A Tale of Two Gardens: Comparing the Aquatic Ecosystems of Two Public Gardens, Greensboro, NC*

9:00-9:15 *__Beaman, Macy__, Lisa Bonner and Patrick Myer. *Effects of Varying Flow, Temperature and Turbidity on Macroinvertebrate Drift and Colonization Patterns*

9:15-9:30 Elliott, Todd F., ***Armin Weise.** *Biodiversity Studies of Ectomycorrhizal Macrofungi of the Guiana Shield*

9:30-9:45 *Dunbar, Matthew G., Nathalie Fortin, Guillaume Meisterhans, Christine Michel, Thomas L. King, Charles W. Greer. *Bioremediation of Oil in the Arctic Sea by Indigenous Bacteria*

Oral Session II

Saturday Morning, ECC, Nature Research Center, specific rooms indicated below

10:15-11:15 am

Environmental Science

Southern Magnolia Rm 4903

10:15-10:30 *__Williams, Stephanie.__ *Comparison of Metal Concentrations between Aquatic and Terrestrial Salamanders across Three Populations to Determine Life History*

10:30-10:45 *__Overbey, Katie__ and Jill Stewart. *Sewage Impacts on Water Quality and Antibiotic Resistance in Beach Waters in the Galápagos Islands*

10:45-11:00 * __Marable, Carmen,__ Barbara McGarrigle, Steven Singleton, and James R. Olson. (NCAT) *Cytochrome P-450 Variants as Potential Biomarkers of Sensitivity to Chlorpyrifos*

11:00-11:15 *__Olearczyk, Avery__. *Fire's Impact on Den Creation for Petaurus australis in Eucalyptus grandis Trees*

Health and Behavioral Sciences

Pecan Hickory Rm 4904

10:15-10:30 *__Bearfield, Logan__. *Effect of a Chemotherapeutic Agent, Rapamycin, on Growth of Lactobacillus acidophilus and Escherichia coli*

10:30-10:45 *__Konzman, Daniel__ and Linda Niedziela__.__ *The Effect of Pentylenetetrazol-induced Seizure and the Antiepileptic Drug Phenytoin on Spatial Learning in Zebrafish*

10:45-11:00 *__Pannier, Katie__. *An Epidemiologic Exploration of Tobacco Smoking at Warren Wilson College*

11:00-11:15 *__Baldwin, Holly__. *The Effect of Negative Stimuli on the Directional Preference of Planaria (Dugesia tigrina)*

Molecular Biology

Red Oak Rm 4905

10:15-10:30 *__Thomas, Phillip__, Richard Graveline, Seyun Choi and Gregory David. *An Unbiased Screen Identifies New Pf1 Interactions, with Biological Significance in Chromatin Modification and Transcriptional Control*

10:30-10:45 *__Khayat, Michael__, James W. Brown, Melanie Lee Brown and Robert Whitnell. *Modeling and Mutagenesis of the FMN Riboswitch*

10:45-11:00 *__Clarke, David__, Tiffany Kennedy and Robert H. Newman. *Development of an in vitro Assay Platform for the Characterization of Genetically-encodable Fluorescent Biosensors of Kinase Activity*

11:00-11:15 *__Sharpe, Iman__, Mehrdad Tajkarimi, Joseph L. Graves and Scott H. Harrison. *Genomic Analysis of Metal Toxin Resistance Phenotypes in Bacteria*

Zoology and Botany

Lane/Smith/Turlington Classroom Rm 4900

10:15-10:30 *__Caine, LaShonda__ and David A. Beamer. *Extreme genetic homogeneity: Phylogeography of the red and mud salamanders*

10:30-10:45 *__Jones, Brittany__, A.L. Lee, __C. S. Tart__, C.L. Martinez, K.M. Lowe and K.A. Malik. *Direct high frequency shoot regeneration from mature seeds in legume*

10:45-11:00 *__Avila, Jessica__, Brenten L. Bottoms and David. A. Beamer. *Morphological Homoplasy within Mountain Dusky Lineages*

11:00-11:15 *__Patton, Austin__, J.J. Apodaca, Lori Williams and Alan Cameron. *Conservation Genetics of the Green Salamander, A. aeneus in Western North Carolina*

Keynote Presentation

Saturday, Daily Planet & ECC, Nature Research Center,

11:30-11:35 **Welcome Remarks**

Dr. Julie Horvath, Local Arrangements Committee Chair

Dr. Michael Kingston, Elon University, President of NCAS

11:35-11:45 Dr. Jim Fuller, Award Presentation

11:45-12:30 **Keynote Address, Daily Planet Theater**

Dr. Rob Dunn, North Carolina State University

"Understanding the Ecology and Evolution of Human Bodies and Homes -- Lessons from Students, Solenodons, and Face Mites"

Student Academy Posters

Saturday, 2nd Floor, Nature Research Center, Windows on Research at Seating Area

12:45-1:45pm

Students will be presenting their posters during this time. Please stop by and chat with them.

James Burcham	Victor Catalan	Lauren Cook
Justin Coye	Indira Gutierrez	Genevie Hernandez
Jeremia Kelly	Sharon Jones	Ricky Jones
Nicholas Rios	Jennifer Wu	Chris Yuan

Special Sessions

Saturday Afternoon, ECC, Nature Research Center, specific rooms indicated below

2:00-3:00 pm

The One Health Challenge: Respecting the Many Interfaces between People, Animals, and the Environment, Southern Magnolia Rm 4903

Physiology of Wearable Robots, Lane/Smith/Turlington Classroom Rm 4900

A Science Comedian's Guide to Communicating Science, Pecan Hickory Rm 4904

Exploring the Solar System: Voyage to the Sun, Asteroid Belt, and Beyond, Red Oak Rm 4905

Oral Session III (NCAS)

Saturday Afternoon, ECC, Nature Research Center, specific rooms indicated below

3:15-4:30 pm

Ecology, Environmental Science and Botany

Southern Magnolia Rm 4903

3:15-3:30 **Locklear, Jared**, and James E. Mickle. Vitis *seeds from the Pleistocene of the Atlantic Coastal Plain of North Carolina*

3:30-3:45 **Fondario Grubbs**, Laura and Parke A. Rublee. *Utilization of qPCR to assess Select Cyanobacteria and Cyanotoxin Abundance in Six Piedmont North Carolina Lakes*

3:45-4:00 **Larsen, Angela**, Jessica Homyack, T. Bently Wigley, Darren Miller and Matina Kalcounis-Rueppell. *Alterations in Behavior Drive Population and Community Dynamics of Rodents Associated with Intercropping Switchgrass in Pine Forests*

4:00-4:15 **White, Joseph C**. and William K. Smith. *Seasonal variation in water sources of the riparian tree species Acer negundo and Betula nigra in the southern Appalachian foothills, USA*

4:15-4:30 **Beamer, David A.** *8000 Salamanders And How I Found Them: A Robust Sampling Strategy For The Southeastern United States*

Evolution and Zoology

Pecan Hickory Rm 4904

3:15-3:30 **Council, Sarah E**., Amy M Savage, Julie M. Urban, Megan E Ehlers, Robert R Dunn and Julie E Horvath. *The Diversity and Evolution of the Primate Skin Microbiome: How different are humans from our closest relatives?*

3:30-3:45 **Perschbacher, Peter W**. and Frank J. Schwartz. *The Marked Goby (Ctenogobius stigmaticus): A Rare North Carolina Fish Enigma*

3:45-4:00 **Granatosky, Michael C**., and Daniel Schmitt. *Mechanical Strategies in Primate Locomotor Switching, and Implications for the Evolution of Locomotor Diversity*

4:00-4:15 **Akond, Masum**, Shiming Liu, Melanie Boney, Stella K. Kantartzi, Khalid Meksem, Nacer Bellaloui, David A. Lightfoot, and My Abdelmajid Kassem. *Identification of Quantitative Trait Loci (QTL) Underlying Protein, Oil, and Five Major Fatty Acids' Contents in Soybean*

Microbiology, Genetics & Molecular Biology

Red Oak Rm 4905

3:15-3:30 **Rumph, Candie** and Karen Katula. *WNT5A Promoter B is Silenced by DNA Methylation in Osteosarcoma Cells*

3:30-3:45 **Harkins, Melissa**, Tamatha Baxley, Margit Schmidt, Eric Anderson, Joseph Chalovich and Jean-Luc Scemama. *Myopodin, an actin binding protein, is localized in the nucleus and nucleoli of cancer cells*

3:45-4:00 **Morgan, Tia**, Ronald R. McMillan, Daniel Williams. *MtrR Regulates Two Major Lytic Transglycosylases Responsible for Peptidoglycan-Derived Cytotoxin Release and Autolysis in Neisseria gonorrhoeae*

4:00-4:15 **Sivaraman, Vijay**, Roger Pechous and William E. Goldman. *Characterization of Early Immune Responses During Primary Pneumonic Plague*

4:15-4:30 **Akindahunsi, Oluwole**, Karen Katula. *The Functional Distinctions between Human WNT5A isoforms A and B*

Chemistry, Math & Science Education

Lane/Smith Turlington Classroom Rm 4900

3:15-3:30 **Guo, Longnail**, Yijun Yu and Naima Naheed. *Theoretical solution of the operations research model for satisfactory highway cruising Speed*

3:30-3:45 **Shtukar, Uladzimir**. *Remark about Simple, Compound and Continuous Interest*

3:45-4:00 **Goller, Carlos C**. *Analyzing big data using simple flowcharts and questioning techniques*

4:00-4:15 **McCombs, Nikolette L.** and Reza Ghiladi. *Peroxygenase and Oxidase Activities of Dehaloperoxidase-Hemoglobin from Amphitrite ornata*

Workshops

Saturday Afternoon, ECC, Nature Research Center, specific rooms indicated below

4:30-5:30 pm

Big Data Science and You, Lane/Smith/Turlington Classroom Rm 4900

Macrophotography of Tiny Arthropods, Red Oak Rm 4905

What Citizens? How Science? Southern Magnolia Rm 4903

Graduate and Professional Panel Discussion, Pecan Hickory Rm 4904

Business Meetings

Saturday Afternoon, ECC, Nature Research Center, specific rooms indicated below

5:30-6:30 **NCAS Business Meeting,*** Pecan Hickory Rm 4904

5:30-6:30 **CANCAS Business Meeting,*** Southern Magnolia Rm 4903

5:30-6:30 **Free time on Floors 2-4, Main Wing of Museum**-Please visit floors 2-4 only during this time as the caterers will be setting up on the first floor

Banquet

Saturday Evening, Main Wing 1st Floor

Entertainment provided by the Graffiti Monkeys

6:30-8:30 pm

Board of Directors Meeting

Saturday Evening, ECC, Southern Magnolia Rm 4903

8:30-10:00 pm

Workshop & Special Session Speakers

Special Session Speakers

Dr. Suzanne Kennedy-Stoskopf
North Carolina State University College of Veterinary Medicine

Special Session: The One Health Challenge: Respecting the Many Interfaces between People, Animals, and the Environment

Suzanne Kennedy-Stoskopf received her DVM from Michigan State University in 1976 and was the first female veterinarian at the National Zoo between 1976 and 1978. She taught at the University of Tennessee, College of Veterinary Medicine before earning her PhD in Immunology and Infectious Diseases from Johns Hopkins University School of Hygiene and Public Health in 1986. She has been at NC State University College of Veterinary Medicine since 1990. Her current research focuses on spatial/temporal interactions of wildlife and disease transmission. She is actively involved with the North Carolina One Health Collaborative and promoting the concept of One Health.

Dr. Gregory Sawicki
North Carolina State University and University of North Carolina at Chapel Hill

Special Session: Physiology of Wearable Robots

Dr. Gregory S. Sawicki is an Assistant Professor in the Joint Department of Biomedical Engineering at North Carolina State University and UNC-Chapel Hill. Dr. Sawicki's research area is Rehabilitation Engineering. He directs the Human Physiology of Wearable Robotics (PoWeR) laboratory focusing on uncovering fundamental principles of locomotion mechanics, energetics and neural control in both healthy and impaired (e.g. stroke and spinal cord injury) populations. The long term vision of the Human PoWeR lab is to exploit useful principles of human locomotion- applying them to motivate bio-inspired designs for state of the art lower-limb prostheses and exoskeletons.

Mr. Brian Malow
North Carolina Museum of Natural Sciences

Special Session: A Science Comedian's Guide to Communicating Science

Brian Malow is Earth's premier science comedian (self-proclaimed). He has worked with the NSF, AAAS, NASA, ACS, AGU, and many other acronyms. He has produced science videos for Time Magazine's website and audio essays for Neil deGrasse Tyson's radio show. Malow has appeared on "The Late Late Show with Craig Ferguson" and co-hosted "Hacking the Planet" and "The Truth About Twisters" on The Weather Channel. He currently blogs for Scientific American and works in science communications at the North Carolina Museum of Natural Sciences.

Dr. Rachel Smith
North Carolina Museum of Natural Sciences & Appalachian State University

Special Session: Exploring the Solar System: Voyage to the Sun, Asteroid Belt, and Beyond

Dr. Smith is an observational astronomer investigating how protoplanetary systems in our Galaxy evolve over time. Using the world's largest ground-based telescopes, Smith observes the ice and gas surrounding forming stars (called protostars), and compares these observations with data from meteorites and the Sun to help understand the chemical evolution of the early solar system. Smith is currently using the 10-meter Keck Telescope in Hawaii to observe the ice and gas surrounding massive protostars. Dr. Smith is Director of the Astronomy & Astrophysics Research Lab at the North Carolina Museum of Natural Sciences, and an Assistant Professor in Physics & Astronomy at Appalachian State University. Dr. Smith received her PhD from UCLA in 2011, and conducted a brief post-doc in Planetary Science at the California Institute of Technology before arriving at the museum in late 2011.

Workshop Speakers

Dr. ClarLynda Williams-DeVane
North Carolina Central University

Workshop: Big Data Science and You

Dr. Williams-DeVane became an assistant professor at North Carolina Central University (NCCU) in August of 2011. Since that time, she has used her passion for students and data to shape her position at NCCU. Her love of data led her to research how to better capture, integrate, and analyze biomedical data. She serves as director of the Bioinformatics, Genomics, and Computational Chemistry Core (BGCCC) at NCCU. She also leads the effort to integrate Bioinformatics into the Undergraduate Curriculum at NCCU. She is developing an independent research program designing data infrastructure analysis pipelines for experimental and clinical biomedical science.

Mr. David Guzman
Entomopixel

Workshop: Macrophotography of tiny arthropods

Mr. Guzman is the owner and principal photographer of Entomopixel LLC, a small independent business with the mission to increase the public's awareness of the importance of preservation of our biodiversity. Mr. Guzman has an MSc in Entomology from the University of Nebraska and an MSc in Electrical and Computer Engineering from North Carolina State University. Over the past 4 years Mr. Guzman has captured more than 30,000 images of insects in the USA and in his native country, Colombia. He has also spent a significant amount of time developing macrophotography techniques for nature and laboratory applications, and presently collaborates with the Universidad Nacional de Colombia to image their extensive museum insect collection.

Dr. Ashalla Freeman
UNC School of Medicine

Workshop: Graduate and Professional Panel Discussion

Dr. Freeman is the Director of Diversity Affairs and the Initiative for Maximizing Student Diversity in the Office of Graduate Education at the UNC-Chapel Hill School of Medicine. She oversees diversity recruitment efforts and serves on the admissions committees for biomedical PhD programs at UNC-CH. As IMSD Director, she develops, administers, and oversees the evaluation of programs and services supporting the success of underrepresented biomedical graduate students. She earned her BS in Chemistry at Tougaloo College (MS) and PhD in Microbiology at the University of Alabama at Birmingham. In 2004, she came to UNC-CH for postdoctoral training as a SPIRE Postdoctoral Fellow (Microbiology/Immunology).

Dr. Maggie Wilson
East Carolina University

Workshop: Graduate and Professional Panel Discussion

Dr. Maggie Wilson currently serves as Vice Dean and Associate Dean for Student Affairs for the East Carolina University School of Dental Medicine. Dr. Wilson joined the faculty of the ECU School of Dental Medicine as a Clinical Professor in November 2009. She is responsible for student recruitment, admission, retention and services. Dr. Wilson also has broader responsibilities in working alongside the Dean of the School of Dental Medicine to ensure the implementation of the school's strategic objectives. Dr. Wilson also coordinates the dental students' professionalism and professional dental ethics curriculum.

Dr. Bernard Roper

Office of Student Inclusion & Diversity Wake Forest School of Medicine

Workshop: Graduate and Professional Panel Discussion

Bernard M. Roper, PhD is the Program Director for the Office of Student Inclusion and Diversity (SID) at the Wake Forest School of Medicine. Prior to joining the SID office, Bernard served as the Health Careers Coordinator at the Northwest Area Health Education Center (AHEC). He received his Bachelor of Art in History degree from Winston-Salem State University, his Masters in School Administration degree from Gardner-Webb University and his PhD in Leadership Studies from North Carolina A&T State University.

Maureen Cullins, A.M.

Duke University School of Medicine

Workshop: Graduate and Professional Panel Discussion

Maureen D. Cullins, A.M. directs the Multicultural Resource Center in the Duke University School of Medicine. The Multicultural Resource Center brings significant resources to bear on the problem of minority underrepresentation in higher education (both student and faculty), specifically in science and medicine. Ms. Cullins work entails student and faculty development, curricular initiatives, community health, and pipeline programs. She is a member of the School of Medicine Admissions Committee and advises students who are interested in the health professions. Ms. Cullins is a proponent of early preparation for education and addressing the challenges to obtaining a health professions degree for underrepresented and disadvantaged students.

Dr. Daphne Rainey
North Carolina Central University

Workshop: Graduate and Professional Panel Discussion

Dr. Rainey is the Director of the Integrated Biosciences Program at North Carolina Central University. Prior to coming to North Carolina Central University, Dr. Rainey served as Executive Director of STEM Advancement at North Carolina A&T State University. She served as Program Director in the National Science Foundation's Division of Undergraduate Education. As Program Director she led the Presidential Awards for Excellence in Science, Mathematics and Engineering Mentoring (PAESMEM) Program. Dr. Rainey used a background in evolutionary genetics to launch into the then new field of comparative genomics. She was a research scientist in plant genomics for twelve years and earned two patents investigating molecular pathways and discovering novel proteins. Eight of those years she lived in The Netherlands working in the plant biotechnology industry before returning to the US to work as research scientist at Virginia Tech University.

Dr. Holly Menninger
North Carolina State University

Workshop: What Citizens? How Science?

Holly Menninger, PhD is the Director of Public Science for Your Wild Life. Your Wild Life is an outreach and science communication program based at NC State University that engages the public in studying, understanding, and celebrating the biodiversity associated with our every day lives - from our belly buttons to our backyards. She earned a PhD in ecology from the University of Maryland, and has worked at the intersection of science and society - in science policy, science communication and natural resource management.

Dr. Michelle Trautwein
North Carolina Museum of Natural Sciences

Workshop: What Citizens? How Science?

Dr. Trautwein is an entomologist whose research interests lie in the continued discovery and understanding of the evolution and diversification of flies and more broadly, insects, through the use of modern phylogenetic and systematic methods.

Questions?

Where do I go for general questions about the conference?

Ask NCAS volunteers positioned around the museum or come up to the information desk of each museum wing located on the first floor near the main entrances.

How will we know if there are changes in the program?

Each room will have an updated list of events in each room.

Is there a phone number for?

The front desk of the Nature Research Center: (919) 707-8081

Will there be a place for me to practice my oral presentation?

Yes. Room 4515 on the 4th floor of the Nature Research Center

Is wireless internet available?

Yes, access will be via wireless internet under "NRC-Visitor"

1. Verify the Wifi setting on your device in enabled
2. Open browser window
3. Enter NRC- Visitor when prompted for a Network Username. You will get the following screens
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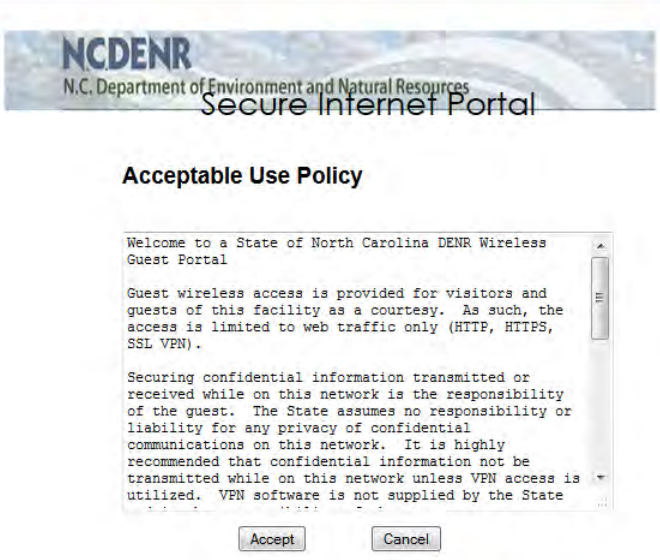

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YARBROUGH UNDERGRAD GRANTS

Fully Funded 2013-2014 Yarbrough Grant Proposals

Student	Institution	Faculty Advisor	Project Title
Jessical Avila	Nash Community College	David Beamer	Morphological Homoplasy Within Mountain Dusky Salamanders
Hannah Billian	Warren Wilson College	Stephen Cartier	Analysis of Berberine and Hydrastine in Rhizomes of Goldenseal (Hydrastis canadesis L.) Grown in Varied Levels of Nitrogenated Soil
Sarah Dietrich	Warren Wilson College	Stephen Cartier	Synergistic Qualities of Goldenseal Hydrastis canadensis versus Bererine Isolate in vitro
Austin Patton	Warren Wilson College	JJ Apodaca	Isolation & Characterization of Polymorphic Microsatellite Loci in the Green Salamander Aneides aeneus

Partially Funded 2013-2014 Yarbrough Grant Proposals

Student	Institution	Faculty Advisor	Project Title
Michi Stewart-Nunez	Warren Wilson College	David Coffeey	Shining a Light on the Physics of Charge Transfer Within Organic Photovoltaics
Stephanie Williams	Warren Wilson College	JJ Apodaca	Analysis of Variation in Venom Within the Western NC Population of Timber Rattlesnakes
Todd Elliott	Warren Wilson College	Mark Brenner	Biodiversity Studies of Ectomycorrhizal Macrofundiy of the Guiana Shield

Student Academy of Science District Winners

The Student Academy winners will present their posters on Saturday during lunch. Their names and titles of their poster presentation appear on the Saturday's Schedule Details, Page 19.

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Duke University

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Gregory Buhrman
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Bianca Bullock
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James Burcham
Wilkes

Sybil Burgess
Brunswick Community College

Kyle Burgett
University of North Carolina at
Greensboro

Thomas Burns
Tekrighter Science Blog

LaShonda Caine
Nash Community College

Gilberto Camilo
Catawba Valley Community College

Russ Campbell
Burroughs Wellcome Fund

Mickael Cariveau
Mount Olive College

Victor Catalan
Charlotte Mecklenburg Schools

Francesca Ceppi
Mount Olive College

Grace Chafin
Guilford College

Tat Chan
Methodist University

Vincent Chen
Guy B Phillips Middle School

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University of North Carolina
at Greensboro

Roxanne Chouinard
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North Carolina A&T
State University

John Clamp
North Carolina Central University

Matthew Clark
Elon University

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Abstracts

Does the Northern Two-Lined Salamander (*Eurycea bislineata*) occur in North Carolina?

NATHANIEL T. AKERS and David A. Beamer

Nash Community College

All populations of two-lined salamanders outside of the Appalachian Mountains in North Carolina are currently assigned to the southern two-lined salamander (*Eurycea cirrigera*). However, recent work suggests that more than one species of two-lined salamander occurs along the North Carolina and Virginia border. One of the species, the northern two-lined salamander (*Eurycea bislineata*), has not previously been recorded in North Carolina. I have sampled five populations from localities further west than previously collected populations of putative northern two-lined salamanders. From each population, I have amplified, purified, and sequenced 1500 base pairs of the mitochondrial gene ND2. Here, I present the results of a Bayesian phylogenetic reconstruction.

The Functional Distinctions between Human *WNT5A* isoforms A and B

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WNT5A, a secreted protein of the WNT signaling pathway, is well known for its role in the cellular differentiation and organ development, as well as proliferation and cellular migration. Studies have shown that it plays a role in many human cancers, acting as both a tumor suppressor, and oncogene. In this study, we analyzed WNT5A regulation from its two major transcription start sites, termed promoter A and promoter B. These promoters give rise to two distinct protein isoforms, identified as isoforms A and B. These proteins differ at the N-terminus; isoform A has an additional 15 amino acids in comparison to isoform B. In osteosarcoma cell line, SaOS-2, isoform A is overexpressed, where as isoform B is not expressed at all. Moreover, both isoforms A and B are expressed in normal osteoblasts. In addition, the colorectal cell line, HCT-116, both isoforms are absent.This leads to the question whether isoform A has a distinct function that from isoform B. It is possible the distinct functions of the WNT5A isoforms are responsible for the oncogenic or tumor suppressor activity of WNT5A in different cancers. To determine this, we analyzed and characterized the functional distinctions between WNT5A isoforms A and B, specifically cellular proliferation, and migration. Together, our data suggest that both isoforms a and B are functional distinct.

The Role of Cardiac Neural Crest in Outflow Tract Septation of *Danio rerio* and *Trachemys scripta*

MARTHA ALONZO-JOHNSEN, Margaret Kirby, Kathleen Smith, and Mary Hutson,

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Throughout vertebrate evolution, the cardiac outflow vasculature has changed from a branchial arch system to a systemic and pulmonary circulatory system. However, all vertebrate hearts and outflow tracts develop from a single heart tube. In the chick and mouse, cardiac neural crest cells divide the single outflow tract into the aorta and pulmonary arteries. Additionally, cardiac neural crest cells provide the smooth muscle of the aortic arch arteries, help to remodel the aortic arch arteries into asymmetrical structures, and contribute cardiac ganglia. Yet, the role of cardiac neural crest cells in vertebrates with an undivided outflow is not well understood. I re-evaluate the role of cardiac neural crest cells in zebrafish *Danio rerio* and hypothesize that neural crest cells in fish contribute to the smooth muscle of gill arch arteries, the ventral aorta and cardiac ganglia, but they do not contribute to myocardium as previously described. I also study the outflow tract development of the turtle *Trachemys scripta* to understand the process of outflow septation in a vertebrate with a divided outflow tract but incomplete ventricle division. I hypothesize that cardiac neural crest cells are also responsible for the outflow tract septation of reptiles. I perform a comparison study of turtle outflow tract formation to that of the chick outflow tract, as much is known about the chick. These results demonstrate that the pattern of cardiac neural crest cell contribution to vertebrate vasculature remains predictable and consistent, enabling future studies to focus on changes in vascular patterning caused by cardiac neural crest cells among different vertebrate lineages.

Glucose starvation induces microautophagy in yeast cells.

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Autophagy is a survival mechanism utilized by all eukaryotic cells during nutrient starvation in which the cell recycles proteins and other cytoplasmic components to the vacuole to degrade and release. Understanding this mechanism can potentially improve cell survival after stroke and heart attack, thus minimizing tissue damage. Macroautophagy, the better known autophagy pathway, involves recycling cellular components via a double-membrane bound vesicle, whereas microautophagy is poorly understood and involves the vacuole directly engulfing the cytoplasmic components. Previous internal studies showed glucose starvation inhibited macroautophagy in *S. cerevisiae* (budding yeast) cells; however, some autophagic activity was still detected by an enzymatic assay. To determine whether this activity was due to microautophagy, we investigated the consequences of deleting a critical gene for macroautophagy, Atg5, and genes that potentially influence microautophagy, Vtc1 and Vtc2, in a Pho8Δ60 strain, allowing us to measure autophagy quantitatively through the Pho8Δ60 enzymatic assay. We starved these strains for nitrogen, glucose, or both nitrogen and glucose for ~30 hours and found that Vtc2, not Vtc1 or Atg5, was required for autophagic activity in glucose-starved cells. We concluded that microautophagy occurs in glucose-starved cells, and some of the genes in the vacuolar transporter chaperone (VTC) complex are more important for microautophagy in glucose-starved cells than others. In the future, we plan to test the impact of deleting all four of the genes in the VTC complex on microautophagy and to test whether selective microautophagy of cell organelles occurs during glucose starvation.

Morphological Homoplasy within Mountain Dusky Lineages (*Desmognathus)*

JESSICA MARIE AVILA[1], Brenten L. Bottoms[2], and David. A. Beamer[1]

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Desmognathus are medium sized lungless salamanders distributed across the Appalachian Mountains. Historically, there has been debate about how many species of Desmognathus there are. Currently there are six recognized species of Desmognathus: ochrophaeus, orestes, carolinensis, apalachicolae, ocoee, and abditus. These six species were recognized in part, based on molecular data. To date, there has not been a comprehensive range wide molecular phylogeny for Desmognathus. Here, we present a range wide molecular phylogeny that reveals the relationships of the six recognized Desmognathus, as well as several, apparently unnamed, lineages. To understand the morphological variation within these lineages we have photographed and measured specimens from twenty one localities. For each of these localities we sequenced a 600 base pair fragment of COX1 mitochondrial DNA. In the 1960's Martof and Rose collected over 4,000 Desmognathus from twenty one localities and made twelve different measurements for the specimens. To leverage their large morphological date set, we collected a series of thirty salamanders from the same localities. We made the same measurements and used our data to supplement their existing data. Here we demonstrate considerable levels of morphological homoplasy in Mountain Duskies. There is both extensive homoplasy and homology with respect to dorsal pigmentation. By supplementing Martof and Rose's large morphological dataset and sequencing each of their populations, I should be able to distinguish between homoplasy and homology.

Acid and Bile Salt Tolerance of Microencapsulated Probiotics Strains

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The objective of this study was to access the acid and bile salt tolerance of microencapsulated probiotic bacteria. Strains of *B. bifidum* NRC and *L. paracasei* 441 with 2% active cells were incubated at 37º C for 18 h in MRS broth. Alginate with NaCl was used for microencapsulation. Survival and viability of microencapsulated strains under different conditions were established. Selected strains in MRS broth cultures were challenged to pH (2 and 1.5) and bile salts (0.5 and 1.0%) for three hours. Our results showed that the total viable counts of the two strains were increased in the different pH levels. Microencapsulated *B. bifidum* NRC and *L. paracasei* 441cells had higher viability (6.90, 6.70 CFU/ml) than free cells (5.69, 5.70 CFU/ml) during exposure to low pH at 1.5. Similarly, the bacterial count of microencapsulated *B. bifidum* NRC cells when exposed to the bile salt for three hours was slightly higher (7.91CFU/ml) than that of non-microencapsulated (7.80 CFU/ml) in the presence of bile salts (1.0%). Bacterial count of microencapsulated and non-microencapsulated for *L. paracasei* 441strain was 7.88 CFU/ml and 7.85 CFU/ml respectively. Our results suggested that the microencapsulated strains have higher viability at 1.5 pH and 1.0% bile salts. These encapsulated strains could be used to improve probiotics viability in food products.

Expression of Oxidative Stress Markers in Zebrafish After Acetaminophen Treatment

KAYLA BABA, Brittani Hodges and Karen Guzman

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Acetaminophen (APAP) is an analgesic commonly administered to both sick children and adults. Studies of APAP toxicity, during embryonic development in the zebrafish (*Danio rario)* as a model organism, have focused on physical changes in development (David, et al. 2009), but an active area of research has begun to focus on its effects at the molecular level. Studies suggest that biomarkers for oxidative stress are elevated in APAP-treated rats (Jin, et al. 2012). To extend these studies, zebrafish embryos were exposed to two concentrations of APAP, 0.37g/L and 0.076g/L, based on previous research that observed anatomical changes in the heart and liver in zebrafish (Xu, et al. 2012 & He, et al. 2012). RNA was isolated at 24, 48, and 72 hours post fertilization (hpf) and mRNA levels of the oxidative stress markers, *heme oxygenase 1a* (*hmox1a*) and *glutathione S-transferase pi 2* (*gstp2*), were measured using RT-PCR. *TATA box binding protein* (*tbp*) was used as an internal reference to normalize the data. At the times and APAP concentrations evaluated, the mRNA levels of *hmox1a* and *gstp2* do not appear to be affected. Since higher doses of APAP were required to cause changes in gene expression in the rat and a longer exposure time to APAP may be required to see changes in the zebrafish at the molecular level, these stress indicators will next be evaluated using a higher dose of APAP, 0.75g/L and 1g/L, and for longer-term effects (96 hpf and 120 hpf).

The Effect of Negative Stimuli on the Directional Preference of Planaria (*Dugesia tigrina*)

HOLLY BALDWIN

Lenoir-Rhyne University

Planaria, *Dugesia tigrina* (freshwater flatworms), are some of the simplest organisms with the ability to learn, and have been used in numerous learning studies. In order to determine the effect of different agents on learning using this model organism, there must first be a reliable method of training. In this study, 54 Planaria were trained for directional preference in a y-maze with negative stimuli: light and electrical shock. They were trained in a specified direction in the maze over a three-day period consisting of ten trials per day. If they attempted to travel in the opposite direction to which they were being trained, they were negatively stimulated. It appeared that Planaria could be successfully trained with the administration of electrical shock; however, light administration was not an effective training method. Since the tactile sensory nervous system is more developed than the visual system in Planaria, this may explain the differences in the success of these training methods.

High-Throughput Screening and Cell-Based Assays to Discover Small Molecules That Overcome Endogenous GTP Inhibition of L-Glutamate Dehydrogenase in Cellular Respiration

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Biomanufacturing Research Institute and Technology Enterprise
North Carolina Central University

This study serves to find a drug that can be used by the body to stimulate fatty acid metabolism to a useable energy source for cellular respiration while overriding the normal mechanism. This conversion process mostly occurs in a starvation state. L-glutamate dehydrogenase (GDH) catalyzes glutamate to alphaketoglutarate (AKG), that feeds into the citric acid cycle. When excess food intake occurs, GDH is inhibited by GTP, ATP and palmitoyl-CoA, slowing the synthesis of AKG. Overcoming endogenous GDH inhibition can have therapeutic benefits including stimulating fatty acid oxidation and basal insulin secretion. This experiment began with designing a kinetic assay that could measure the reversal of inhibition of the GDH enzyme as done by the body when converting fatty acids to glucose. Once this was accomplished, the assay was miniaturized and automated for high-throughput small molecule screening in 384-well microtiter dishes to discover potential GDH activators. To start, plates are loaded with a buffer solution containing l-Glutamate, NADH and +/-GTP (no GTP for control wells), then test compounds or dimethyl sulfoxide (DMSO) as the vehicle control. Next, a buffer solution containing GDH is applied to all wells and it is immediately read on a BMG Pherastar fluorospectrometer at 30-second intervals for five minutes to detect NADH. The points are plotted, the slope determined, and wells with slopes 50% greater than negative control (relative to the uninhibited positive control) are selected. After confirmation, these hits will be used in a cell-based assay to determine effects on cellular respiration. Any molecules showing desired results will be considered for future animal studies. Should this prove successful, this will provide a novel approach to helping people with Diabetes lose excess weight in a controlled manner.

Effects of Varying Flow, Temperature and Turbidity on Macroinvertebrate Drift and Colonization Patterns

MACY BEAMAN, Lisa Bonner and Patrick Myer

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Hester-Dendy samplers were placed in riffles and pools to map colonization and drift patterns along a reach of Crabtree Creek that historically has dramatic fluctuations in flow as a result of rainfall. Data showed the species that colonized the samplers, primarily mayflies, caddisflies and dipterans, represent a small proportion of biota that typically inhabit the area. Riffle samplers were consistently colonized by more individuals than pool samplers on any given sample day with a higher proportion of *Baetis* and *Hydropsyche* relative to pools, which showed greater numbers of *Maccaffertium*. In riffle habitats there was a clear positive relationship between the increase in flow and the increase in colonization. However, when flow rates reached scouring levels little to no colonization occurred. At high flow rates a sampler, which was previously occupied, was stripped of colonizers. There was also a strong negative relationship between species colonization and turbidity, however temperature did not appear to be a factor.

8000 Salamanders And How I Found Them: A Robust Sampling Strategy For The Southeastern United States

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The success of any phylogenetic survey depends on a sampling regime of appropriate geographic scope and scale. Unfortunately how to design an appropriate sampling regime is often obscure. In the case of morphological conservative species it can be even more difficult to design an adequate sampling regimen because all populations look similar and there is nothing to suggest where the most informative collections should be made. Here I report the results of a robust sampling regimen that I believe is of broad utility, especially for low vagility organisms. As a case study we sampled ~700 populations across the distribution of all described dusky salamander (*Desmognathus*) species. Because most species of *Desmognathus* are semiaquatic, upland dispersal is probably minimal; instead, most inter-population movement likely occurs via streamside (and/or other wetland) conduits that are eventually circumscribed within a given river drainage system. Thus, river drainages provide a logical starting point for investigating distribution patterns and evolutionary relationships of *Desmognathus*. To enhance resolution and repeatability, I have identified a second-level sampling component to standardize finescale geographic coverage: level IV ecoregions, which denote areas of general similarity in ecosystems as well as in the type and quantity of environmental resource. I compare and contrast the results of our survey with previous work in the genus to highlight some of the unique findings uncovered with this sampling regime.

Effect of a Chemotherapeutic Agent, Rapamycin, on Growth of *Lactobacillus acidophilus* and *Escherichia coli*

LOGAN NICOLE BEARFIELD

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Rapamycin is a drug used to suppress the growth of tumors in many different types of cancer. Rapamycin inhibits the signaling of the mammalian target of Rapamycin (mTOR) and causes a decreased proliferation rate of eukaryotic cells. In this study the growth effects of Rapamycin (40μM, 20μM, 10μM, 5 μM, 0.5 μM, 50 nM, 5 nM) on *Lactobacillus acidophilus* and *Escherichia coli* were tested to see if the drug might inhibit the gut microbiota when administered to cancer patients. *Lactobacillus acidophilus* and *Escherichia coli* are essential components of the gut microbiome; they increase the functioning of the immune system and help to prevent invasion of pathogens. While there have been many studies conducted on the tumor suppressive effects of Rapamycin, this study tested the effects this chemotherapeutic agent had on two components of normal microbiota in the human gastrointestinal tract. The hypothesis that Rapamycin inhibits the growth of *Lactobacillus acidophilus* and *Escherichia coli* in a dose-dependent manner was rejected. Other chemotherapy drugs are now being investigated.

Multi-locus-sequence-typing of *Azotobacter zettuovi:* an organism without a place

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Azotobacter sp. ATCC 49359 (known informally as *"Azotobacter zettuovi*"), was isolated in Holland in 1960 by S. Rowinski and deposited in the American Type Culture Collection (ATCC) by P. Jurtshuk of the University of Texas Houston. *Azotobacter sp.* ATCC 49359 is a gram-negative, nitrogen-fixing, free-living, soil bacterium that remains officially unrecognized and uncharacterized. Phylogenetic analysis of the 16S and RNase P genes, from this lab, placed *Azotobacter sp.* ATCC 49359 in the genus *Azomonas,* most closely related to *Azomonas insignis*. *Azomonas*, *Azotobacter*, *Azorhizophilus* and *Pseudomonas* are all genera of the family *Pseudomonadacea.* The taxonomy of this family is based on superficial morphological and biochemical traits, and as a result is a poor match to the phylogenetic relationships between member species. This study will use multi-locus sequence typing (MLST) of nine genes, consisting of house-keeping and DNA repair, as well as genes for traits specific to this family, to further support the placement of *Azotobacter sp.* ATCC 49359 in the genus *Azomonas*. Additional methods of characterization will be used according to the specifications of the International Journal of Systematic and Evolutionary Microbiology (IJSEM) to support the placement of *Azotobacter sp.* ATCC 49359 in the genus *Azomonas* and to formally recognize it as "*Azomonas zettuovi*".

Serpentinizing Environments as a Potential Source of Novel Antibiotic Compounds

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Antibiotics are usually the strongest and last line of defense against bacterial pathogens and the vast majority of antibiotics today originate from soil microbes. In particular, actinomycetes have been an exceptional source of novel antibiotics and other bioactive compounds. Actinomycetes are Gram-positive, saprophytic bacteria found in soil, water and colonizing plants. Of all the bioactive compounds that have been obtained from microbes, slightly less than half originated from actinomycetes. Actinomycetes have been intensively studied in a variety of places, including previously unexplored habitats, extreme environments, yet there is currently no publication characterizing the antibacterial activity of actinomycetes and other microbes collected from soils affected by serpentinization.

Serpentinization occurs on a global scale and has been observed in marine and terrestrial environment; this metamorphic process occurs when ultramafic rocks, characteristic of the Earth's lower crust and upper mantle, are uplifted by tectonic activity and interact with water. This interaction creates an environment rich in electron donors such as hydrogen and methane; short-chain hydrocarbons and small organic acids are also produced to a lesser extent. Serpentinization leads to alkaline conditions in excess of pH 10, limited access to dissolved inorganic carbon and terminal electron acceptors, and low biological diversity. These factors make serpentinizing environments unique and challenging for inhabiting microbes. Microbes, especially actinomycetes, which can withstand the unique niche caused by serpentinization and warrant further study because of the potentially undiscovered bioactive compounds they produce. Recent surveys of serpentinized soils indicate a significant portion of

microbes found there belong to the group actinomycetes. This further advocates the notion that serpentinized soils may contain unidentified bioactive compounds.

Thermal Stability and Refolding Capability of Shark VNAR

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Camelids contain heavy chain antibodies (hcAbs) from which single domain antibodies (sdAbs) are derived. Sharks contain hcAbs from which VNAR are derived. For this reason, Shark sdAbs have not been specifically focused on greatly in the past and we wished to determine if they would also prove to be thermally stable. Twenty clones were transformed and cultured and the melting temperatures of 19 out of the 20 clones were determined. We transformed the cells using pHelp, Turner DE3 strain of *E. coli*, and plasmid DNA encoding one of the 20 VNAR from one of the 20 different cell lines of each type of shark. We inoculated the cells and grew them in terrific broth (TB) media. From these cells we extracted the protein and determined the melting point using a dye melt technique. Using about half of the samples, we performed circular dichroism (CD) as another, more accurate way to test the melting temperatures and to observe whether or not the shark proteins refolded. Using the dye melts, we found melting temperatures ranging from 42 ºC to 77 ºC. When we performed CD on the samples, we found that the melting temperatures usually fell within a 5ºC range of those from the dye melt. We concluded that shark VNAR do not refold in the same manner as camelid sdAbs. Some of the proteins were shown to have above a 70% refolding ability, but it was not conclusive for a majority of the samples that were tested.

Degeneracy and Reassignment of Instruction Sets in a Digital Evolution Experiment

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Evolving software systems have instructions that are typically encoded by symbols or commands that do not change their meaning in terms of their corresponding machine functions. Here we explore the before-and-after scenarios for how instruction symbols may be reassigned during digital evolution experiments. We made adjustments to the digital evolution software AVIDA codebase to implement two basic steps. We configured AVIDA to support multiple forms of an instruction that have different symbolic encodings but the same machine function. We then substituted all copies of one of the redundant symbolic encodings in an evolved organism to correspond to a different machine function and examined the consequences. Our analysis workflow enabled us to identify different scenarios where substitutions of redundant symbolic encodings reduced fitness. There are broader implications for how this digital evolution experiment may relate to codon degeneracy in biological systems.

Extreme genetic homogeneity: Phylogeography of the red and mud salamanders

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The salamander genus *Pseudotriton* (family Plethodontidae) has several races , yet red salamanders (*Pseudotriton ruber*) and mud salamanders (*Pseudotriton montanus*) remain the only identified species of the genus. *Pseudotriton* is widely distributed across the eastern United States, ranging from New York in the north to Florida in the south westward to the Mississippi River. While the distinctiveness of the two species of *Pseudotriton* has long been recognized, the genetic variation and structure of populations has never been analyzed. In general plethodontid salamanders are characterized by a pattern of extreme geographic partitioning and cryptic speciation but to date no studies have addressed these issues in *Pseudotriton*. We have sampled 110 populations spanning the extent of this genera's distribution. For each population sampled, we extracted DNA, amplified and sequenced a 1686 base pair fragment of the mtDNA genome. Here we present the results of a Bayesian phylogenetic reconstruction for this genus.

Mutagenesis of the FMN Riboswitch of *Photorhabdus luminescens* and an analysis of mutagenic effects on its pathogenicity towards *Caenorhabditis elegans*

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Riboswitches are an important aspect of the regulation of some genes in bacteria. The binding of effector molecules to mRNA leader regions specifies their secondary structures, which modulates transcription termination and therefore the expression of downstream genes. This study examines the FMN riboswitch located upstream of the *rib* operon in *Photorhabdus luminescens,* and seeks to determine if this region of regulatory RNA is a potential target for novel antimicrobials. The pathogenicity of this bacterium on its host, *Caenorhabditis elegans*, will be examined via mutagenesis to lock the riboswitch in the "on" or "off" conformations using the lambda-Red recombineering system. If the bacterium loses virulence when the FMN riboswitch is locked in the "off" confirmation, it may mean that the bacteria cannot bypass the *rib* pathway and can therefore not sequester FMN from its host. This finding would focus attention on the FMN riboswitch as a potential target for novel antimicrobials for use against multi-drug resistant bacteria.

Composting in School and the Community: Experience of Trash Terminators 2.0

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Chapel Hill landfill closed in July 2013 resulting in transportation of city trash to a remote site, thus adding cost and creating pollution. Since ~50% of school cafeteria trash and ~35% of city trash are compostable, composting in schools and communities will significantly reduce greenhouse gases and transportation cost. Four objectives were developed by the Trash Terminators team: to practice and educate composting at our school, to duplicate the program in other district schools, to conduct community outreach, and to propose municipal composting to local governments. We first set up composting bins in the school cafeteria, and contracted with a composting company for collecting compostable waste. In this school year, 10.24 tons (>80%) of trash will be either recycled or composted, and thus 574 pounds of CO2 and $550 of cost will be reduced for our school. We reached out to the leadership in our school district, and successfully convinced all district schools to compost their cafeteria trash by the end of this year. In the community, our outreach activities included composting demonstrations in public places and events, local newspaper interviews, infomercial aired on TV, and information and surveys posted through social media. We have presented to governing bodies of Chapel Hill Township, Carrboro Township and Orange County to promote municipal composting. Two State Senators have highlighted our initiatives in their newsletters. In summary, we have successfully launched a composting program in our middle school and expanded it into our communities with the goal of promoting an eco-friendly society.

Acrolein toxicity in endothelial cells involves lipid peroxidation, protein damage, reduced cellular GSH, and augmented monocyte adhesion

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Cardiovascular diseases (CVD) account for a large number of mortality all over the world. Although the pathogenesis of CVD includes numerous factors, environmental toxin exposure from air pollution could elevate the risk of CVD. Acrolein, a highly reactive aldehyde species, is a major air pollutant. It is generated in high quantities from automobile exhaust and tobacco smoke. However, the action of acrolein in endothelial cells remains to be investigated. The present study examined acrolein-induced cellular injury, oxidative modifications of cellular constituents, altered intracellular glutathione (GSH), and adhesion of monocytes to endothelial cells on EAHY926 cells, a widely used endothelial cell line for study of endothelial cell dysfunction. Incubation of cells with acrolein at pathophysiological concentrations for 24 hours caused a significant decrease in cell viability, as measured by the MTT assay and an increase in the release of LDH, which further confirms a significant change in the cell morphology. Acrolein also increased the amount of thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, and protein carbonyl levels, a marker of protein damage in the cells. Incubation of cells with acrolein also resulted in a significant depletion of cellular GSH and augmented monocyte adhesion to human endothelial cells, an important step in the development of atherosclerosis. The results of this study may contribute to our ability to assess the cardiovascular risk of human exposure to acrolein.

Comparison of Physiological Reactions to Stress in College Freshman and Senior Student-Athletes

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University of Mount Olive

This research measured and compared the levels of physiological reactions to stress of freshman and senior student-athletes. The hypothesis was that senior student-athletes would have more physiological reactions to stress compared to freshman student-athletes. To measure the level of stress experienced by the student-athletes, a survey that measured the physiological responses to stress was distributed to 20 freshmen and 20 seniors student-athletes of the University of Mount Olive. These student-athletes were currently engaging in athletic activities such as practicing and competing. The results supported the hypothesis and showed that seniors experience more physiological reactions to stress than freshmen. There was a significant correlation between class status and physiological responses to stress. To our knowledge, this was the first study that compared physiological responses to stress between freshman and senior student-athletes. Further research should examine other factors that influence physiological responses to stress in student-athletes.

Development of an *in vitro* assay platform for the characterization of genetically-encodable fluorescent biosensors of kinase activity.

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Protein phosphorylation, mediated by protein kinases, underlies nearly all cell signaling processes. Recently, it has become apparent that, in addition to direct kinase-substrate interactions, cells achieve signaling specificity by differentially activating distinct pools of a given kinase within the cell. To monitor the spatiotemporal regulation of kinases within the endogenous cellular environment, we are developing a series of genetically-encodable kinase activity reporters that can be targeted to specific subcellular regions. These reporters, which rely on the phenomenon of fluorescence resonance energy transfer (FRET), are based on a modular design in which a switching domain composed of a "substrate region" (e.g., a consensus phosphorylation motif) and a "phosphoamino acid binding domain" is sandwiched between complementary fluorescent protein (FP) color variants, such as CFP and YFP. Phosphorylation-dependent conformational changes alter the relative orientation of the FPs, causing a change in FRET. We are currently developing a generalizable *in vitro* assay platform to characterize these reporters.

Here we report the expression, purification and initial characterization of one such reporter, the A-kinase activity reporter 3 (AKAR3). AKAR3 was expressed in JM109(DE3) *E. coli* and purified by Ni-NTA affinity chromatography. The purified product was analyzed by gel electrophoresis and visualized by silver staining. We are currently using active human PKA purified from yeast to measure its specificity and dynamic range using a multimode plate reader. To demonstrate the generality of this approach, we also describe the construction of a second reporter, BtkAR1, which is designed to measure the activity of Bruton's tyrosine kinase (Btk), a primary mediator of antigen receptor signaling in B lymphocytes

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Investigating Peroxidation of Lipid by Heme-Artemisinin Adducts *in vitro*

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Malaria, a devastating mosquito-borne disease caused by *Plasmodium* parasites, infects roughly 219 million people annually, resulting in 660,000 deaths. Trioxane-based antimalarials, including the well-known natural artemisinin (ART) compound, are among the most effective pharmaceuticals to date. The endoperoxide pharmacophore in the antimalarial trioxane ART has great potency against the *Plasmodium* parasites, including *P. falciparum*, the deadliest parasitic species infecting humans. Despite a multitude of research efforts the mechanism of action of this drug class remains unresolved. Our previous research demonstrates that fluorescent trioxane derivatives (12C) localize within digestive-vacuole associated neutral lipid bodies of *P. falciparum in vitro* (Hartwig *et al*., 2009). Based on these findings and others we proposed a mechanism of action for trioxane antimalarials that involves activation by heme iron and formation of heme-ART adducts capable of lipid peroxidation; ultimately resulting in parasite death. To explore this theory further we designed an *in vitro assay* based on methods by Berman and Adams (1997) and Messouri *et al.* (2006) to explore the effects of heme-ART adduct formation in the presence of various lipid species. *In vitro* assays using various combinations of lipid standard (1-oleoyl-rac-glycerol, 1,2-Dilinoleoyl-rac-glycerol, oleic, linoleic or palmitic acid), ART and ferrous heme were allowed to react for 24 h at 37 °C, followed by chloroform:methanol:water (8:4:3) extraction. The resulting extracts were analyzed under UV illumination and thin layer chromatography (TLC). Our studies provide evidence of a novel fluorescent reaction product resulting from the formation of heme-ART adducts in the context of a lipid environment; thus further supporting lipid peroxidation as a potential mechanism of ART in the parasite.

Drug discovery efforts to enhance neuroprotective endocannabinoid signaling through dual inhibition of FAAH and MAGL enzyme

CORONA A, Quizon PMP, Romine H, Kelly W, Naidoo V, Ruiz S, Suggs S, Mbugua B, Jackson R, Elliott L, Long C, Ellerbe J, Stephens K, Showers J, Makriyannis A, and Bahr BA

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Excitotoxic brain injuries such as seizures and strokes are challenging with respect to the slow recovery of normal brain function. The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are members of the endogenous cannabinoid system, a system with on-demand response that protect against excitotoxic injuries. To modulate the endocannabinoid response during events of excitotoxicity *in vivo,* a non-covalent dual fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibitor, AM6642, was screened to test for neuroprotection from kainic acid (KA)-induced seizures. KA is an agonist of ionotropic glutamate receptors and KA injection lead to excessive glutamatergic activity, mitochondrial dysfunction, and low brain function. KA-exposed rats were treated immediately with vehicle or AM6642 injections, resulting in reduced seizure severity mediated by AM6642. Behavioral paradigms conducted 24 to 48 h after all injections consisted of rotarod test. AM6642-treated mice exhibited dramatic recovery of rotarod, performance time, and performing similar to the level as in non-seizure control rat. Brains were then dissected and further evidence of AM6642-mediated protection was found when measuring spectrin breakdown product and pre and post

synaptic products. In conclusion, FAAH and MAGL inhibition after excitotoxic brain injuries leads to recovery in synaptic markers, cytoskeletal damage, and behavior integrity.

The Diversity and Evolution of the Primate Skin Microbiome: How different are humans from our closest relatives?

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It is becoming increasingly evident that the skin microbiome plays a key role not only in body odor but also human health and disease. However, little is known about the diversity of microbial inhabitants of the skin, nor of how these assemblages may persevere or turnover throughout evolutionary time. By taking a comparative approach and studying the microbial associates of non-human primates, we seek to broaden our understanding of host dependent effects on microbial composition and provide insights into the extent to which the modern condition of our skin microbiome is similar to that of our closest relatives or is uniquely human. To answer these questions, we characterized the microbes living on the skin of humans, chimpanzees, gorillas, rhesus macaques and baboons. We evaluated the bacterial and archaeal residents associated with the axilla of these primate species through high throughput sequencing of the 16S rRNA gene. These axillary samples provide a glimpse into the evolution of the primate skin microbiome and are the first study of the skin microbiome of non-human primates using non-culture dependent methods. In our study, we found that human skin microbial communities were unique relative to other primates, both in terms of which lineages were present and which were absent. In part these differences may reflect the broad story of primate evolution. Alternatively, some of the unique attributes of the human skin microbiome may reflect modern shifts in the skin microbiome as a function of human hygiene.

Independent nuclear markers reveal cryptic diversity within seepage salamanders (*Desmognathus aeneus*)

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Phylogenetic reconstruction based on mtDNA data show both patterns of diversification and genetic homogeneity across different portions of the range of the seepage salamander (*Desmognathus aeneus*). To further evaluate the specific status of these salamanders we sequenced independent nuclear markers. Analyses of these markers suggest the presence of multiple lineages within seepage salamanders. This finding has important conservation implications and provides a strong case for the importance of molecular systematic techniques in revealing the biodiversity of the southeastern United States.

Phenotypic Analysis of Transcription Factor Mutants In *Neurospora crassa*

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While the genome of many organisms may be mapped, there are still many unknowns as to the roles and functions of specific genes. Understanding individual genes on a functional level has a multitude of implications in both medicine and agriculture. This study uses the non-pathogenic, eukaryotic model organism, *Neurospora crassa.* This organism is useful for studying human biology and cellular processes due to the convenience in culturing, and it has closer relation to mammals than yeast does. In this ongoing study, about 252 (eventually 283) gene-specific transcription factor mutants previously generated by a high throughput knockout project are analyzed. Defects were specifically looked for in the sexual cycle and major asexual cycle. Phenotypic characterizations of all uncharacterized transcription factor mutants available were performed, testing for defects in linear and aerial growth of hyphae, asexual sporulation, female fertility and general morphology. The number of transcription factor mutants phenotyped and their representative families include 121 Zn2Cys6 binuclear cluster, 61 C2H2, 25 bZIP, 20 Myb, 15 bHLH, and 6 GATA factors, as well as others in smaller numbers. Unique phenotypes were discovered such as the production of multiple perithecial beaks, ectopic melanin production, temperature sensitivity, substantial growth rate inhibition, substantial aerial hyphae inhibition, sexual cycle arrest, and infertility of male structures.

Bioremediation of Oil in the Arctic Sea by Indigenous Bacteria

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The Arctic sea is an important ecosystem and recent oil discoveries and melting sea ice have lead to increased marine traffic, increasing the risk of an oil spill. The colder climate of the Arctic sea and its natural bacterial populations have not been studied as extensively as warmer seas, so the hydrocarbon degradation potential is relatively unknown. To determine this potential we examined a series of microcosms containing seawater and sea ice taken from Resolute Bay in the high Arctic and incubated under ambient conditions in the presence of crude Arabian light oil. The microcosms were incubated at -1°C for 15 days, with gentle shaking. Chemical analysis was performed on the microcosms to determine the removal efficiency of the hydrocarbons, and the microcosms were filtered at time zero and after 15 days to collect the microorganisms that had developed during the incubation. Total DNA was extracted from the filters and 16S rRNA gene fragments were amplified and sequenced to determine the taxonomy of the bacteria present and metagenomic sequencing was performed to identify some of the functional genes involved in hydrocarbon degradation. The results showed a significant increase in known bacterial hydrocarbon degraders in Arctic seawater containing oil compared to the controls and the

chemical data corroborated this shift in bacterial diversity. Sea ice, however, did not show significant changes between controls and oiled samples. The results suggest that Arctic seawater does contain an active hydrocarbon degrading bacterial population that responded rapidly to the presence of oil.

Biodiversity Studies of Ectomycorrhizal Macrofungi of the Guiana Shield

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Warren Wilson College

With nearly 200 recognized species, the mono-dominant *Dicymbe* legume forests of the Guiana Shield are one of the epicenters of lowland tropical ectomycorrhizal fungal biodiversity. Previous pioneering work by Dr. Terry Henkel (Humboldt State University) and his research teams opened up the opportunity for intensive field work in the upper Potaro river basin of Region 8, Guyana. This 2013 expedition provided an additional data set on the biodiversity of many ectomycorrhizal fungal groups, as well as an additional year of masting data from mono-dominant stands of *D. corymbosa*. We sampled the diversity of ectomycorrhizal fungal species within these areas, making dried herbarium collections and full taxonomic descriptions of the specimens collected. Many of the fungi collected were undescribed taxa that we are currently preparing for publication. Preliminary data was also collected to support the hypothesis that *Guyanagaster necrorhiza* is a virulent root pathogen in this forest ecosystem and is possibly being dispersed by an entomological symbiont. The diversity of hypogeous fungi collected was far less than previously anticipated; however, we suspect this may be due to the timing within the rainy season. This research project has opened larger research questions about the diversity and distribution of disjunct populations. A 2014 expedition to the Guineo-Congolian rainforest of Cameroon will allow us to study these disjunct populations in the similarly mono-dominant lowland tropics of Africa.

Expression of a Novel Scorpion Venom Metalloprotease in *Escherichia coli*

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The ability for cells to communicate is imperative for multicellular organisms to respond to a variety of stimuli. These responses are performed via paracrine interactions, which require vesicle trafficking to direct a signal to be exocytosed. Once complete, nearby cells can receive and act upon said signal. A crucial component of vesicle trafficking is the interaction between the vesicle and cell membrane, facilitated by SNARE proteins. v-SNAREs, found on the vesicle, and t-SNAREs, found on the membrane, interact with each other to form the complex required to mediate fusion and exocytosis.

There are many toxins that disrupt SNARE complex formation inhibiting the release of signaling molecules and, eventually, disrupting the inability to respond to stimuli. Common toxins utilizing this mechanism include: *Bacteroides fragilis* toxin (BFT), Botulinum neurotoxin, and tetanus toxin. A novel metalloprotease with a similar mechanism was identified within the

venom of the Brazillian scorpion *Tityus serrulatus*. It has been named Antarease. Structurally and mechanistically, Antarease resembles BFT with one key difference, the ability to be targeted to any cell in the body, not just the neurons. This atypical targeting indicates that Antarease may be an effective means of suppressing a variety of medically relevant cell to cell signaling pathways, including those involved in inflammatory responses.

Our project is focused on producing the scorpion-derived Antarease protein in an *Escherichia coli* expression system. By generating large quantities of active protein we will be able to further evaluate the properties of Antarease that distinguish it from other characterized metalloproteases, and examine how this protein's novel properties can be utilized in both medical and research applications.

Novel Approach to a Generalized Theory on Nanostructure Devices

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Nanoscience comprises a significant area in science's immediate frontier. Because of its prospects and its relevance to a wide variety of research fields, it is important to engage this area head-on, and from a variety of perspectives. In this investigation, some fundamental, salient properties of modern quantum devices (i.e. quantum dots, wells and wires) will be expounded upon. From there it will be shown that a generalized, yet very simple, model may be constructed without straying far beyond the rudimentary assertions made in classical rotational dynamics. This integrative approach is not only simple, but cogent enough to provoke deeper and purposefully unconventional thinking for both physics and chemistry majors. 1D-confined quantum wells, 2D-confined quantum wires, and 3D-confined quantum dots will ultimately be described mathematically, resulting in a basic framework with which to consider nanoscale devices.

Engineering and expressing an upstream insulin promoter-binding fusion protein.

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Insulin is produced in pancreatic beta cells in human in response to glucose in blood.
For insulin production, the insulin gene is turned on by multiple transcription factors. Three transcription factors, Pdx1, MafA and Beta2, are necessary for synergistic activation of insulin transcription, which is an important function of a normal pancreatic beta cell.
The insulin promoter contains multiple binding sites for all of these factors, which are in close proximity to each other. We have created a novel transcription factor fusion protein with the DNA binding domains of Pdx1 and MafA, linked to each other by a flexible linker. This fusion protein was over-expressed in E. coli followed by purification using a nickel affinity column. The fusion protein is tested for DNA binding affinity to a sub-site of the human insulin promoter that contains the GG2, GG1 and C1 sites, responsible for binding to Pdx1 and MafA, respectively. Experiments of the DNA binding specificity and affinity of the fusion protein, when compared to those of individual transcription factors to DNA binding domains, will help to

shed light on the mechanism of synergistic activation of insulin transcription and provide an insight for potential gene therapy to diabetic patients.

Expression of Chondrocyte Markers in ATDC5 Cells Exposed to Osmotic Stress

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Chondrocytes are the cells found in cartilage that are critical to maintaining the cartilaginous matrix of healthy joints. As is true for most cells, the expression of certain genes can be used as markers of that cell type and to assess if they are functioning normally. Chondrocytes are bathed in synovial fluid, which contains a variety of ions, including sodium, potassium, and calcium, creating a hypertonic environment (van der Windt, Anna E et al. 2010) and affecting the normal physiology of these cells. If this external environment is disturbed by a disease state or other environmental effectors, the normal ion concentration may be altered and impact the ability of these cells to function. To determine if alterations in the concentration of sodium chloride, potassium chloride, and calcium chloride can impact the function of chondrocytes, we used a chondrocyte cell line that was derived from mouse embryonic carcinoma cells (ATDC5) and evaluated the expression of the chondrocyte markers SOX9, Collagen 2, and Syndecan 4, while also using β-actin as an internal control for normalizing the data. To first understand the normal expression of these markers, we evaluated the time-dependent expression of their mRNAs using RT-PCR. Surprisingly, we found that our results differed from previous descriptions in the literature (Shukunami, Chisa. et al. 1997), for example, expression of Collagen 2 was observed earlier and throughout the entire time course. We also saw expression throughout the time course of a chondrocyte marker, Syndecan 4, which had not previously been evaluated in these cells. Based on these results, we are currently evaluating if changes in the concentrations of sodium chloride, potassium chloride, and calcium chloride in the culture media alter the expression of these markers.

Analyzing big data using simple flowcharts and questioning techniques

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Proficiency with bioinformatics tools to manipulate large sequence datasets is now a critical component of the toolkit of modern-day molecular biologists. The metagenomics course offered by the North Carolina State University Biotechnology Program is an 8-week lab module combining wet labs, computer activities using local and cloud-based software, and interactive lectures. Offered for the first time in the fall of 2013, this module was designed to familiarize upperclassmen, graduate students, and postdoctoral fellows with approaches for the analysis of genomic data. Concept mapping and diagramming processes have been shown to promote meaningful learning; thus, throughout the course participants created flowcharts depicting the procedures required to prepare and analyze genomic data. Participants used Lucidchart web-based software to create and share diagrams. After each laboratory activity, pairs of students were asked to submit their depiction of the processes conducted and personal written reflections of the implications of the tools they applied. Anonymous student self-assessments that were administered at the beginning and end of the course revealed increased student confidence in the

knowledge and skills gained from completing this course. Results of the survey are presented in light of the influence of diagramming and reflective writing on students' attitudes toward process-driven bioinformatics instruction.

Mechanical strategies in primate locomotor switching, and implications for the evolution of locomotor diversity

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Primates appear to have the greatest locomotor diversity of any other mammalian group. This has been attributed to a high degree of locomotor versatility, which is the ability to rapidly switch between disparate locomotor behaviors and/or alter certain aspects of gait characteristics in order to effectively adjust for substrate variation. Currently, little information is available concerning the mechanical strategies utilized by primates when switching between distinct locomotor behaviors. We use the transition from above- to below-branch (suspensory) quadrupedal walking to examine the underlying mechanisms of locomotor versatility. Three primate species that vary in their frequency of below-branch movement--*Lemur catta, Varecia variegata*, and *Propithecus coquereli* --were encouraged to walk both above and below an instrumented arboreal runway in order to collect kinetic, kinematic, and spatiotemporal gait variables. Kinematic and spatiotemporal values were similar during above- and below-branch locomotion, with the exception that during below-branch walking there was an overall tendency for increased limb flexion. In contrast, during below-branch compared to above-branch walking animals demonstrated relatively higher peak vertical forces in the forelimb, and the forelimb, rather than the hindlimb, served a net propulsive role. The combination of conserved kinematics and timing and clear differences in gait kinetics suggests that primates experience a very different loading regime during below-branch compared to above-branch walking and likely developed a musculoskeletal or neuromuscular mechanism to effectively execute such locomotor shifts. Selection towards anatomical and neurological features that favor locomotor versatility may represent an adaptation important for the diversification of primate locomotion.

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Utilization of qPCR to assess Select Cyanobacteria and Cyanotoxin Abundance in Six Piedmont North Carolina Lakes

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Cyanobacteria species are distributed worldwide and comprised ≥ 90% of phytoplankton in many NC reservoirs. Extensive growth can result in cyanotoxin production, hypoxic zones, human health risk, and mortality of fish, domesticated animals, invertebrates, and plants. Quantitative PCR (qPCR) can be used to help water quality managers and regulatory agencies assess and manage cyanobacteria and cyanotoxin abundance to reduce environmental and health risk. This study determined the presence and relative abundance of 4 potentially toxic

cyanobacteria taxa (*Microcystis aeruginosa, Cylindrospermopsis raciborskii, Lyngbya wollei,* and *Aphanizomenon/ Anabaena* spp.), total cyanobacteria, and three toxin genes (microcystin *mcyH+/A-*, cylindrospermopsin *PKS*, and anatoxin *PKS*). Additionally, concentrations of microcystin and cylindrospermopsins were determined in selected samples using ELISA assays. All species were found in all lakes, but abundance of individual taxa SSU rDNA and toxin genes differed among lakes. Microcystins were found at low levels (≤0.31ppb) in 4 of the 6 lakes sampled, but were not correlated with *mcyH+/A-* abundance. The cylindrospermopsin *PKS* gene and cylindrospermopsins were not found in any of the lakes sampled. The anatoxin *PKS* gene was found in all lakes sampled and was significantly correlated with the presence of *Aphanizomenon/ Anabaena* species in two of the lakes. Anatoxin may be an emerging problem in North Carolina lakes.

Theoretical solution of the operations research model for satisfactory highway cruising Speed

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Due to the impracticality of optimal highway cruising speed for fuel economy, based on the speed versus miles per gallon curve and data, by using the cubic spline curve fitting, an operations research model is established to find the satisfactory highway cruising speed for private cars. The model can be successfully solved theoretically to obtain the satisfactory speed that is an optimum balance between fuel economy and highway speed limit.

Myopodin, an actin binding protein, is localized in the nucleus and nucleoli of cancer cells.

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Myopodin is a member of the synaptopodin 2 superfamily of actin binding proteins. Several studies showed that these proteins act as a tumor promoter while other studies demonstrated that synaptopodin 2 overexpression leads to a decrease in invasiveness in myoblast cells. These differences have been associated to a nuclear-cytoplasmic translocation of myopodin. It has been suggested that the tumor suppressor function was associated with nuclear location and tumor promotion with cytosolic location. Four synaptopodin 2 isoforms, which differ in their C termini, have been characterized to date (isoforms A, B and C, and Myopodin). Myopodin is a truncated version of isoform B that lacks the N-terminus. The specific involvement of the different isoforms is still unclear. We investigated the distribution of myopodin and isoform B in several mammalian cell lines using an antibody that recognize specifically these two isoforms as well as fusion GFP protein for each isoforms. We report that the cells in our study expressed primarily myopodin rather than synaptopodin 2 isoform B. Myopodin expression was largely confined to the nuclei. We observed intense nucleolar staining. An analysis of the nucleolar expressed proteins suggested that the protein product may be a truncated form of myopodin.

Dissecting the underlying mechanisms involved in brain hemorrhage in cilia mutants.

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is an inherited disease that primarily affects the kidneys causing it to enlarge due to the array of cyst forming clots and can also affect the brain and artery blood vessels. ADPKD is the most common life-threatening single gene disease and impacts over 500,000 individuals in the Unites States. In brain hemorrhage, ADPKD is characterized by the role cilia plays in Intraflagellar Transport (IFT) and defects in this cilia mediated transport has been correlated to many vascular deficiencies such as an Intracranial Hemorrhage (ICH). Although new therapeutic approaches are being developed for treatment of ADPKD and ciliopathies, the underlying pathophysiology, function and role of the cilia is not fully understood involving ICH. We hypothesize that hemorrhage in IFT mutants results from vascular leakage. The objective of the current study was to assess the ability to measure and better understand the vessel development in ICH embryos and IFT mutants. Developing a zebrafish line with a transgenic Fli:GFP *(Tg (fli:GFP))* marker and IFT mutants will help determine how vascular stability plays a role in IFT mutants. For the present study, three different types of IFT mutant fish lines were crossed to *Tg(fli:GFP)* ; ift81, ift172 and a qilin. Previously in the lab, a full length mRNA experiment was successful in effectively decreasing the ICH and body curvature present in mutants however; a better conclusion can be drawn by examining additional cilia and non-cilia mutants to determine if the hemorrhaging phenotype is specific to the absence of cilia and by providing information on the role hedgehog signaling plays in cilia regulating vascular stability.

Solvent Effects on the Copper(I)/TEMPO-Catalyzed Aerobic Oxidation of Primary Benzylic Alcohols

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Recently, a homogeneous catalyst system utilizing copper(I) bromide, bipyridine (bpy), TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl), and N-methylimidazole (NMI) to oxidize primary alcohols to the corresponding aldehydes was reported. Acetonitrile and acetone were used as the solvents in these initial studies. The creators of the copper(I)/TEMPO system reported the rate-limiting step of this oxidation reaction to be slower than comparable literature values, attributing this discrepancy to solvent and ligand effects. The results of this oxidation reaction with a variety of benzylic alcohols in a variety of solvents will be reported. The effects of replacing bipyridine with a more electron-donating copper ligand will also be reported. Baseline reaction times and yields were gathered from using acetonitrile and acetone for comparison. The purity of the aldehydes produced by this catalyst system was evaluated by IR and NMR spectroscopy.

The phylogeography of the Southern seepage salamander (*Desmognathus aeneus*)

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The seepage salamander, *Desmognathus aeneus*, reaches the southern terminus of its range near the fall line of Alabama. In general this species is more widespread in the northern portion of its distribution. The southern populations tend to be isolated and disjunct. Here we present the results of a molecular phylogenetic survey comprised of fourteen populations from the southern range extent. We collected mitochondrial DNA sequence data (~650 bps COX1) that was used as the input of a Bayesian phylogenetic reconstruction. The southern populations appear to represent the earliest diversification events in this species, but otherwise there is surprisingly little genetic variation within these populations.

Studies of the signal transduction pathways regulating myosin II localization and activity in the social amoeba *Dictyostelium discoideum*

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In *Dictyostelium discoideum*, four related myosin II heavy chain kinases (A, B, C, and D) regulate myosin II-mediated contraction of actin filaments during cell division (cytokinesis) and cellular migration. In addition, there is evidence suggesting that two other proteins, a DYRK family protein kinase – YakA and alpha kinase I (AK1), may also function in regulating myosin II filament turnover in the cell. YakA is responsible for the phosphorylation of PI3K, resulting in actin polymerization, suggesting involvement in myosin II recruitment. In order to understand the potential role of YakA in myosin II cortex localization, we used a YakA null cell line, electroporated with GFP-tagged myosin II to visualize localization when stimulated with cAMP. Additionally, the AK1 protein shares sequence homology with the catalytic domains of the MHCK family, suggesting a possible role in regulation of myosin II function in the cell. To explore this possibility, we have engineered recombinant plasmids both for the inducible expression of AK1 as a GFP-tagged fusion protein within *Dictyostelium* as well as for the purification of AK1 as a FLAG-tagged fusion protein for biochemical studies. Collectively, these studies have the potential to impact our understanding of the basic cellular functions of these two novel proteins in a variety of cellular contexts, including cellular division and cell migration, both of which are impaired in cancer cells that exhibit uncontrolled multiplication and metastasis.

Exploring charge dependence of the strong force by modeling neutron-proton scattering

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In nature we find four fundamental forces; the strong nuclear, weak nuclear, electro-magnetic, and gravitational. The strong force, as the name implies is the strongest at nuclear range.

We now know that Quantum ChromoDynamics (QCD) is the underlying theory of the strong nuclear interaction. However, the strong force in the low energy regime is mediated by the pion (the lightest meson). The strong nuclear force is largely charge-independent but this symmetry is broken in nature as evidenced for example: by the existence of

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Examination of Mediators of Interleukin-1 Receptor Antagonist (IL-1RA) during *Yersinia Pestis* Pulmonary Infection

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Yersinia pestis is an extremely virulent bacterium responsible for hundreds of thousands of deaths throughout the course of modern history. The respiratory route of *Y. pestis* transmission causes a robust pneumonia that is 100% lethal. This high lethality rate of the infection may stem largely from the organism's ability to evade the hosts innate immune response for up to 36 hours post-infection, thus giving *Y. pestis* the time necessary to reproduce and resulting in the death of the host. *Y. pestis* is able to evade early immune activation, at least in part, by activating host protein interleukin 1 receptor antagonist (IL-1RA). Our group has shown that this protein inhibits the activity of interleukin 1, a pro-inflammatory cytokine that is a key component of the host's innate immune response.

Our group aims to first identify, and then further mechanistically decipher the contributions of both the pathogen and the host in the innate immune response to an infection of the lung by *Y. pestis.* We hypothesize that *Y. pestis* activates a toll-like receptor (TLR) on the surface of cells in the lung, which in turn secrete IL-1RA. We will use both an in-vivo mouse model, as well as an in-vitro model with specific cell types to characterize this host cell response. Our preliminary data suggest that neither TLR 4 nor TLR 5 are involved in this process. Further research will consist of using the systems mentioned above to further characterize this response.

Direct high frequency shoot regeneration from mature seeds in legumes.

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University of Mount Olive

A novel method for a high frequency shoot regeneration was established in Pisum sativum var. Sabre using N6-benzylaminopurine(BAP) and Thiadizuron (TDZ). Mature seeds cultured on Murashing and Skoog medium (MS) supplemented with BAP (100 um) or TDZ (10 um) induced multiple shoots within 4-6 weeks. Our data demonstrated that TDZ induced a higher frequency of shoot regeneration compared to that of BAP. One to two cm long shoots were excised and cultured on MS medium. Shoots developed roots in 4-6 weeks. Successful acclimatization of pea in vitro plants was achieved in the soil. No genotypic variation in multiple shoot regeneration was observed and the results were consistent with previous studies. However, BAP and TDZ failed to regenerate multiple shoots from mature seeds of Phaseolus lunatus. The regeneration system developed in this investigation for this important crop could be a useful tool for the genetic modification through Agrobacterium-mediated or particle bombardment genetic

transformation.

Identification of Quantitative Trait Loci (QTL) Underlying Protein, Oil, and Five Major Fatty Acids' Contents in Soybean

My Abdelmajid Kassem, Akond, Masum, Shiming Liu, Melanie Boney, Stella K. Kantartzi, Khalid Meksem, Nacer Bellaloui, and David A. Lightfoot.

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Improved seed composition in soybean [*Glycine max* (L.) Merr.] for protein and oil quality is one of the major goals of soybean breeders. A group of genes that act as quantitative traits with their effects can alter protein, oil, palmitic, stearic, oleic, linoleic, and linolenic acids percentage in soybean seeds. The objective of this study was to identify Quantitative Trait Loci (QTL) controlling protein, oil, and fatty acids content in a set of $F_{5:8}$ RILs derived from a cross between lines, MD 96-5722 and 'Spencer' using 5,376 Single Nucleotide Polymorphism (SNP) markers from the Illumina Infinium SoySNP6K BeadChip array. QTL analysis used WinQTL Cart 2.5 software for composite interval mapping (CIM). Identified, were; one protein content QTL on linkage group (LG-) B2 or chromosome (Chr_) 14; 11 QTL associated with oil content on six linkage groups LG-N (Chr_3), LG-A1 (Chr_5), LG-K (Chr_9), LG-F (Chr_13), LG-B2 (Chr_14), and LG-J (Chr_16); and sixteen QTL for five major fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids) on LG-N (Chr_3), LG-F (Chr_13), LG-B2 (Chr_14), LG-E (Chr_15), LG-J (Chr_16), and LG-G (Chr_18). The SNP markers closely linked to the QTL reported here will be useful for development of cultivars with altered oil and fatty acids compositions in soybean breeding programs.

Keywords: Soybean; SNP Linkage Map; QTL; RIL; oil; major fatty acids; MD96-5722; 'Spencer'.

Modeling and Mutagenesis of the FMN Riboswitch

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Riboswitches are RNA structures that bind metabolites, and via alternative secondary structures modulate downstream gene expression in bacteria. We are using homology modeling to identify key nucleotides involved in the binding of flavin mononucleotide (FMN) to the FMN riboswitch of *Chromobacterium violaceum*. Proposed mutations will be studied computationally and promising mutations will be created molecularly at key positions in the aptamer/ligand-binding domains of the riboswitch. The affect of the mutations on ligand binding will be compared through biochemical and phenotypic analysis.

The Effect of Carbohydrates on Cell Morphology in *Halobacterium salinarum*

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Halobacterium salinarum is an extremely halophilic archaeon, thriving in environments 5 – 10 times the salinity of sea water. These organisms possess a complex DNA repair system, acidic proteins to minimize denaturation, and other adapted molecular characteristics that enable them to survive in such adverse conditions. However, an understanding of their metabolic pathway is vague at best. More specifically, the link between cell shape and nutrients is unclear. One of the central regulators of metabolism in *H. salinarum* is the transcription factor, TrmB. To give insight on how and why TrmB regulates metabolism, we studied a mutant strain lacking the gene, Δ*trmB*, and its parent strain, Δ*ura3*. Under normal conditions, *H. salinarum* is rod shaped. However, the Δ*trmB* mutant is spherical and grows significantly slower. We supplemented Δ*trmB* with carbohydrates and assayed cell shape in order to determine which compounds complement the shape phenotype and are likely of intrinsic value. Results indicate that glycerol and glucose solutions can cause Δ*trmB* to resemble its parent strain. This suggests that they are of some importance to central metabolism.

Synthesis of Peropyrene Derivatives

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Peropyrene crystals have exhibited no evidence as a suitable molecule for singlet fission, mainly due to the herringbone packing structure observed in the crystal structure. By varying the substituents on the molecule, the packing of the crystal structure can be disrupted and possibly allow singlet fission to occur. The goal of this project is to synthesize a range of peropyrene derivatives that will be assessed for singlet fission activity. The synthesis of the final peropyrene derivative that will be evaluated for singlet fission is a five-step synthesis that starts with 2,7 – dihydroxynaphthalene. This project reports the successful synthesis of 2-amino-7-hydroxynaphthalene and the attempted synthesis of 7-hydroxy-2-iodonaphthalene, which are the products of the first two steps.

The effect of pentylenetetrazol-induced seizure and the antiepileptic drug phenytoin on spatial learning in zebrafish

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Impairments of cognitive function can be caused by both epileptic seizures and antiepileptic drugs, complicating the management of epileptic disorders. The present study investigated the

cognitive impairments due to seizures to those caused by the antiepileptic drug phenytoin. Adult zebrafish (*Danio rerio*) were used as the model system, as they have been shown to be reliable model systems for the research of epilepsy and operant conditioning. In order to assess the extent of cognitive impairment, fish were trained in a T-maze over 16 trials using positive punishment. Seizures were kindled using pentlyenetetrazol (PTZ), a compound that has been established for its utility in seizure research in adult zebrafish. Three chemical treatment groups were tested to compare the effects of seizures with those of the phenytoin and their interaction: PTZ alone, phenytoin alone, PTZ with phenytoin pretreatment, and an untreated control. Learning was assessed primarily by the number of trials it took each fish to reach a total of three correct responses. Results showed learning speed was significantly ($p<0.01$) reduced for the PTZ only condition and for the doubly-treated condition as compared to control. A trend toward increased impairment was seen when fish received the combination treatment compared to the PTZ only group. These results indicate that phenytoin does not ameliorate the cognitive impairment associated with seizures, as was expected based on similar studies using different antiepileptic compounds. The observed effect poses a potential problem for those managing their epilepsy with phenytoin and warrants further investigation.

Alterations in Behavior Drive Population and Community Dynamics of Rodents Associated with Intercropping Switchgrass in Pine Forests

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Although habitat alterations have been shown to impact rodent populations and communities, individual behaviors will be impacted initially. 'Alamo' switchgrass (*Panicum virgatum*), a native biofuel feedstock, was intercropped in loblolly pine (*Pinus taeda*) plantations in Kemper County, MS, on land owned and managed by Weyerhaeuser Company within stands established and maintained by Catchlight Energy LLC (CLE), a Chevron|Weyerhaeuser joint venture. We hypothesized that population and community dynamics would differ between traditional pine stands and pine intercropped with switchgrass, driven by alterations in individual rodent habitat selection (home range size and suitability) and communication (amount of ultrasonic vocalizations (USVs)) through effects on survival, recruitment, and reproduction. To test our hypothesis, we are assessing effects of three treatments (switchgrass monoculture, switchgrass intercropped in loblolly pine, and control loblolly pine) on rodent individual behaviors, populations, and community structure. Our methods include live-trapping, radio telemetry of individual *Sigmodon hispidus*, thermal video and acoustic recording, and vegetation surveys during 2013-2015. We will use NMDS and ANOVA models to compare dependent variables within and among treatment plots. Among treatments, preliminary results do not show separation in rodent community assemblages ($R=0.06$, $p=0.25$), but indicate microhabitat characteristics differed ($R=0.61$, $p<0.01$). We expect individual home range area to decrease where grasses are abundant, and subsequently, we predict that individuals will interact more frequently using USVs. We expect that populations whose individuals have smaller home ranges and interact more, will have higher survival, recruitment, and reproduction and therefore higher abundances. Lastly, we expect plots with abundant grass cover to be dominated by populations of herbivorous species and therefore to have the lowest rodent diversity. Results from this study

will improve understanding of how individual responses to habitat alteration affect changes at population and community levels.

Cellular studies of *Dictyostelium* myosin II heavy chain kinase D

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Dictyostelium is a simple eukaryote with a small genome (34Mb) that encodes many genes with structural and functional homologues in higher eukaryotes. Previous studies have shown that myosin II turnover via phosphorylation is regulated by at least three myosin heavy chain kinases (MHCKs) –A, -B, and –C; all of which share homologous catalytic and WD-repeat domains. A fourth kinase, MHCK-D, also contains these homologous domains and shares the ability to phosphorylate and drive myosin II filament disassembly in vitro, though it's presence is only detected in developing cells. Our studies using fluorescence microscopy to examine live cells expressing GFP-tagged MHCK-D revealed that the kinase undergoes robust translocation to the cell cortex in response to stimulation with the chemoattractant cyclic-AMP. To examine the in vivo role of MHCK-D in myosin II filament turnover, the localization of GFP-tagged Myosin II was examined in cell lines lacking MHCK-D, as well as in cell lines lacking MHCK-A/B/C in both vegetative and developing conditions. Fractionation studies indicate that developing cells lacking MHCK-D show an increase in Myosin II associated with the cytoskeleton, 34%, when compared to wild-type cells, 28%, under the same conditions. Taken together, our data indicate that MHCK-D, presumably through its ability to drive myosin II disassembly in the cell, plays a central role in controlling the tightly regulated and highly specific changes in shape required for the movement of cells in the contexts of chemotaxis and migration.

Does ESAT-6 Elicit an Immunological Effect on Multi-Wall Carbon Nanotube Instilled Mice?

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Background. Pulmonary granuloma formation represents a complex and poorly defined response involving environmental and host factors that can culminate in persistent and chronic inflammatory disease. We have described a murine granuloma model (AJRCMB 2011, 45: 858) in which multiwall carbon nanotubes (MWCNT) elicit a granulomatous disease markedly similar to that found in Sarcoidosis, a prototypical human granulomatous disease. MWCNT elicited granulomatous disease is chronic (granulomas persist up to 90 days), and characterized by elevated pro-inflammatory cytokines together with T cell and macrophage recruitment – all traits found in Sarcoidosis. ESAT-6 is a *Mycobacterium tuberculosis* secreted protein and T cells from patients with Sarcoidosis have also been reported to react to ESAT-6 peptides. Our lab has shown that granuloma formation is greater in MWCNT+ESAT-6 mice compared to MWCNT instilled mice. **Hypothesis.** We hypothesized that ESAT-6 will elicit an increase in inflammatory response induced by MWCNT+ESAT-6. **Methodology**. MWCNT (100 μg) +/- ESAT-6 peptide 14 [NNALQNLARTISEAG] (20 μg) were instilled into wild-type C57Bl/6

mice. Controls consisted of (a) sham-instilled and (b) ESAT-6 alone. Animals were sacrificed after 10, 30, and 60 days. The spleens of the mice were used in order to analyze the inflammatory response of the lymphocytes. Spleens were extracted and perfused with PBS. Cell pellets were washed with PBS and resuspended in media. Cells were counted and checked for viability. Cells were washed, and then cultured for 24 hours with different stimuli, PHA and ESAT-6. Fresh cells were also used for Flow Cytometry using the following antibodies: CD3, CD4, and CD154 and analyzed via FACSDIVA software. **Results.** FACS analysis was performed on fresh and cultured isolated spleen cells. Freshly isolated spleen cells revealed no difference in the cell surface markers, MHC, CD4, and CD8 within the 10D, 30D, and 60D post MWCNT instillation. Upon stimulation with PHA, or ESAT-6, the expression of CD3, CD4 and IFNγ were unchanged as compared to unstimulated. No changes were observed at different time points (10D, 30D, and 60D). **Summary and Conclusions**. Data suggest that there was no change in the extra pulmonary response induced by MW+ESAT-6. There was no recruitment of CD3 or CD4. Further study will need to be conducted to determine the specific effects of ESAT-6 on MW-instilled mice.

The Effect of Valproic Acid on Notch Signaling in Intestinal Cell Development in *Danio rerio*

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Inflammatory bowel disease (IBD), a classification of autoimmune disorders characterized by chronic GI distress, is known to be caused by an interaction between environmental factors and a genetic predisposition. However, an exact mechanism explaining the onset of IBD has yet to be uncovered. Recent studies have found that a reduced number of mucus-secreting cells is typical in people affected with IBD. Furthermore, the differentiation of intestinal cell types into either absorptive or secretory is governed by a signaling pathway, known as Notch, wherein an intracellular domain behaves as a coactivator for transcription. Despite extensive research, there remain gaps in knowledge of how this pathway is activated or inhibited in people who experience chronic IBD. The purpose of this study was to induce the Notch pathway with the expectation that a corollary shift in the expression of secretory versus absorptive cell types in the gut would be observed. An unrelated study showed that Valproic acid (VPA), an organic compound commonly used in the treatment of neurological disorders, was discovered to activate the Notch pathway in the regulation of neuronal cells. Commonly reported side effects of VPA treatment include many GI dysfunctions, indicating the possibility that VPA affects Notch signaling in the intestines. Zebrafish (*Danio rerio*) models were used to observe this effect. A dosage curve was generated to determine the optimal concentration of VPA to administer to the zebrafish. Four concentrations—100 μM, 500 μM, 1000 μM, 1500 μM—were delivered twice a day over the course of three days. The results showed that 500-1000 μM was the optimum level to have an effect without overdosing the zebrafish. Further testing is currently in progress. Following VPA treatment, intestinal tissue will be extracted and a PCR analysis will be run to determine any fluctuations in the expression of the Notch receptor.

Vitis seeds from the Pleistocene of the Atlantic Coastal Plain of North Carolina

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The Martin Marietta locality, recently discovered in an open aggregate mine pit near Wilmington, North Carolina (34° 22. 368' N, 77 ° 50.356' W), has produced abundant macrofossil remains. Palynological records from the site indicate a Pleistocene age and suggest that the sediments are from the Sangamon Interglacial period. The flora shows a wide range of diversity compared to other Pleistocene sites. Among these, *Vitis* seeds have been identified and described. Comparison to extant and extinct species of the Vitaceae was conducted, with similarity most prevalent with modern *Vitis* located in the local extant flora. Seed length, ventral infold length and width, chalaza width, and basal groove angle were the main characters used to determine species. Taxa identified include *Vitis* subspecies *Vitis,* and *Vitis rotundifolia*. This represents one of the few records of *Vitis* in the Pleistocene, North Carolina, and the Sangamon Interglacial.

A Clearance Pathway for Treating Age-related Diseases: Enhancing the Lysosomal Pathway with PADK Promotes Protein Clearance in Models of Alzheimer's and Parkinson's Disease

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Lysosomes are components inside of cells that clear out mutated and misfolded proteins. Alzheimer's diseases (AD), Parkinson's disease (PD), and other age-related brain disorders linked to dementia have a key pathogenic similarity: they all exhibit abnormal protein accumulation events intracellularly and extracellularly. Cathepsin-B (Cat-B) is an enzyme created by the lysosomes that has been shown to degrade mutated and misfolded proteins. Amyloid Beta (Aβ) is a protein that accumulates in the brain and forms plaques that are associated with AD pathology. A-synuclein (α-syn) is a protein that also accumulates in the brain and in associated with PD. Therapies are being developed to target a broad range of protein accumulations in order to treat a wide array of protein folding pathologies. A compound, Z-Phe-Ala-diazomethylketone (PADK) is one of those treatments that has been shown to modulate lysosomes, facilitating the production of Cat-B. Three separate mouse models were used for this experiment: two models with AD and one model with PD. Both models were treated with PADK (ip, 18 mg/kg/d for 9-14 days). Based on these treatments of AD and PD, significant results were observed and obtained. In the AD mouse models a substantial reduction of Aβ and an increased production of Cat-B were observed. Furthermore, a reduction of α-syn proteins and an increase of Cat-B were observed in the PD mouse model. In both the AD and PD mouse models, visible improvements in clearance pathways and improved synaptic integrity were observed. The reduction of the Aβ and α-syn are shown to encourage synaptic recovery and lessen the effects of protein pathologies.

Developing a novel class of antibiotics: targeting essential metabolic pathways to combat antibiotic resistance

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The most common class of antibiotics used to treat bacterial infection are β-lactams, which inhibit cell wall synthesis. The treatment of antibacterial infections has become a major problem in the clinical setting due to the widespread development of antibiotic resistance. The β-lactamase enzyme confers resistance to β-lactam antibiotics in many strains of bacteria by cleaving and degrading β-lactams. Our goal is to develop a unique class of antibiotics which will counter these mechanisms of resistance by taking advantage of β-lactamase processing to release the active component of a pro-drug. We developed a scheme to identify compounds that would work as possible drugs, identified target pathways (such as ATP synthesis), and we tested suggested inhibitory drugs. Each compound was tested in culture conditions that mimic the *in vivo* environment. We hypothesized that inhibition of essential metabolic pathways would effectively prevent growth of pathogenic bacteria. Using the plague bacterium *Yersinia pestis* as a test case, we performed minimum inhibitory concentration (MIC) tests to determine the effectiveness of each compound. All the tested compounds significantly inhibited *Y. pestis* at low micromolar concentrations. Once an effective delivery system is in place, these findings will be useful in administering the compounds as drugs with limited toxicity in mammals.

Encapsulation of Derived Bicellular Vascular Population in Type I Collagen Gel

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Vascular diseases, such as Coronary Artery Disease, Hypertrophic Cardiomyopathy, and heart failure continue to be the leading cause of death in the United States. Current treatment therapies employ catheters, stents, and grafts to help preserve arteries and vessels affected by vascular disease. These therapies are often associated with negative attributes such as, complications during surgery, immunogenicity**,** and inability to recover the functionality of vasculature. Cell-based therapies, such as engineered vascular networks, allow for a more complete and sustainable treatment to vascular diseases and is a crucial branch of regenerative medicine. The vasculature's main component, Endothelial Cells (ECs), have the ability to form complex networks when cultured in 3 Dimensional (3D) scaffolds; however, these networks are not sustainable and are shown to regress over time. This regression is due to the absence of Pericytes, structural cells which surround the microvessel endothelium. Pericytes play an active role in angiogenesis such as sensing the physiological needs of the tissue, the presence of angiogenic stimuli, and the hemodynamic forces within the vessel. Pericytes have also been shown to help degrade the extracellular matrix, act in cell-cell contact-dependent control of EC proliferation and signaling, and to migrate toward EC tubules to reach the abluminal surface on which they are found. Current in vivo microvasculature studies employ individual cell sources of EC's and pericytes; however this methodology proves to be clinically irrelevant due to limited human cell sources. Here, we develop a protocol for encapsulation of a derived bicellular vascular population of ECs and Pericytes from human pluripotent stem cells in a 3D type I collagen gel matrix. When encapsulated in collagen gel we observed increased sustainable network formation of early vascular cells (EVCs). When supplemented with growth factors stem cell factor, stromal cell derive factor, vascular endothelial growth factor, and interleukin-3, network formation was shown to last up to one week. In conclusion, the protocol developed for

encapsulation of a derived bicellular population will provide a more relevant option for engineering vascular network.

Cytochrome P-450 (CYP) Variants as Potential Biomarkers of Sensitivity to Chlorpyrifos

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Genetic variants in CYP 2B6 and CYP 2C19 are useful biomarkers of susceptibility to chlorpyrifos toxicity. Chlorpyrifos (CPF) is a prevalent organophosphate pesticide (OP), used in agriculture throughout the world. Following exposure, CPF is metabolized by cytochrome P-450 2B6 (CYP2B6) to an active oxon metabolite that inhibits acetyl cholinesterase activity, and contributes to neurotoxicity. Previously, Egyptian agriculture workers have been reported to have neurobehavioral deficits which are associated with high occupational exposures to CPF (Farahat et al., 2011). The purpose of this study is to assess the prevalence of know polymorphisms (genetic variations) of CYP2B6 (activates CPF) and CYP2C19 (detoxifies CPF) in these Egyptian agriculture workers, which may serve as biomarkers for the susceptibility of individuals to the neurobehavioral deficits reported with occupational exposures to CPF. The experiment began using saliva that was collected from a previous study of participants, and DNA was isolated according to manufacturer's protocol. Two separate rounds of multiplex PCR on genomic DNA samples. The first round is referred to as the CYP Exon round which amplifies specific regions of the target genes CYP 2B6 and CYP 2C19. The multiplex PCR takes place on ice. CYP 2B6 exons: 5+6, 9 & 4 and CYP 2C19 exons: 2+3 &5 will be amplified. The second round is referred to as the CYP Single nucleotide polymorphism (SNP) round which is specific for the CYP 2B6 and CYP 2C19 polymorphism of interest. SNP is the most common type of genetic variation among people. This round uses the product from the Exon product template diluting it (1:100). The polymorphisms of interest are now distinguished as either dominant or variant and prepared accordingly. The PCR products were ran on an agarose gel to determine CYP genotypes. Multiplex polymerase chain reaction (PCR) was used to simultaneously amplify multiple regions of these target genes and assign genotype. Multiplex PCR was successfully used to simultaneously amplify multiple known polymorphic variants of CYP 2B6 and CYP 2C19 in Egyptian pesticide applicators. CYP2C19 produces the greatest formation of TCP following chlorpyrifos metabolism, thus supporting its role in the detoxification (dearylation) reaction. The prevalence of CYP 2B6 variants in Egyptian pesticide applicators suggests that genetic variants of CYP2B6 will serve as biomarkers of susceptibility to chlorpyrifos. Future studies will then investigate the association of CYP2B6 and CYP2C19 genotype with the neurobehavioral deficits observed in occupational cohorts exposed to CPF.

Effects of Varying Agricultural Practices on Mammal Diversity

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Wildlife habitat is increasingly being converted to agricultural land to meet the demands of a growing human population. Determining how different agricultural practices will influence

biodiversity is thus critically important. Although significant literature has explored the relationship between farming practices and insect diversity (particularly for pests and pollinators) less work has focused on the impacts of farming practice on vertebrate species. We are investigating the relationship between specific agricultural practices (including pesticide use, farm size, and crop diversity) and mammal biodiversity (measured through species diversity, abundance, and Shannon Index) on farms throughout the Piedmont of NC. We are collecting mammal samples from fifteen farms in Central North Carolina. Twenty Sherman live traps and three Bushnell Trophy Cams will be used at each site to measure both small and large mammal diversity. We have collected preliminary data from two study sites, but will continue sampling through June 2014. Sampled species to date include the white-footed mouse, hispid cotton rat, white-tailed deer, and Virginia opossum. Results will be analyzed using a MANOVA. Use of pesticides is anticipated to be correlated with lower mammal diversity, and high crop diversity with the higher mammal diversity. Results aim to inform future agriculture in a direction that facilitates the greatest natural biodiversity, thus preserving ecosystem services. Due to its demonstrated interest in boutique agriculture, North Carolina is excellent candidate for applying this study's findings.

Peroxygenase and Oxidase Activities of Dehaloperoxidase-Hemoglobin from Amphitrite ornata

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The marine globin dehaloperoxidase-hemoglobin (DHP) from Amphitrite ornata was found to catalyze the H2O2-dependent oxidation of monohaloindoles, a previously unknown class of substrate for DHP. Using 5-Br-indole as a representative substrate, the major monooxygenated products were found to be 5-Br-2-oxindole and 5-Br-3-oxindolenine. Isotope labeling studies confirmed that the oxygen atom incorporated was derived exclusively from H2O2, indicative of a previously unreported peroxygenase activity for DHP. Peroxygenase activity could be initiated from either the ferric or oxyferrous states with equivalent substrate conversion and product distribution. It was found that 5-Br-3-oxindole, a precursor of the product 5-Br-3-oxindolenine, readily reduced the ferric enzyme to the oxyferrous state, demonstrating an unusual product-driven reduction of the enzyme. As such, DHP returns to the globin-active oxyferrous form after peroxygenase activity ceases. Reactivity with 5-Br-3-oxindole in the absence of H2O2 also yielded 5,5'-Br2-indigo above the expected reaction stoichiometry under aerobic conditions, and O2-concentration studies demonstrated dioxygen consumption. Non-enzymatic and anaerobic controls both confirmed the requirements for DHP and molecular oxygen in the catalytic generation of 5,5'-Br2-indigo, and together suggest a newly identified oxidase activity for DHP.

Inhibition of Peroxynitrate-Induced DNA Damage by Caffeine

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Caffeine is a widely consumed beverage in much of the world. Recent epidemiological studies have demonstrated that coffee consumption is inversely associated with neurodegenerative

disorders such as Parkinson's disease and Alzheimer's disease. However, the mechanism for this protection is unknown. This study was undertaken to investigate the effects of caffeine in peroxynitrite-induced DNA strand breaks, an important event leading to peroxynitrite-elicited neurotoxicity. Incubation of φX-174 plasmid DNA with the peroxynitrite generator 3-morpholinosydnonimine (SIN-1) led to the formation of both single- and double-stranded DNA breaks in a concentration-dependent manner. Caffeine as low as 50 μM significantly inhibits SIN-1-induced DNA strand breaks in a concentration-dependent manner. The protective effects of caffeine on peroxynitrite-mediated oxidative DNA damage may shed some light on the mechanisms of its neuroprotective activities observed in human clinical trials.

Comparing the Dot chromosome of *Drosophila biarmipes* to *Drosophila melanogaster* based on similarities in the amino acid sequences of the exons.

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The purpose of this project is two-fold: 1.) to provide GeneBank with information on the *Drosophila biarmipes* Dot chromosome and 2.) to document the evolutionary divergence between two species of *Drosophila*: *D biarmpies* and *D. melanogaster*. The Dot chromosome is the smallest and most heterochromatic of the four *D. melanogaster* (Dmel) chromosomes. Recent advances in sequencing technology have enhanced research of this region. Specifically, this project focuses on a ~40kB stretch of *D. biarmapies* Dot chromosome known as 'contig13'. Bioinformatic blastx results suggest that contig13 contains nineteen translated exons similar to Dmel genes: CG31999 isoform A and *Crk*, isoforms A-F. Since the function of both gene products is essential for proper organismal development, it is hypothesized that exon sequence and usage will be conserved between the two species of fruit flies. In general, methods used to compare the *Dmel* and *Dbiarm* Dot DNA regions included bioinformatics analyses of amino acid sequences and splice-site phase alignment for each unique gene isoform. In addition, analysis of Dbiarm RNA-seq expression patterns was necessary to locate small exons. The culmination of gene annotation is a proposed gene model for the unstudied *D. biarmapies* genes of the Dot chromosome. Based on the data gathered both genes are conserved in both species, they both have thirteen exons for CG31999 and six exons for Crk. Overall the exons were the same sizes, but differed slightly within the amino acid sequence, indicating significant conservation, but variances suggest evolution and adaptation has occurred in these genes contributing to speciation.

Cycad cuticle micromorphology: What role does ecological pressure play?

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Classically the phylogeny of cycads have been mainly based on macro-morphology. Recent works based on molecular data have rearranged the cycads phylogeny, and suggested that the extant cycads diversity is the result of a Miocene-Pliocene radiation, probably triggered by increased aridity and seasonality. This study was carried on all extant cycads genera to investigate whether leaf cuticle micromorphology reflects phylogenetic relationships among species, and if it is influenced by ecological pressures. Whole leaf and isolated cuticle specimens from 42 cycads species were examined using SEM for features of inner and external surfaces. Samples were collected from the middle region of leaflets of mature leaves. For external surfaces, samples were air dried or fixed in FAA (10:5:50) and critical-point dried. For the inner cuticle surface, isolated cuticles were obtained using 20% CrO_3. The stomata and cuticle micromorphology as well as the distribution of different kinds of epicuticular waxes in the species considered coarsely reflect the phylogenetic patterns emerged from molecular analyses. Besides, it was possible to detect additional patterns in cuticle micro-morphology characters probably related to ecological pressures. In particular, epicuticular wax occurs as granules to ridges bordering the stomata pits in *C. euryphyllidia*, *C. miqueliana*, *C. norstogii* and as reticulate ridges in *C. hildae*, *C. kuesteriana*, *C. latifolia*, *C. mexicana*. The differences in cuticle morphology closely follow the geographic distribution of studied species and their habitat. Moreover, the closeness of *Stangeria eriopus* to the other taxa is not supported by cuticle micromorphology. However, the peculiarities detected in this monotypic genus could be explained as the consequence of the adaptation of leaf surface to water repellency.

A Tale of Two Gardens: Comparing the Aquatic Ecosystems of Two Public Gardens, Greensboro, NC

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Water is a critical component of any ecosystem and is necessary for sustaining and connecting all life. Runoff from terrestrial habitats carries contaminants and sediments into rivers, lakes, and streams where they accumulate. These aquatic environments are an essential component of assessing the overall condition of the surrounding ecosystem. The species of macroinvertebrates living in the stream are important bioindicators of ecosystem integrity. The Bicentennial Garden and the Bog Garden are located in an urbanized floodplain in Greensboro, along a stream that feeds into the local drinking water supply. We compared the aquatic ecosystems of both gardens using data collected between September 2013 and February 2014. The physical habitats were assessed using the Environmental Protection Agency's Rapid Bioassessment Protocols (RBP). Nine water quality parameters were evaluated based on the North Carolina Department of Environment and Natural Resources standards for surface waters. Macroinvertebrate populations were sampled using the RBP's Benthic Macroinvertebrate Protocols. Artificial leaf packs were created to compare macroinvertebrate colonization of different plant species found in each garden. Physical evaluations placed both gardens in the low suboptimal range. Average water quality measurements for both gardens fell within NC state standards, but the Bicentennial Garden scored significantly better in two key parameters that impact macroinvertebrates; dissolved oxygen and turbidity. Preliminary results show that the Bicentennial Garden has a greater diversity of macroinvertebrate species. This suggests that the sedimentation and lower oxygen levels in the Bog Garden may be adversely impacting the variety of macorinvertebrate habitats available resulting in lower species diversity.

MtrR Regulates Two Major Lytic Transglycosylases Responsible for Peptidoglycan-Derived Cytotoxin Release and Autolysis in *Neisseria gonorrhoeae*

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The multiple-transferable resistance protein (MtrR) is a transcriptional repressor of the *mtrCDE*-encoded drug efflux system and Type IV pilus biosynthesis genes and activator of PBP1 expression in *Neisseria gonorrhoeae*. More recently, published microarray data showed that MtrR is an activator of *ltgA* expression in gonococci. LtgA is a lytic transglycosylase responsible for recycling approximately half of the cell wall in GC and releasing peptidoglycan-derived cytotoxins, which cause epithelial damage and elicit specific inflammatory cytokine responses. Based on the previous microarray data, we decided to further define MtrR's capacity to regulate *ltgA* and *ltgD*. Although *ltgD* was not detected by the microarray, we decided to determine whether it is MtrR regulated since *ltgD* is responsible for the remaining portion of recycled cell wall. To this end, we examined *ltgA* and *ltgD* mRNA-transcription and LtgA protein levels. We determined that MtrR directly increases *ltgA* expression, resulting in increased LtgA levels and increased *ltgD* expression in gonococci. Disruption of *mtrR* reduced PG monomer release and cell lysis, which suggests that MtrR regulation of *ltgA* and *ltgD* may impact gonococci's ability to recycle and release peptidoglycan and modulate autolysis during growth. Thus, the study provides insight as to how lytic transglycosylases regulate cell growth and peptidoglycan-derived cytotoxin release.

Basal mRNA Expression of Clock Gene Bmal in the Hippocampus and the Amygdala of Male and Female Rats

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Clock genes are differentially expressed throughout the day and their temporal expression profiles are associated with circadian rhythms in mammals. Glucocorticoids (GCs) are lipid soluble steroid hormones secreted with a basal circadian rhythm for daily functioning and at increased levels in response to stress. GCs are involved in modulating clock gene expression in peripheral tissue. While little is known about clock gene expression in the brain, GC's ability to modulate the clock gene *per1*, via the glucocorticoid response element in *per1*'s promoter region, has been demonstrated in peripheral tissue. Stress-released GCs may shift *per1* expression within the brain, and serve as a mechanism for altering typical function of brain regions outside the Suprachiasmatic Nucleus (SCN) of the hypothalamus. Interestingly, the SCN is the "body's master clock" and it has no glucocorticoid receptors (GRs). Understanding both basal and stress-induced GC's effects on clock genes expression is essential in understanding the mechanisms underlying stress's effect on circadian rhythms. The expression of the essential clock gene *bmal* is regulated in part by *per1* expression. We characterized the mRNA expression of *bmal,* over the course of 24 hours under basal 12:12 hour light: dark conditions. We focused on the hippocampus and the amygdala, stress-related brain regions with abundant GR expression. Using in situ hybridization, *bmal* mRNA levels were measured in male and female rat brains.

Significant rhythms were found in both the hippocampus and some amygdala sub regions. This characterization provides in part the foundation to further study alterations of circadian rhythms by stress.

Contribution of Thyroid Hormone Levels to Estrogen Receptor Activation in Breast Cancer Cells

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The involvement of estrogens in breast cancer development and growth has been established. Evidence from epidemiologic studies has revealed a possible association between thyroid dysfunction and breast cancer. Hyperthyroidism is usually found in postmenopausal breast cancer patients. However, neither the epidemiologic or in vitro data provide a definitive picture of the significance of thyroid hormone on the etiology of breast cancer. In this project, we investigated the response of human breast cancer cells to thyroid hormone and to agonists of the estrogen receptor. Using a tetrazolium-based colorimetric assay (MTT), we demonstrated that triiodothyronine (T_3) reduced the viability of MDA-MB-231 cells but enhanced the viability of T47D cells. In addition, both estrogen receptor agonists DPN and PPT enhanced the proliferation of T47D cells but had little to no effect on the viability of MDA-MB-231 cells. Based on these studies, we suggest that T_3 may play a role in the activation of the estrogen receptors in breast cancer cells. Studies are currently being carried out to determine the effects of simultaneously treating with T_3 and agonists of the estrogen receptor on the viability of breast cancer cells.

Fire's Impact on Den Creation for *Petaurus australis* in *Eucalyptus grandis* Trees

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The Yellow-Bellied Glider, *P. australis*, of northeastern Australia dens in the hollows of *E. grandis* trees over 99.5 cm diameter at breast height in Wet Sclerophyll Forests. This study compared *E. grandis* trees in this size class possessing potential *P. australis* dens (den trees), with *E. grandis* trees of the same size without any potential of dens (non-den trees). This study investigates the role fire may play in the development of dens since it is known that fire increases tree susceptibility to termite and fungal infestations, facilitating tree hollowing which creates suitable *P. australis* dens. Den and non-den trees were compared for differences in respect to the number and size of fire hollows. Den trees had significantly more fire hollows than non-den trees. Fire hollows were also significantly larger in den than in non-den trees, indicating that den trees were subjected to much higher intensity fires. The average number of trees found in the canopy was significantly higher around den trees, and the average number of trees in the sub-canopy was significantly lower around den trees than non-den trees. Results of this study imply necessity for high intensity fires in the Wet Sclerophyll Forest for the creation of suitable den habitats for *P. australis* gliders.

Sewage Impacts on Water Quality and Antibiotic Resistance in Beach Waters in the Galápagos Islands

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Tourism and residential population growth are increasing on the Galápagos Islands, yet the effects on environmental quality are not well understood. This study provides a baseline characterization of water quality on San Cristóbal, one of the inhabited Galápagos Islands. Five sample beaches, and the mouths of two sewage effluent pipes were selected to represent recreational water with and without the presence of sewage effluent. Baseline water quality was characterized by quantifying *Enterococcus* concentrations using the IDEXX Enterolert kit. *Escherichia coli* was also isolated from samples using membrane filtration and tested for susceptibility to five antibiotics using Kirby-Bauer disk diffusion. Levels of *Enterococcus* and antibiotic resistance were compared across sample sites. These results revealed significantly higher *Enterococcus* concentrations and levels of antibiotic-resistant bacteria near sites subjected to sewage effluent ($p < 0.01$). This research provides evidence that humans are contributing to the degradation of water quality in the Galápagos Islands. The high concentrations of *Enterococcus* and elevated levels of antibiotic resistance near sewage discharge sites indicate that the release of raw sewage into recreational waters may be affecting environmental and public health. This study provides insight into how humans impact their environment in an area where economic and developmental demands compete with environmental and public health concerns. Additionally, this research was the first to evaluate antibiotic resistance in recreational waters in the Galápagos and evaluate the potential relationship between human activity and antibiotic resistance on the Islands.

This research was supported by the UNC Summer Undergraduate Research Fellowship and the UNC Center for Global Initiatives Vimy Global Team Award.

Title

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Insulin is produced in pancreatic beta cells in human in response to glucose in blood.
For insulin production, the insulin gene is turned on by multiple transcription factors. Three transcription factors, Pdx1, MafA and Beta2, are necessary for synergistic activation of insulin transcription, which is an important function of a normal pancreatic beta cell.
The insulin promoter contains multiple binding sites for all of these factors, which are in close proximity to each other. We have created a novel transcription factor fusion protein with the

DNA binding domains of Pdx1 and MafA, linked to each other by a flexible linker. This fusion protein was over-expressed in E. coli followed by purification using a nickel affinity column. The fusion protein is tested for DNA binding affinity to a sub-site of the human insulin promoter that contains the GG2, GG1 and C1 sites, responsible for binding to Pdx1 and MafA, respectively. Experiments of the DNA binding specificity and affinity of the fusion protein, when compared to those of individual transcription factors to DNA binding domains, will help to shed light on the mechanism of synergistic activation of insulin transcription and provide an insight for potential gene therapy to diabetic patients.

An Epidemiologic Exploration of Tobacco Smoking at Warren Wilson College

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Warren Wilson College

The aim of this study was to address an area of campus wellness at Warren Wilson College by monitoring students' tobacco smoking patterns, context, and stimuli as well as attitudes surrounding tobacco smoking on campus. During November of 2013, a 44 question cross-sectional survey was administered to students on a volunteer basis in the college's cafeteria. Of the 831 students enrolled at the college, 196 (smokers and nonsmokers) completed the anonymous survey. Main outcome measures include time of smoking initiation, number of days cigarettes were smoked per month, number of cigarettes smoked per day, risk perception, smoking context, and comprehension and assessment of the college's current tobacco use policy. The collected data was normalized to the college's gender and class distribution and analyzed using standard epidemiologic statistics. The 30 day prevalence of tobacco smoking for the population was 40% with freshman males having the highest prevalence and sophomore males having the lowest. Older students were more likely to be classified as light smokers. Nonsmokers were more likely than smokers to be bothered by and view secondhand smoke as a health risk. Smokers were more likely to associate smoking with stress relief and "coolness" while expressing resistance toward implementation of tobacco smoking prevention and cessation initiatives at the college. Understanding student tobacco use can help the college decide what, if any, policy measures it should take to improve student's health. As this study was a cross-sectional study, it is important to conduct similar studies on a regular basis to observe how tobacco use changes over time.

Conservation Genetics of the Green Salamander, *A. aeneus* in Western North Carolina (WNC)

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Warren Wilson College

In human modified environments, patterns of dispersal and gene flow among populations may be significantly altered. Thus understanding patterns of genetic connectivity and the features that influence them are crucial to the conservation and management of imperiled species. This is especially true for Plethodontids, which tend to display notoriously low levels of migration. In particular, habitat specialists are particularly susceptible to the detrimental effects of habitat fragmentation and disturbance, as their specialization effectively isolates individuals in a matrix

of unsuitable habitat types. One such habitats specialist is the Green salamander (Aneides aeneus). In this study, we used twelve polymorphic microsatellite loci to investigate geospatial patterns of genetic variation in populations of the Green salamander (*A. aeneus*) found within the Blue Ridge Province of North Carolina. Additionally we used our locality information in conjunction with digital elevation models (DEM's), and David Theobald's natural landscapes layer in ArcGIS to develop habitat suitability models for *A. aeneus* in nine counties in WNC. Baysian clustering indicated that three well-supported lineages exist within this relatively small geographic area. This evidence, along with tests of inter-population gene flow indicates that these clusters are experiencing low levels of migration and consequently high levels of inbreeding.

The Marked Goby (*Ctenogobius stigmaticus*): A Rare North Carolina Fish Enigma

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The marked goby (*Ctenogobius stigmaticus* Poey, 1860) is a diminutive, uncommon, tropical-subtropical, estuarine Atlantic coast fish. It is considered rare in North Carolina. In the past it has been confused with the similar darter goby (*Ctenogobius boleosoma* Jordan and Gilbert, 1882) and considered common in North Carolina. Recently, some of the confusion has been eliminated and characters to separate these two similar gobies identified. These characters were used to determine that 6 specimens were collected and cataloged from the state (one is missing, but a photograph has the marked goby coloration). When examining the remaining 5, it became obvious that only one is *C. stigmaticus*. The four others are presumably the darter goby, as anal fin ray counts (which are now used to separate them) may overlap. Differences in head shape, maxillary length relative to eye, and the presence of tusks in *C. stigmaticus* compared to the darter goby could be considered in future keys. Color patterns are also strikingly different in specimens were color still exists. Unfortunately, the four specimens in question lack color patterns from preservation. Thus, only two specimens from the North Carolina are known. Fortunately, these specimens were captured with substantial environmental data during the most intensive inshore aquatic survey in the state to date: daily sampling from March to Nov. 1968-1977 with varied gear in the lower Cape Fear River and adjacent ocean. The range of the marked goby thus extends from SE North Carolina to the border of Brazil and Uruguay, roughly 34 N to 34 south. All specimens from these studies have been taken in large estuarine bays or coastal lagoons, in shallow to intertidal waters, on muddy-sand substrates, and in moderate salinities. The difficulty in sampling these small, slender fishes ($\leq$80 mm) may also contribute to their scarcity and lack of knowledge.

Testing of a Rat LDL Receptor Minigene in Hypothyroid Medium

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Hypothyroidism is a condition in which the thyroid gland does not produce enough thyroid hormones. Hypothyroidism has the ability to negatively affect the expression of the low density

lipoprotein (LDL) receptors causing hypercholesterolemia. A minigene version of the rat LDL receptor gene has been prepared and is currently being tested in the hepatocyte-like cell line C3A treated with a hypothyroid/hypercholesterolemic (THD/LDL) medium. In this project, the minigene was first transient transfected into the cells grown in the presence of the THD/LDL medium. Treatments with 3 and 10 nM of triiodothyronine (T_3) were then carried out for 24 hours, and the levels of mRNA produced from the minigene were confirmed using real-time PCR. Analysis of the mRNA levels corresponding to the endogenous LDL receptor was also done. Thus far, we have been able to demonstrate the effectiveness of the THD/LDL medium in reducing the expression of the endogenous LDL receptor, an effect that was reverted when treating with T_3. Further optimization of the transfection with the minigene plasmid is currently ongoing. If the minigene is proven to be responsive to hypothyroidism and T_3 treatment, it could be employed in the screening of thyroid mimetic drugs to fight hypercholesterolemia.

The phylogeography of the northern seepage salamander (*Desmognathus aeneus*)

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The seepage salamander, *Desmognathus aeneus*, ranges from southwest of the Little Tennessee River in North Carolina and Tennessee, along the Blue Ridge Mountains, to their terminus in northern Georgia and Alabama. Populations then become disjunct to at least fall line of Alabama. Here we present the results of a molecular phylogenetic survey comprised of those populations occupying the northern, continuous portions of the distribution. We collected mitochondrial DNA sequenced data(~650 bps COX1) from forty three populations. Our Bayesian phylogenetic reconstruction reveals the presence of several well-supported, distinct evolutionary lineages. Some of these lineages occur in very close proximity of one another. This distributional pattern of mtDNA haplotypes suggests that these populations are worthy of continued investigation with respect to their specific status.

Effects of habitual loading on bone material properties in the mammalian post-cranial skeleton

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It is well-documented that primates exhibit unusual biomechanical patterns during quadrupedal locomotion, relative to most other mammals, that are thought to be associated with arboreal movement in a terminal branch environment. Perhaps most notable is a pattern of peak force distribution in which greater loads are experienced by the hindlimbs than the forelimbs, a pattern opposite of that observed in most other non-primate mammalian species. Currently, it is not known whether this pattern of limb loading is reflected in the bone material properties in the limb bones of primates and other mammals. We test the hypothesis that elastic modulus will vary according to load in the limb bones of primate (*Lemur catta*, *Varecia variegata*, and *Macaca mulatta*) and non-primate (*Dasypus novemcinctis*, *Felis domesticus*, and *Didelphis virginiana*) species using microindentation. We sampled Vickers hardness from one transverse

section from both the distal radius and tibia of each specimen. Hardness values were converted to elastic modulus via regression equations specific for bone tissue. Analysis of variance (ANOVA) reveals that primates tend to have significantly more compliant bone in the forelimb (9.77 GPa) when compared to the hindlimb (12.13 GPa). The pattern is reversed in non-primate species (15.38 versus 13.41 GPa). This finding fails to reject our hypothesis that differences in habitual loading patterns are reflected in material heterogeneity between the forelimbs and the hindlimbs. The overall more compliant bone observed in the primate forelimb suggests a reduced ability to withstand high compressive loads when compared to other mammals.

WNT5A Promoter B is Silenced by DNA Methylation in Osteosarcoma Cells

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Cancer is the second leading cause of death in America, with an estimated 1,600 Americans dying of cancer every day. Cancer is caused by both mutations and nongenetic (epigenetic) changes in gene expression. WNT5A, a secreted ligand involved in Wnt signaling, is often misregulated in cancer. In this study, we analyzed WNT5A regulation from its two major transcription start sites, termed promoter A and promoter B. The levels of promoter B transcripts were reduced in the osteosarcoma cell line U20S, and in primary tissue of three individuals, compared to normal osteoblasts. To determine if this decrease in promoter B activity is due to DNA methylation, we analyzed six CpG islands associated with promoter B by bisulfite sequencing. Results show that regions 1 and 2 are unmethylated, regions 3, 4, 5 are methylated and region 6 is generally unmethylated. Since region 6 includes the promoter B transcription start site, these data suggest that methylation of CpG's in regions 3, 4, and 5 influence promoter B transcription, or else the low level of region 6 methylation is the cause. Together our data suggest that during transformation of osteoblasts to osteosarcoma promoter B is being inactivated by DNA methylation.

Analyzing DNA sequences through Graph Entropy and Chaos Game

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A DNA sequence is comprised of different nucleotides : adenine(A), cytosine(C), guanine(G), and thymine(T). Since the DNA molecule contains plentiful biological, physical, and chemical information, it has become very important to analyze DNA sequences statistically. Now the nucleotides stored in GenBank have exceeded hundreds of millions of bases and the increasing rate is considerably rapid. Therefore, biologists, physicists, mathematicians , and computer specialists have adopted different techniques to research DNA sequences in recent years , including the statistical methods and some mapping rules of the bases.

In our project, we analyzed DNA sequences using statistical method such as graph entropy and mapping rules such as Chaos Game Representation. The general aim was to analyze DNA sequences and find interesting sections of a genome using a new formulation of Shannon like graph entropy and to understand the characteristics of genome through visualization.

We developed a Graph Entropy tool to identify Tandem repeats and Direct repeats of genome.We have done experiment on 26 species and found many tandem repeats and direct repeats(CRISPR for bacteria or archaea) ; some of them are new and some of them are already known.There are several existing separate CRISPR or Tandem finder tools but our entropy can find both of these features if present in genome. We developed a Mathematica program to compute CGR graphics and it's fractal dimension. We observed similarity of CGR and dinucleotide probability matrix within chromosome and dissimilarity among genomes.

Protection of HepG2 Cells from Acrolein Toxicity by CDDO-Im via Glutathione-Mediated Mechanism

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Acrolein is an environmental toxicant, mainly found in smoke released from incomplete combustion of organic matter. The compound is ubiquitously found in endogenous as well as exogenous environment. Several studies showed that exposure to acrolein can lead to liver damage. The mechanisms involved in acrolein-induced hepatocellular toxicity, however, are not completely understood. This study examines the toxic effects and cytotoxic mechanisms of acrolein on HepG2 cells. Acrolein at pathophysiological concentrations was shown to cause a concentration-dependent decrease in cell viability. Acrolein exposure was also found to cause apoptotic cell death and an increase in levels of protein carbonyl and TBARS (thiobarbituric reactive acid substances), markers of protein damage and lipid peroxidation, respectively, in HepG2 cells. Acrolein also rapidly depleted intracellular glutathione (GSH), phase II enzyme GSH-linked glutathione-S-transferases (GST) and aldose reductase (AR), three critical cellular defenses that detoxify reactive aldehydes. Results further showed that depletion of cellular GSH by acrolein preceded the loss of cell viability, which suggests that cellular GSH depletion may be an important event in acrolein-induced cytotoxicity. To further determine the role of cellular GSH in protecting against acrolein-mediated cytotoxicity, buthionine sulfoximine (BSO) was used to inhibit cellular GSH biosynthesis. It was observed that depletion of cellular GSH by BSO led to a marked potentiation of acrolein-mediated cytotoxicity in HepG2cells. Furthermore, induction of GSH levels by CDDO-Im, a triterpenoid compound, afforded protection against acrolein toxicity in a concentration-dependent manner via induction of GSH mechanism. This study may provide understanding on the molecular action of acrolein which may be important to develop novel strategies for the prevention of acrolein-mediated toxicity.

Genomic Analysis of Metal Toxin Resistance Phenotypes in Bacteria

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Bacterial resistance to environmental toxins has been extensively investigated for specific pathways encoded in bacterial genomes. Many of which have been attributed to horizontal transfer of resistance genes across independent evolving lineages. We propose that a comprehensive analysis of resistance would examine more mechanisms of direct resistance that are often spread through horizontal transfer. In addition this analysis may examine for changes across complex interacting systems of pathways, some of which we hypothesize may be due to vertical change. We implemented a workflow to investigate variations in the genomic encoding for metabolic pathways through the use of the Department of Energy Joint Genome Institute Integrated Microbial Genomes resource (IMG). We complemented this analysis further with KEGG Pathway data. We identified those fully sequenced genomes of strains with a phenotype of metal toxin resistance based on the literature and presence of direct resistance pathways. We then established a paired set of genomes from metal toxin sensitive strains based on phylogenetic relationship, literature, and the absence of direct resistance pathways. Our overall findings included how biomass-generating pathways were more likely to be found in metal toxin sensitive strains. We generally found that our computation of these pathway differences helped to further quantify metal toxin resistance pathways previously proposed in the literature, even from the pre-genomic era.

The Informed Consent Process: The Effectiveness of Alternative Delivery Methods

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Obtaining informed consent is required in most research and medical settings that utilize human subjects. It is designed to inform individuals of important information including potential risks and benefits. Failure to administer informed consent properly may lead to legal and moral dilemmas, and could possibly endanger the subject. To investigate the effectiveness of the informed consent process, we tested participants' memory of key points from the informed consent document itself immediately after accepting its conditions. We also investigated alternative methods of issuing informed consent, including an oral presentation and alternative written format, to see if one method produced a more favorable result than the others. Data collection remains ongoing, however tentative analysis has suggested that oral presentation could possibly be a more effective method of issuing informed consent.

Characterization of Early Immune Responses During Primary Pneumonic Plague

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The pathogenesis of *Yersinia pestis* respiratory infection is significant for its severity, and the pathogen/host interactions that lead to disease are incompletely characterized. Increasing evidence suggests that *Y. pestis* employs multiple levels of innate immune evasion to produce an early "pre-inflammatory" phase of respiratory infection, after which the disease is highly inflammatory in the lung and 100% lethal.

In this study, we aimed to investigate the contribution of "inflammasome" activation during respiratory infection by *Y. pestis*. We find that "inflammasome" activation within the lung occurs at early times post-inoculation, and this activation eventually contributes to pulmonary pathology. Interestingly, we demonstrate that although inflammasome activation of cytokines occurs early during lung infection, *Y. pestis* also activates IL-1 receptor antagonist (IL-1RA) to preferentially occupy the IL-1 receptor. The result is evasion of early immune activation, allowing for bacterial replication and dissemination to occur unchecked by the host innate immune system.

Development of a Calibration Scheme for *In Vivo* Microiontophoresis

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Ionotophoresis is a technique for the transdermal administration of drugs that is commonly used in various clinical and laboratory settings. In neuropharmacology, the related technique of microionotophoresis utilizes very small implanted pipets to allow for precise ejection of drugs and related compounds into specific regions of the brain. Despite its many applications, microionotophoresis is a semi-quantitative approach to drug delivery since there is presently no means of directly measuring the amount of compound ejected. Research in our group aims to develop a calibration protocol suitable for *in* vivo applications of microionotophoresis using popular multi-barrel carbon fiber probes. In our approach, absolute ejection rates will be determined *in vitro* by photometric measurements and are correlated with simultaneous voltammetric measurements. This protocol will allow accurate quantification of molecules ejected from the probes during *in vivo* use when photometric measurements are not possible. We have used methylene blue to provide proof of concept for the photometric measurements, confirming the ejection rate follows a linear trend with time for a given ionotophoresis current. Present work is directed towards characterizing the electrochemical traits of methylene blue at carbon fiber electrodes, and future work will combine and correlate electrochemical and photometric measurements to construct an accurate and effective quantifiable model of microionotophoretic ejection. This work was supported in part by both the Pembroke Undergraduate Research and Creativity Center and the Research Initiative for Scientific Enhancement Program.

Isotopic Investigation of Mycoheterotrophy in the Southern Blue Thread (*Burmannia capitata*)

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The Burmanniaceae contain several lineages of achlorophyllous mycoheterotrophic plants that may associate with arbuscular mycorrhizal fungi (AMF). Here we investigate the isotopic profile

of a green and potentially mycoheterotrophic wetland plant in situ, *Burmannia capitata*, the Southern Blue Thread, and associated vegetation. We generated 13C and 15N stable isotope profiles of a population of *B. capitata* from the Sand Hills Game Lands in Scotland County, North Carolina. The shoots of *B. capitata* are indistinguishable from other C3 reference vegetation but did show significant depletion in 13C relative to C4 reference vegetation. The highest 15N values were observed in the *B. capitata* shoot. The 13C signal of *B. capitata* root fraction was significantly enriched relative to the root fraction, suggesting a signal from mycorrhizal associates. Within the genus *Burmannia* transitions to full mycoheterotrophy have occurred numerous times suggesting that some green *Burmannia* species are likely partially mycoheterotrophic. Further investigations of mycorrhizal associate using isotopic, molecular and microscopic methods are planned.

Abundance of Select Cyanobacteria OTUs in Six North Carolina Drinking Water Supply Reservoirs

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Cyanobacteria (blue-green algae) are photosynthetic prokaryotes found in aquatic habitats worldwide, including North Carolina drinking water supply reservoirs (DWSRs). Common in eutrophic systems, cyanobacteria are important to monitor and understand because of their influence on water quality and their ability to release toxins. Cyanobacteria can form harmful algal blooms (CHABs) that are aesthetically unpleasing, can cause odor and taste problems, and may lead to oxygen depletion when CHABs decompose. Cyanobacteria identification generally relies on time and resource intensive visual analyses. Improved methods for quick and reliable identification can help in understanding CHABs. Six NC DWSRs were sampled 18 times from June 2011 to November 2012 to evaluate the abundance of selected cyanobacteria OTUs (groups of sequences >97.5% identical that did not match known species) using targeted primers in quantitative polymerase chain reactions (qPCR). OTUs were present in varying abundance in most DWSRs sampled and followed expected seasonal trends with peak abundance occurring in warmer months. Based on available data, three DWSRs were evaluated for correlations of OTU abundance and select environmental parameters (pH, conductivity, dissolved oxygen, phycocyanins, chlorophyll, and turbidity). All three DWSRs showed significant correlations of multiple OTU abundances with one or more parameter. These results suggest that qPCR has potential for monitoring cyanobacteria and can contribute to understanding and management of cyanobacteria in North Carolina drinking water supply reservoirs.

Development of mercury methylating communities in contaminated sediments.

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Mercury is a widespread environmental contaminant, predominantly from the burning of fossil fuels and artisanal gold mining. Inorganic mercury can be methylated by anoxic bacteria to a toxic form that can bioaccumulate in food webs and become a human health risk through fish

consumption. We are conducting a microcosm experiment to test for the development of mercury bioindicators and mercury methylating microorganisms. Microcosms consisted of glass dishes with sediment collected from a North Carolina Piedmont lake and soil from an urban woods. Microcosm treatments were run in triplicate and consisted of control and three levels of inorganic mercury addition. Microcosms were sampled at scheduled intervals for total and methyl-mercury, the presence of potential mercury bioindicator microbes, and mercury methylating genes by quantitative PCR. Successful detection of mercury bioindicator microbes and mercury methylating microbial communities should be useful for monitoring pollution events such as the recent coal ash spill on the Dan River and may aid in developing mitigation strategies.

Shining a Light on the Physics of Charge Transfer within Organic Photovoltaics

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The development of new energy technologies is essential for environmental and global security. So far, solar photovoltaics have not been utilized to the extent of its potential due to high costs in production. Organic photovoltaics (OPVs) show promise as a low cost alternative to the traditional inorganic photovoltaics currently available. Presently, the maximum achievable efficiencies are unknown due to the lack of understanding their underlying charge transfer mechanisms. The aim of this study was to construct a steady-state photoinduced absorption (PIA) set-up to probe the physics of charge transfer within bulk heterojunction OPV materials and investigate the creation of long lived charges. An indication of promising solar cell material, long-lived charges are a crucial component of higher efficiency OPVs. A prominent emphasis in this study included building a PIA set-up and establishing instrument control through LabVIEW, a visual programming language. Several preliminary experiments were conducted to test the integrity of the equipment, prior to OPV film studies. PIA spectra of a MDMO-PPV polymer and $PC_{60}BM$ fullerene control film, typical materials for an OPV, were acquired using the home-built set-up and analyzed for long-lived charges. Current work includes the study of a F8T2 polymer and $PC_{60}BM$ fullerene blend and expansion of current data acquisition programs. Constructing this PIA set-up opens the doors for future development of new experiments that can delve into the underlying process of charge transfer in OPVs.

Investigating Mutant suppressor of synthetic lethality between *htz1D* and *RPB2-2*^{SL} in *Saccharomyces cerevisiae*

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Histone H2A.Z is a H2A variant form of the highly conserved Histone H2A.Z/F family, which is found among most vertebrates. In yeast *Saccharomyces cerevisiae* H2A.Z (encoded by the *HTZ1*) is not necessary for life, but *htz1* mutants exhibit many different phenotypes including transcriptional regulation, gene silencing, and preventing the spread of heterochromatin, and mitotic chromosome transmission. Transcription regulation by Htz1 has been the focus of

numerous studies Htz1 containing nucleosomes have been shown to poise quiescent genes for activation and transcriptional initiation. We have also provided evidence for a role of Htz1 in transcription elongation. In order to elucidate the mechanism involved in regulating transcription elongation, we are using a dominant allele of the Pol II gene, *RPB2-2* ^{SL} identified in an unbiased screen for mutations synthetic lethal with deletion of the histone H2A.Z gene. Extragenic suppressors of the double mutant were isolated and efforts to identify them by complementing the suppressor phenotype have yielded the *SET2* gene. *SET2* encodes a histone methyltransferase, which has shown to play roles in transcription elongation. We are working to formally prove or disprove that a mutation in *SET2* is indeed suppressing the synthetic lethality of *htz1*/ *RPB2-2* ^{SL}. We are also investigating if *htz1*/ *RPB2-2* ^{SL} may display a cryptic initiation phenotype and if it does, what effect the suppressors have on it.

Isolation and Characterization of Fluoroquinolone Resistant Gram-Negative Bacteria From Hog Fecal Samples

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There are more commercial hogs in NC than people; therefore, the swine industry has an enormous impact on the state. Due to their large numbers, environmental and public health concerns have resulted. These concerns are related to the effects of treating hogs with fluoroquinolones (FQ) for infections and the disposal of their fecal waste which may contain antibiotics. Hog waste can be sprayed on agricultural fields; however, this may result in human exposure to antibiotic resistant bacteria. Exposure may occur through ingestion of contaminated food which may transfer resistance to human commensal bacteria. The potential for FQ resistance to be gained and transferred to other species of bacteria is especially concerning when it occurs through plasmid transfer. Since FQs are a second-line antibiotic for many human diseases, FQ resistance bacteria would be more expensive and difficult to treat. This research is designed to determine the prevalence of FQ resistance and then characterize potential genetic mechanisms of acquired resistance in Gram negative bacteria.
Hog fecal samples were collected both before and after pigs were treated with enrofloxacin, a licensed FQ. Samples were grown with and without selective pressure. Colony counts show no significant difference in number of resistant bacteria found before and after treatment, suggesting low acquisition of resistance. PCR was used to screen resistant gram negative bacteria for the presence of common plasmid mediated FQ resistance genes including: : *qnrA, qnrB, qnrS,* and *aac-(6')-lb-cr.* Out of 27 Gram negative resistant colonies, 7 showed positive results for *aac-(6')-lb-cr* gene, two for *qnrA*, and one for *qnrB*. The above PCR results will be sequenced to confirm resistance and characterize the causative genes.

An Unbiased Screen Identifies New Pf1 Interactions, with Biological Significance in Chromatin Modification and Transcriptional Control

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The PHD domain-containing protein Pf1 is a specific component of a multiprotein complex that also contains the scaffolding protein Sin3B, the histone deacetylases HDAC1/2, and the chromodomain containing MRG15 protein. This complex is recruited to actively transcribed regions where it serves to mitigate RNAPII progression. Studies in yeast showed that Sin3, the homologue of Sin3B in mammals, is associated with two distinct histone deacetylase complexes, namely Rpd3S and Rpd3L. Rco1, the homologue of Pf1, however, is specific to Rpd3S. This suggests that Pf1 harbors specific functions in the regulation of transcription. Using co-immunoprecipitation of flag-tagged Pf1 stably expressed in HeLa cells followed by Mass Spectrometry, we previously identified components of the Nuclear Pore Complex (NPC) as well as for DNA damage/repair response (DDRR) susceptible to interact with Pf1. In this study, we sought to confirm the interactions between Pf1 and a wide range of proteins. We transiently co-transfected HEK 293T cells with tagged Pf1 and with Sec13 or NABP2, two proteins corresponding to the NPC and the DDRR, respectively. After co-immunoprecipitation and detection by western blotting using the appropriate antibodies, our results uncover Sec13 and NABP2 as specific interactors of Pf1. Since the NPC is the major gateway between the cellular genome and the cytoplasm, this result is consistent with studies that demonstrate the functionality of the NPC in regulating gene expression. Moreover, the association of Pf1 with NABP2, a central component of the DDRR, could reveal a new role for Pf1 in the DNA damage response pathway.

Printing Cell-Laden Alginate Fibers

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Corneal implants are often required for patients who have experienced shallow to deep scratches or infections from contact lenses or everyday injuries. While current implants are known to have relatively high success rates there are still drawbacks to consider. Current implants require a supply of donor tissue that is limited and at times rejected by the patient. The hard clear plastic core that functions as the replacement cornea in many of these implants also shows flaws as far as the mechanical properties and efficiency. In this study I perform material tests on sodium alginate gels in hopes of determining a viable hydrogel substitute for current corneal implants. Prior use of alginate shows that it may maintain its flexible yet durable properties, specifically in the form of fibers, while also acting as a highly biocompatible material. Various experiments observing the concentration of alginate and the alginate crosslinking solution, calcium chloride, were performed in an attempt to determine the most efficient material for use in the printing process. A 3-dimensional inkjet printer was utilized to determine if alginate could be printed as a specifically ordered or structured scaffold for implantation. Encapsulation and growth of cells within alginate fibers was also observed, however further tests investigating the viability of cells within the alginate gels over extended periods of time are still to be implemented. The use of a 3-dimensional inkjet printer proved to print fibers with less variation and more ordering than basic manual extrusion techniques. Future work includes testing various materials that may induce cell growth and proliferation within alginate gels. A particular interest would be the material fibrin. All material tests hope to apply the use of a 3-dimensional inkjet printer for reproducible hydrogel scaffold designs.

Comparative Muscle Sarcomere Lengths

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Sarcomeres are the functional units of skeletal and cardiac muscle and are found in vertebrates and invertebrates. Anatomically, a sarcomere can be defined as the length of muscle that extends from one Z line to the next. There are no conclusive reports on whether or not sarcomere lengths are tailored to a particular physiological ecology. Furthermore, previously reported studies on sarcomere lengths have utilized transmission electron microscopy (TEM) that requires fixation and staining. The purpose of our research was twofold: 1) to develop a method to measure sarcomere lengths in fresh, unfixed and unstained tissue; and 2) to measure sarcomere lengths in four ecologically diverse vertebrates – salmon, tuna, tilapia, and chicken. Due to the anisotropic (A) and isotropic (I) light refraction characteristics of muscle actin and myosin, sarcomeres were observed in unstained fresh tissue at 400X and 1000X. With the use of a mm rule, we could measure linear sarcomeres on a printed-to-scale digital photograph of the muscle tissue. Then using the raw data, we could calculate sarcomere mm lengths based on ocular units calibrated with a stage micrometer. Results were surprisingly similar to previously reported sarcomere lengths measured with TEM; and our initial studies suggest a relationship between sarcomere lengths and physiological ecology.

Seasonal variation in water sources of the riparian tree species *Acer negundo* and *Betula nigra* in the southern Appalachian foothills, USA

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Determining which water sources a plant accesses throughout a year is an important step in understanding how changes in source characteristics, such as availability and water quality, affect utilization by a plant. The current study examined the primary water sources of selected riparian species common the foothills of the southern Appalachians, *Acer negundo* and *Betula nigra*, during spring leaf bolt, flowering, and leaf senescence. Plant water sources were examined over a 12-month period and divided into early and late growing season and fall dormancy. Source utilization was monitored monthly by comparing the stable isotopic composition of water samples taken from woody tissue to those of possible water sources. Throughout the year, both species used a combination of deep ground and shallow soil water sources, with a greater contribution from deeper sources during the late growing season. Water extracted from *B. nigra* was typically more depleted in δ^2H than all collected sources, while values from *A. negundo* were more variable throughout the study period. Isotopic values did not vary on a monthly or seasonal scale for either species ($P>0.56$). Interspecific values also showed consistency, with differences only at December, January, and July samplings ($P<0.02$). Strong positive relationships between air temperature and isotopic values of both species ($P<0.04$) were also found and may be due to increased evaporation of moisture from the upper soil layers which both species appeared to use, to some degree, most of the year.

Comparison of metal concentrations between aquatic and terrestrial salamanders across three populations to determine life history

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Metal pollution, a growing concern in developed nations, can result from a variety of industrial practices, for example: the burning of coal, fertilizer production, and nuclear industry. When introduced into waterways, metals can accumulate and begin to affect populations of small amphibians. These species are good indicators of environmental health and, in some cases, can be used to illustrate pathways in which bioaccumulation can occur in humans. This study examines metal concentrations in the livers of two salamander species: *plethodon shermani* and *desmognathus ocoee* across three populations. The livers were dissected, dried, and digested in nitric acid followed by analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES).

Characterization of *Pseudomona sp.* (ATCC 55646)

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The *Pseudomonadaceae* family comprises several genera of Gram-negative, free-living gamma proteobacteria including the genera *Pseudomonas*, *Azotobacter*,*Azophizophilus, Azomonas, Mesophilobacter, Cellvibrio, Rugamonas, Serpens* and *Rhizobacter*. Historically, organisms were assigned to genera within this family based on morphological and biochemical traits, including the ability to fix N_2 or form cysts. In the last 10 years, phylogenetic analysis of 16S rDNA and other stable RNAs has lead to reconsideration of the taxonomy of this poorly defined phylogenetic group. The research conducted in this study focuses on metabolic characterization and phylogenetic analysis of *Pseudomonas* sp. (ATCC 55646). This organism was isolated from a soil sample in Bridgewater, New Jersey during a study on aerobic degradation of aromatic and aliphatic compounds in waste materials and deposited in the American Type Culture Collection (ATCC) by Cytec Industries. Upon completion of this study it will be determined whether *Pseudomonas* sp. (ATCC 55646) can be identified as a member of a preexisting species in the family *Pseudomonadaceae*or whether it is a novel species worth further characterization.

The Role of Cysteine Desulfurase (NifS) of *Bacillus subtilis* in the Assembly of the [4Fe-4S] Cluster of Quinolinate Synthase (NadA)

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Nicotinamide adenine dinucleotide (NAD) plays an essential role in biosynthetic pathways of all living organisms. The biosynthetic scheme of NAD can be achieved through two main pathways: *de novo* or salvage biosynthetic pathways. In the *de novo* biosynthetic pathway in most bacteria,

quinolinic acid is an important precursor, which is converted from L-aspartate by a two-step reaction catalyzed by L-aspartate oxidase (NadB) and quinolinate synthetase (NadA) respectively. Previous work in *B. subtilis* has shown that deletion of cysteine desulfurase *nifS* gene, which is upstream of NadA and NadB, caused a nicotinic acid auxotrophic phenotype. Later research revealed that NadA requires a Fe-S cluster cofactor to perform its functionality in the nicotinic biosynthesis pathway. Since sulfur mobilization by cysteine desulfurase is the first step of Fe-S biosynthesis, we hypothesize that NifS is essential to the production of the 4Fe-4S cluster in NadA. Initial experiments devoted towards the characterization of NifS showed an optimum pH of 8.0 with an associated pKa of 7.36 and a K_m for cysteine of 20 μM. Moreover, in this study investigated the kinetic profile of alanine formation by NifS when in the presence of components of the NAD pathway, NadA and/or NadB. Later studies focused on the reconstitution rate of Fe-S cluster on NadA under various conditions to explore the involvement of NifS on the direct assembly of Fe-S cluster onto the quinolate synthase NadA.

Morphological and Molecular Identification of Mosquito Diversity in the Fred Stanback Jr. Ecological Preserve at Catawba College, Salisbury, North Carolina.

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The species diversity of mosquito populations in the Fred Stanback Jr. Ecological Preserve located in the central piedmont of North Carolina is currently unknown, primarily due to a lack of surveillance. Since its establishment in 1999 this 68 hectare natural area, bracketed by the Catawba College campus, a riparian greenway, and residential areas, has reverted from primarily agricultural use to a seasonally inundated early successional piedmont flood plain forest. In order to investigate species richness, relative abundance, and invasive species establishment we conducted a survey of mosquito populations from April – August 2013 using standard protocols. Samples were identified morphologically to create a database and reference collection. To further confirm species identity we performed PCR of 18S rDNA, a standard DNA barcoding locus used to generate the phylogeny of *Culicidae* of the northeastern United States (Shepard *et al.,* 2006). We morphologically identified 21 mosquito taxa in the Preserve, with 5 species confirmed through bioinformatic analysis. Of those 5 we have conclusively demonstrated the presence of *Ochlerotatus japonicus*, an invasive disease vector species relatively new to North Carolina. Ongoing efforts will continue morphological and molecular identification of our current collections along with expanded sampling to further develop our understanding of mosquito diversity.

Epigenetic Modulation of WNT5A Expression in the Human Colorectal Cell Line HCT-116

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WNT5A is a secreted glycoprotein that is involved in the non-canonical WNT/β-catenin signaling pathway. WNT5A plays a role in development, differentiation, and tissue function. WNT5A misregulation has been associated with various human conditions, particularly cancer. This project is focused on the lack of WNT5A expression from its two major promoters A and B in the human colorectal cancer cell line HCT-116. Promoter B transcript levels were found to be over 10-fold higher than promoter A transcripts in normal colon epithelial cells (FHC). To determine if inactivation of promoter B is due to DNA methylation, we analyzed six CpG islands associated with promoter B for DNA methylation using bisulfate sequencing. Regions 1, 2, and 6 were found to be methylated, whereas Regions 3 and 4 showed variable methylation and Region 5 was unmethylated. We then investigated the effects of epigenetic drugs to reactivate promoters A and B. 5-Aza, a DNA methyltransferase inhibitor; SAHA, a histone deacetylase inhibitor; and DZNeP, a histone methyltransferase EZH2 inhibitor, were used alone and in combination. Our results show that a combination of 5-Aza and SAHA result in the highest levels of reactivation of WNT5A promoters A and B. These data suggest that WNT5A expression can be modulated by epigenetic drugs, possibly leading to a novel therapeutic treatment for human colorectal cancer.


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