Molecules in the Mountains

2015 Meeting

Western Carolina University

North Carolina Biotechnology Center

9 April 2015

Schedule		
All Talks are in the University Center Theater and Posters, Lunch, and Breaks are in Illusions (down the hall)		
7:30	Registration Poster and Talk Set Up Coffee Break	
9:00	Welcome by Seán O'Connell, Department Head of Biology, Western Carolina University	
Short Talks 1 (General Science): Timothy Driscoll, Chair		
9:15	Larry Daniel	Hydrogen Peroxide, a Key Molecule in Cellular Growth Control
9:35	Jamie Wallen	Using Bacteriophage T7 as a Model System to Understand How Proteins Work Together to Copy DNA
9:55	Tom Hollis	TREX1: The Connection Between Nucleic Acid Processing and Autoimmunity
10:15	Poster Session I and Coffee Break Sponsored by PHENIX Research Products	
Short Talks 2 (General Science): Kelly Grisedale, Chair		
11:15	Adam Brown	New Methods for Studying Bacterial Efflux Pump Inhibitors, and Studies on Their Distribution in Land Plants
11:35	Maria Gainey	Cowpox Viral Evasion of CD8+ T Cell Effector Responses
11:55	Timothy Driscoll	Genomic Diversification in Strains of *Rickettsia felis* Isolated from Different Arthropods
12:15	Lunch	
Short Talks 3 (Fermentation … And Beyond): Jamie Wallen, Chair		
1:30	Rusty Bryant	Hop Acid-Rich Spent Craft Brewer's Yeast Modulates Gut Bacterial Growth
1:50	Eric Schmitt	The Role of Microbiology in Chocolate Production

Schedule		
2:10	Kevin Sandefur	Cultural Impact of the Craft Beer Industry Past, Present and Future
2:30	Chris Cooper	The Politics of Beer: Exploring State Legislative Decision-Making on Beer Bills
2:50	Poster Session II and Coffee Break Sponsored by Western Office of NC Biotech Center	
Keynote Address (Ray Daniels): Introduction by Seán O'Connell		
3:50	Ray Daniels	Applied Biochemistry and the Flavor of Beer: Why Your Bartender Understands Enzymes
4:50	Seán O'Connell	Student poster awards and closing remarks
5:00	Adjournment	Optional post meeting get together

Abstracts for Talks (arranged in order of presentation)

1.1) Hydrogen Peroxide, a Key Molecule in Cellular Growth Control

Larry Daniel
Department of Biochemistry, Wake Forest School of Medicine

H_2O_2 is a highly reactive species frequently associated with cellular damage and bacterial killing. However, H_2O_2 is increasingly recognized as an important component of cellular signaling pathways. Lysophosphatidic acid (LPA) is a growth factor for many cells including prostate and ovarian cancer-derived cell lines. LPA stimulates H_2O_2 production which is required for growth. However, there are significant gaps in our understanding of the spatial and temporal regulation of H_2O_2-dependent signaling and the way in which signals are transmitted following receptor activation. We have developed two tools, DCP-Bio1 and DCP-Rho1, to determine the localization of active protein oxidation after LPA stimulation by detection of protein sulfenic acids. We found that LPA stimulation causes internalization of LPA receptors into early endosomes that contain NADPH oxidase components and are sites of H_2O_2 generation. DCP-Rho1 allowed visualization of sulfenic acid formation, indicative of active protein oxidation, which was stimulated by LPA and decreased by an LPA receptor antagonist. Protein oxidation sites co-localized with LPAR1 and the endosomal marker EEA1. Concurrent with the generation of these redox signaling-active endosomes (redoxosomes) is the H_2O_2- and NADPH oxidase-dependent oxidation of redoxosome localized proteins detected using DCP-Bio1. These new approaches enable detection of active, H_2O_2-dependent protein oxidation linked to cell signaling processes.

1.2) Using Bacteriophage T7 as a Model System to Understand How Proteins Work Together to Copy DNA

Jamie Wallen
Department of Chemistry & Physics, Western Carolina University

Bacteriophage T7 has long served as a model system to study the processes of DNA replication, as only four proteins are required to accurately copy DNA. Our laboratory is interested in understanding how these four proteins interact and communicate in order to efficiently copy both strands of DNA in a coordinated manner. We have recently determined X-ray crystal structures of complexes of these proteins that are providing the first images of how these proteins interact at the molecular level. We are currently generating point mutations in these proteins to attempt to disrupt the interactions observed in the crystal structures. We will test the consequences of these mutations on the ability of these proteins to interact using a combination of biochemical approaches such as in vitro DNA replication assays and in vivo bacteriophage T7 complementation assays. Our ultimate goal is to understand how all four proteins assemble into a single *machine* to carry out a process that is essential for life.

1.3) TREX1: The Connection Between Nucleic Acid Processing and Autoimmunity

Tom Hollis
Department of Biochemistry, Wake Forest School of Medicine

TREX1 is a 3'-deoxyribonuclease that degrades single- and double-stranded DNA (ssDNA and dsDNA) to prevent inappropriate nucleic acid-mediated immune activation. TREX1 participates in a cell death process, implicating this major 3' --> 5' exonuclease in genomic DNA degradation to minimize potential immune activation by persistent self DNA. More than 40 different disease-causing TREX1 mutations have been identified exhibiting dominant and recessive genetic phenotypes in a spectrum of autoimmune disorders. Our structural and biochemical studies of TREX1 have revealed the dimeric structure that is relevant to its function and to disease mechanisms in individuals carrying mutant alleles. Analysis of heterodimer TREX1 dominant mutant proteins reveals they have selectively dysfunctional

dsDNA degradation activities, but not ssDNA. The aberrant dsDNA degradation activities of these disease-related TREX1 mutations could account for the persistent dsDNA from dying cells leading to activation of innate immune responses.

2.1) New Methods for Studying Bacterial Efflux Pump Inhibitors, and Studies on Their Distribution in Land Plants

Adam Brown
Department of Chemistry and Biochemistry, University of North Carolina Greensboro

Multiple plants have been identified that inhibit bacterial toxic compound efflux pumps, thus synergizing the activity of antimicrobial compounds. This study sought to develop improved methods for the study of these phenomena, and to investigate the prevalence of efflux pump inhibitors in land plant lineages. Two improved assays were developed using both fluorimetry and mass spectrometry. These were employed to evaluate efflux pump inhibitory activity for a set of plant extracts and pure flavonoids. The fluorimetry-based assay was effective and rapid for some samples, but was confounded by quenching effects inherent in many of the samples tested. The mass spectrometry based assay circumvented these quenching issues, and was successful in quantifying the efflux pump inhibitory activity of a wide array of plant extracts and pure compounds. The data produced using the mass spectrometry-based assay when applied to plant extracts and to pure flavonoid standards was useful in demonstrating that the production of efflux pump inhibitors is more widely distributed in land plants than the previous literature suggests.

2.2) Cowpox Viral Evasion of CD8+ T Cell Effector Responses

Maria Gainey
Department of Biology, Western Carolina University

Cowpox virus (CPXV) is an orthopoxvirus that encodes a plethora of immunomodulatory proteins. While CPXV causes zoonotic infections of humans and assorted other animal species, the natural reservoirs of CPXV are wood mice and bank voles. As CPXV has co-evolved with its rodent hosts, CPXV infections of rodents represent an excellent opportunity to study virus-host interactions. MHC class I-restricted CD8+ T cells can recognize and rapidly kill virus-infected cells. We and others previously showed that wild type (WT) cowpox virus (CPXV) potently downregulates MHC class I molecules during infection. We found that survival of mice is greatly increased after intra-nasal infection with a virus (Δ12Δ203) lacking the two open reading frames responsible for MHC class I downregulation. Attenuation was abrogated by CD8+ T cell depletion during Δ12Δ203 infection. Our current studies revealed that there was no defect in initial generation of CPXV-specific CD8+ T cells during WT CPXV infection. Moreover, these virus-specific CD8+ T cells did not show major impairment in cytokine production or target cell killing. However, we found that there were more CD8+ T cells actively producing IFNgamma in Δ12Δ203-infected lungs as compared to WT CPXV infected lungs at a time when viral titers were similar. Furthermore, WT CPXV-induced CD8+ T cells were defective in directly controlling WT CPXV but not Δ12Δ203 skin lesions in vivo. Taken together, our studies indicate that CPXV-induced MHC class I down-regulation allows CPXV to evade CD8+ T cells at the level of effector responses rather than priming.

2.3) Genomic Diversification in Strains of *Rickettsia felis* Isolated from Different Arthropods

Timothy Driscoll (Joseph J. Gillespie[1], Timothy Driscoll[2], Victoria I. Verhoeve[3], Tadanobu Utsuki[3], Claudia Husseneder[4], Vladimir N. Chouljenko[3], Abdu F. Azad[1], and Kevin R. Macaluso[3])
University of Maryland School of Medicine[1]; Western Carolina University[2]; Louisiana State University, School of Veterinary Medicine[3]; Louisiana State University Agricultural Center[4]

Rickettsia felis (*Alphaproteobacteria*: Rickettsiales) is the causative agent of an emerging flea-borne rickettsiosis with worldwide occurrence. Originally described from the cat flea, *Ctenocephalides felis*, recent reports have identified *R. felis* from other flea species, as well as other insects and ticks. This diverse host range for *R. felis* may indicate an underlying genetic variability associated with host-specific strains. To determine a potential genetic basis for host specialization, we sequenced the genome of *R. felis* str. LSU-Lb, which is an obligate mutualist of the parthenogenic booklouse *Liposcelis bostrychophila* (Insecta: Psocoptera). We also sequenced the genome of *R. felis* str. LSU, the second genome sequence for cat flea-associated strains (c.f. *R. felis* str. URRWXCal2), which are presumably facultative parasites of fleas. Phylogenomics analysis revealed *R. felis* str. LSU-Lb diverged from the flea-associated strains. Unexpectedly, *R. felis* str. LSU was found to be divergent from *R. felis* str. URRWXCal2, despite sharing similar hosts. While all three *R. felis* genomes contain the pRF plasmid, *R. felis* str. LSU-Lb carries an additional unique plasmid, pLbaR, nearly half of which encodes a unique 23-gene integrative conjugative element. Remarkably, pLbaR also encodes an RTX-like type 1 secretion system and associated toxin, heretofore unknown from other Rickettsiales genomes, which likely originated from lateral gene transfer with another obligate intracellular parasite of arthropods, *Cardinium* (Bacteroidetes). Collectively, our study reveals unexpected genomic diversity across three *R. felis* strains, and identifies several diversifying factors that differentiate facultative parasites of fleas from obligate mutualists of booklice.

3.1) Hop Acid-Rich Spent Craft Brewer's Yeast Modulates Gut Bacterial Growth

Rusty Bryant (Robert W. Bryant[1], Seth Cohen[2], Michael D. Flythe[3], Brittany E. Harlow[3], Sean P. O'Connell[4], Rebecca J. Truitt[5], and Langdon J. Martin[5])
Asheville Flavor Innovations, LLC[1]; Appalachian State University[2]; USDA ARS Forage-Animal Production Research Unit (Lexington, KY)[3]; Department of Biology, Western Carolina University[4]; Department of Chemistry, Warren Wilson College[5]

Alpha and beta hop acids (humulones and lupulones) from *Humulus lupulus* are inhibitors of Gram-positive organisms and important natural antibiotics for beer fermentation and carbohydrate feed stocks for biofuel production. Recent observations (Bryant and Cohen) of high levels of hop acids in spent yeast from craft beer brewing suggested studies to evaluate the antibiotic properties of hop acid rich craft brewers yeast regarding their influence on gut bacteria. Pasteurized spent yeast from craft beer brewing caused zones of inhibition on agar lawns of Gram-positive *Bacillus cereus* and *Clostridium sticklandii* SR. Hop-free baker's yeast was inactive. Biochromatography by TLC demonstrated that the majority of the antibiotic activity in craft brewer's yeast resided in hop acids. Craft yeast inhibited ammonia production 60% in mixed ruminant anaerobic bacterial cell suspensions and reduced numbers of hyper ammonia producing bacteria by 10 fold versus baker's yeast. Additional studies with craft yeast are in progress looking at its effect on the anaerobic gut metabolites methane and short chain fatty acids. The gut microbiome influences GI health, immune response and brain function. Modulators of the microbiome are seen as new ways to influence health and hop acid-rich craft brewer's yeast could play a role in gut health.

3.2) The Role of Microbiology in Chocolate Production

Eric Schmitt (Eric K. Schmitt and Seth Cohen)
Fermentation Sciences, Appalachian State University

Microbiology plays a crucial role in the production of chocolate. The successful fermentation of raw cocoa beans by a consortium of microorganisms is a key factor in the ultimate quality of the final chocolate products manufactured. The results of this fermentation step have historically been highly variable. This variability has a direct impact on the quality of the farmer's product and is reflected in the price at which they are able to sell. The overall fermentation process and the microorganisms involved, as well as the current research plan, will be discussed. The goal of this research is to investigate the viability of preparing standard stock cultures to be used to inoculate the cocoa fer-

mentation beds and ultimately produce a consistently high quality cocoa bean product. This has the potential to directly and positively affect the cocoa farmer's livelihood. The first step is to isolate microorganisms from daily samples collected during the entire 7 to 8-day fermentation process. Once isolated, the microorganisms will be identified using standard DNA sequencing methods. The isolated microorganisms will then be evaluated to determine their necessary growth conditions for large scale production. Each microorganism's tolerance of standard processes for long-term storage will also be investigated. Ultimately, a standardized stock culture will be developed and tested under actual fermentation conditions to test performance against the current natural fermentation process.

3.3) Cultural Impact of the Craft Beer Industry Past, Present and Future

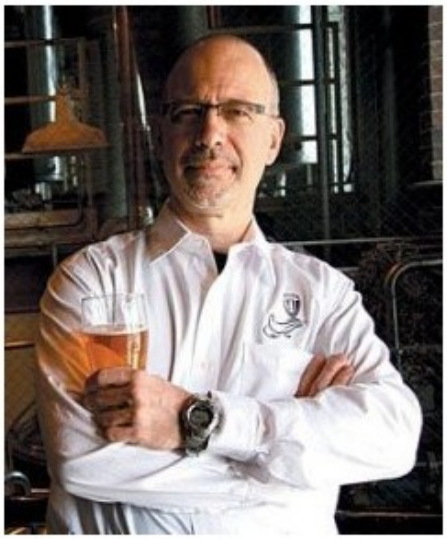
Kevin Sandefur
Founder/President, BearWaters Brewing Company

The craft beer industry started an American revolution in beer starting in the early 1980's. Since then it has grown into a significant industry that continues to shift market share away from the big three brewing companies. In 2009 there were just over 1400 craft breweries in the United States, today the number has surpassed 3000. With all this explosive growth how is the industry handling it's growth? Is the founding culture of the industry sustainable and where is it heading as the big three continue to redefine their roles in the beer market.

3.4) The Politics of Beer: Exploring State Legislative Decision-Making on Beer Bills

Chris Cooper
Department of Political Science and Public Affairs, Western Carolina University

It is no secret that our politics are defined by polarization. Indeed, the vast majority of state legislative votes today (as compared to just a decade ago) can be predicted with just one variable: partisanship. Bills of interest to the craft beer industry, however, are a rare exception to this trend. In this exploratory study, I analyze state legislative decision-making patterns on craft-beer related bills. These results should be of interest to social scientists, and craft beer advocates who wish to change policies in their state.



Ray Daniels is the founder and director of the Cicerone Certification Program — the standard for beer sommeliers — and teaches classes at the Siebel Institute of Technology, America's oldest brewing school. He is a veteran beer educator and promoter who has traveled the world in search of great beer. Ray learned to homebrew in 1989, initiating his transformation from beer drinker to beer expert. He is the author, editor, or publisher of more than two dozen books on beer and brewing. Examples include *Designing Great Beers* (1998), *Best of American Beer and Food* (1997), and *The Brewers Association's Guide to Starting Your Own Brewery* (2006). Other titles have covered specialties like smoked beer and farmhouse ales, monastic brewing, and the art of English pub tending. In 1998, Ray was named Beer Writer of the Year by the North American Guild of Beer Writers. Ray became certified as a beer judge in 1992 and has since accumulated thousands of beers worth of judging experience. He was first invited to judge professionally at the 1996 Great American Beer Festival and soon after at the World Beer Cup. In addition to judging and organizing competitions, he has trained consumers, servers and brewers in beer appreciation.

Adapted from promotional materials from Brewers Publications

Applied Biochemistry and the Flavor of Beer: Why Your Bartender Understands Enzymes

Ray Daniels
Cicerone Certification Program

Twenty-five years ago, Ray Daniels finally started putting his biochemistry degree to use by making beer at home. Those early fermentations (plus many more years of study) led him to become an influential author and leading educator in the beer community worldwide. Eight years ago, he began developing what would become the Cicerone Certification Program, the world's leading beer sommelier program. In this keynote address he'll talk about the connections between the ageless biochemistry of beer and the modern fascination with flavor.

Poster presentations

Posters 1-11 will be presented in the morning session and posters 12-23 will be presented in the afternoon session.

1	Joshua Boggs	Identification of Virulence Related Factors in *Cryptococcus neoformans* by RNAi
2	Amber Dyson	A Genetic Screen for RNA-processing Genes that Control Sensory Neuron Function in *Drosophila melanogaster*
3	Mike Mutchler	Genetic Screen for G Protein Signaling Components Involved in Nociception in *Drosophila melanogaster*
4	Timothy Driscoll	Invasive Species and the Soil Microbiome I: Assessment of Microbial Community Diversity and Functional Metagenomics
5	Charley Kelly	Invasive Species and the Soil Microbiome II: Assessment of Soil Properties, Nitrogen Mineralization and Microbial Biomass
6	Amy Griffin	Change in Bacterial Phage with Presence of Invasive Species
7	Joe Egan	Endophytic Fungi of *Hydrastis canadensis* and Their Potential Role in the Antimicrobial Activity
8	J.P. Gannon	Dissolved Organic Carbon (DOC) Transport Patterns in a Headwater Stream in Cullowhee, NC
9	Christian Munoz-Pineda	Characterization of Genetic Diversity in Demes of Purple Pitcher Plants (*Sarracenia purpurea* L.) from Western North Carolina
10	Marietta Shattelroe	Determining Genetic Variation Among Western North Carolina Ginseng (*Panax quinquefolius* L.) Populations
11	Paul Rice	Selective Reduction of Diesters using Lithium Borohydride
12	Britt Bintz	Development of a Multiplex Quantitative PCR (qPCR) Assay for Simultaneous Quantification of Human Nuclear and Mitochondrial DNA from Forensically Relevant Samples
13	Sherri Deaton	Optimization of a Method for the Extraction of DNA from Human Skeletal Remains
14	Chequita Brooks	Comparing the Microbiology of Brands and Flavors of Kombucha
15	Monesha Harris	Determination of Nutraceuticals, Aroma, and Flavor Compounds in Kombucha
16	Rebecca Truitt	Anaerobic Growth of Equine GI Bacteria at Warren Wilson College
17	Jamie Rowell	Statistical Analysis of Fermentation Rate in Hard Cider Brewing
18	Lindsey Burleson	Keratin Biomaterials Attenuate Hypoxia-mediated Cell Death

19	Mariah James	Effects of Keratin on Melanoma Cancer Cells
20	Yeng Vang	Investigation of the Effects of Oleuropein, an Antioxidant Found in Olive Leaves, on the Biosynthetic Folding of the NBD1 Domain of CFTR
21	Jessica Moore	Genomic Annotation and Analysis of the Draft Sequence of the Pathogenic Microorganism, *Elizabethkingia meningoseptica*
22	Amanda Haile	Identifying ABC Multidrug Efflux Pump Genes in the Pathogenic Microorganism, *Elizabethkingia meningoseptica*
23	Laol Vang	Identifying Antibiotic Resistance Genes from the Major Facilitator Efflux Pump Superfamily in the Pathogenic Microorganism, *Elizabethkingia meningoseptica*

Abstracts for posters (arranged in alphabetical order by presenting author)

Britt Bintz
Forensic Science Program, Western Carolina University

Development of a Multiplex Quantitative PCR (qPCR) Assay for Simultaneous Quantification of Human Nuclear and Mitochondrial DNA from Forensically Relevant Samples

In forensic casework, multilocus short tandem repeat (STR) typing is often the preferred method of analysis due to its high power of discrimination. However, many evidentiary samples contain low amounts of DNA, or degraded DNA that is not suitable for STR typing. In these cases, mitochondrial DNA sequence analysis is typically performed. Determining which investigative approach is most suitable can be challenging, especially in cases where the sample or extract is limited. Here, we describe a powerful multiplex 5' nuclease real-time PCR assay that enables simultaneous quantification of both human nuclear and mitochondrial DNA from a sample extract. This tool provides specific quantitative data that can be used to determine the most appropriate analytical workflow without consumption of additional sample or increase in labor compared to methods currently used in crime laboratories. The nuclear target for this custom qPCR assay is the 143 bp Alu Yd mobile element originally described by Xing et al. High sensitivity of nuclear DNA quantitation using the multicopy Alu Yd marker has previously been reported, and a qPCR assay designed for this target has been used successfully. The mtDNA target sequence corresponds to a 105 bp segment of the NADH dehydrogenase subunit 5 gene, and is described by Kavlick et. al. for use in an assay that is utilized routinely. Both targets have been shown to exhibit little to no cross-reactivity with non-human sources. In addition to these primary targets, an internal positive control (IPC) has also been included for assessment of possible PCR inhibition.

Joshua Boggs
Department of Biology, Western Carolina University

Identification of Virulence Related Factors in *Cryptococcus neoformans* by RNAi

The basidiomycetous fungus *Cryptococcus neoformans* is an opportunistic pathogen that is responsible for the most common fungal infection in the Central Nervous System, cryptococcal meningoencephalitis. This is a very common disease in AIDS patients and can be found less commonly in patients with other opportunistic diseases. This organism has three very important virulence factors that include melanin production, a polysaccharide capsule, and the ability to grow at high temperatures. Using an RNAi library that was developed using ~2 Kb fragments of Cryptococcal DNA, various genes have been silenced in the fungus to determine if the silenced genes have an effect on the phenotypes related to virulence. In *Cryptococcus* transformants that showed decreased production of melanin from

the wild type on Niger seed agar, PCR reactions and DNA sequencing of the produced DNA were used to amplify the DNA and then determine the sequence of the gene fragment that potentially effected the phenotype. Using the genome sequence of the fungus these produced sequences were used to determine if the gene in question's function has been identified, and if so what function it has in the virulence of the fungus. Identification of unknown virulence factors can potentially provide a great deal of understanding in the pathway that leads to pathogenicity of *C. neoformans*. Work is still underway to amplify and sequence the genes potentially responsible for the production of melanin in *C. neoformans*. A total of 12 gene fragments have been sequenced and identified at this point with more to be sequenced in the near future. One of the genes that have been identified is known as CNLAC1, this gene codes for the enzyme laccase that is known to be crucial to the production of melanin in *C. neoformans*. Finding this gene shows that this method is working to identify genes that are critical to virulence. Other proteins have been identified that have no known function, these potential virulence factors functions still need to be identified.

Chequita Brooks
Department of Biology, Western Carolina University

Comparing the Microbiology of Brands and Flavors of Kombucha

Consumption of kombucha, a drink which is the product of fermenting sweetened black tea, has recently experienced a dramatic rise in popularity. However, questions have been raised regarding the potential benefits and risks of drinking kombucha. A mixture of microorganisms, including *Saccharomyces* spp. and lactobacilli, are involved in making kombucha and are classified as probiotic in nature. Buchi, a local business in Asheville, NC, bottles a variety of flavors of kombucha, each with different additives. Two to twelve agents including plant-based dyes, spices, medicinal plants, and fruit juices are added to each product. The purpose of this study was to compare the microbiology of Buchi's raw kombucha to four of its flavored products and to compare the microbiology of raw Buchi to a raw product from another kombucha producer, For Life Kombucha. Microbial cultures were assessed using most probable number (MPN) of acetic and lactic acid producing microbes and via carbon source utilization profiles ("CSUPs" using Biolog EcoPlates). The number of acetic acid producing microorganisms was approximately three orders of magnitude higher than lactic acid producers (10^9 versus 10^6 cells per milliliter). The CSUP responses of kombucha from different brands was maintained in a comparison of Buchi and For Life Kombucha raw products. Changes in the response of the communities based on additives were seen, but more research on the effects of additives on microbial composition needs to be conducted. More research is also needed in order to elucidate the effects of kombucha on human metabolic health. Work in our lab is ongoing to identify the microbial species and define their roles in these kombucha products.

Lindsey Burleson
Department of Biology, Western Carolina University

Keratin Biomaterials Attenuate Hypoxia-mediated Cell Death

Myocardial infarction (heart attack) is a leading cause of death in the US that occurs when a coronary artery becomes occluded (blocked), leading to localized oxygen deprivation (hypoxia) within the heart muscle. The resulting hypoxia can lead to cardiac cell death (apoptosis). Regenerative medicine research in this area is aimed at 1) providing healthy cells to the damaged region of the heart and 2) rescuing pre-apoptotic cells from death. In this study, we tested the feasibility of using a human hair-derived keratin biomaterial to attenuate hypoxia-mediated cell death. To achieve our objective, we developed an in vitro model of hypoxia using BD's Anaerobic Gas Paks, exposed human umbilical vein endothelial cells (HUVECs) to long-term hypoxia (to model myocardial infarction), and assessed the ability of keratin supplements to rescue cells from death (measured using WST-1 toxicity assays). Using our hypoxia-model we reduced O_2 dissolved in the medium to 1.45%. We determined that cell numbers were lower in cells exposed hypoxia compared to the normoxia control ($p=0.006$), indicating that hypoxia resulted in cell toxicity. We also observed a trend in cells treated with keratin and exposed to hypoxia that suggests that these cells proliferated in response to keratin treatment. These data suggest that hypoxia reduces cell numbers, potentially by inducing apoptosis, and that keratin

supplemented in the medium may rescue HUVECs from hypoxia-mediated death. This study provides preliminary evidence to support the further study of keratin as a treatment for hypoxia-mediated cell death.

Sherri Deaton
Department of Biology & Forensic Science Program, Western Carolina University

Optimization of a Method for the Extraction of DNA from Human Skeletal Remains

Obtaining full DNA profiles from bone can be challenging due to the inherently low quantity and quality of nuclear DNA. Although STR profiling is preferable due to its higher discriminatory power, mitochondrial DNA (mtDNA) analysis is often utilized because of its higher copy number per cell. The current focus of mtDNA analysis is two hypervariable regions where the majority of differences between individuals are found; however, it can be challenging to discriminate between individuals who share common polymorphisms within these regions. Whole genome mtDNA analysis has been shown to increase resolution between common haplogroups. Amplification of the entire mitochondrial genome, combined with current-generation sequencing technologies allows for rapid generation of whole genome sequence data. In order to utilize these methods, sufficient quantities of amplifiable mtDNA must be obtained. An efficient extraction protocol is required to maximize DNA recovery from bone samples while minimizing the coextraction of the PCR inhibitors naturally present in bone. In this work, different stages of DNA extraction were evaluated and modifications made in an attempt to enhance DNA recovery from bone. Maximizing DNA recovery from bone will make whole genome mtDNA analysis possible allowing in greater discriminatory power of mtDNA sequence analysis.

Timothy Driscoll
Department of Biology, Western Carolina University

Invasive Species and the Soil Microbiome I: Assessment of Microbial Community Diversity and Functional Metagenomics

Introduction of non-native (invasive) plant species in North American have caused a change in the biodiversity of many forest ecosystem across the country. *Ligustrum sinense* (Chinese privet) is an aggressive, invasive woody shrub which has successfully colonized riparian forests in the Southeastern United States. In this study, the impact of *L. sinense* on soil microbial diversity is being assessed by comparing microbial communities found in privet-dominated forest soils versus communities from soil with primarily indigenous plant species. Microbial diversity analysis will be performed by PCR amplification of bacterial 16S rRNA and fungal 18S rRNA sequences, in conjunction with Illumina MiSeq sequencing. Additionally, we will carry out metagenomic analyses using the MiSeq to assess the full functional repertoire of each community. Previous work has linked invasive plant colonization with changes in soil biogeochemical properties (e.g., pH and nutrient content); therefore, we anticipate detecting differences in microbial community makeup and functional capacities between privet-dominated and privet-free soils.

Amber Dyson
Department of Biology, Appalachian State University

A Genetic Screen for RNA-processing Genes that Control Sensory Neuron Function in *Drosophila melanogaster*

Approximately 100 million Americans are affected by chronic pain, generating costs of up to $600 billion per year, according to the Institute of Medicine. The need for safer, more effective clinical interventions poses a significant hurdle in addressing this pervasive problem. To this end, researchers seek to identify mechanisms governing molecular signaling pathways in sensory neurons. We are using the larvae of *Drosophila melanogaster* as a model organism for identifying genes that control sensory neuron function by measuring nocifensive escape locomotion (NEL), which is a distinct and quantifiable behavioral response to noxious stimuli in *Drosophila*. Additionally, *Drosophila* are particularly amenable

to genetic manipulation using the GAL4/UAS system for cell-specific expression. Recent studies have indicated that the mRNAs transcribed from several genes involved in sensing pain are alternatively spliced to control their function. RNA processing is also integral in sensory neuron development and morphology. We are conducting a cell-specific RNA interference (RNAi) screen in which we systematically knock down RNA-processing genes in nociceptor neurons of *Drosophila* larvae and test for changes in nocifensive behavior relative to wild-type larvae. We have compiled a list of 119 putative RNA-processing genes and obtained fly lines targeting each of these for RNAi knockdown. We have successfully tested 50 of these and found that 6 of them (12%) show a potential defect in NEL behavior. Once the initial screen is complete, we will further characterize the best candidates through morphological analysis of sensory neurons and RT-PCR analysis of identified splicing targets.

Joe Egan
Chemistry and Biochemistry, The University of North Carolina at Greensboro

Endophytic Fungi of *Hydrastis canadensis* and Their Potential Role in the Antimicrobial Activity

Medicinal plant chemistry often looks to traditional medicine for new potential activities against growing issues, such as the development of antimicrobial resistance. Goldenseal (*Hydrastis canadensis* L. Ranunculaceae), a plant native to the Appalachian region of the United States has often been used in traditional medicine to treat topical infections. However, much of the evaluated activity relates directly to the botanical itself, the major bioactive component of which, berberine, is well-studied weak antimicrobial. It was proposed that perhaps the antimicrobial activity of berberine was not the only component of the botanical's antimicrobial activity, and that in part, some bioactivity may be the result of endophytic fungi that live asymptomatically within the plant tissues. Seventy isolates of endophytic fungi were evaluated for antimicrobial potential against *Staphylococcus aureus*. Several underwent structure elucidation via MS and NMR and yielded compounds with stronger antimicrobial activity than that of berberine, and may play a role in the overall bioactivity of *H. canadensis*.

J.P. Gannon
Department of Geosciences and Natural Resources, Western Carolina University

Dissolved Organic Carbon (DOC) Transport Patterns in a Headwater Stream in Cullowhee, NC

Dissolved Organic Carbon (DOC) dynamics in freshwater ecosystems have long been a topic of interest and have yet to be fully understood. Furthermore, recent studies have shown significant and not fully explained increases in DOC through time in many headwater streams around the globe. DOC is an important part of the carbon cycle and is the primary source of energy within aquatic food webs. Too much DOC, however, increases acidity, light attenuation, and enhances the mobility of trace metals. DOC is derived from humic substances both in plants and soil and can be contributed to a stream ecosystem both allochthonously and autochthonously. For instance, soil and hydrological processes interact to create source areas in the landscape, also called hotspots. To better understand the processes creating these DOC hotspots, and thereby controlling stream DOC concentrations, streamwater concentrations will be measured every 50 m throughout the entire stream network of the Upper Long Branch watershed in Cullowhee, NC. These data will be used to identify DOC hotspots. Once hotspots are identified, the topographic, hydrologic, and soil chemical conditions leading their formation will be studied. We hypothesize that the frequency of water table incursion into shallow soil horizons, topographic wetness index, and variations in the chemical composition of soil carbon will play large roles in determining mobility and transport of carbon to the stream network. In this poster we present our initial synoptic DOC dataset and propose experimental methods to test potential controls on the spatial patterns identified.

Amy Griffin (Amy Griffin, Maria Gainey, and Timothy Driscoll)
Department of Biology, Western Carolina University

Change in Bacterial Phage with Presence of Invasive Species

Bacteriophage in forest soils are numerous, acting as important mediators of bacterial growth and diversity and potentially facilitating gene transfer between species. The invasive plant Chinese privet (*Ligustrum sinese*) is known to affect macro-diversity, as well as bulk soil properties such as pH and nutrient content; however, the effect of invasive plant colonization on the diversity and abundance of soil bacteriophage is largely unknown. In this study we are assessing phage isolated from privet-dominated soil compared to matched plots with no privet. Abundance of phage will be measured using modified plaque assay techniques and several different target bacteria, including *Escherichia coli* and *Bacillus cereus*. We anticipate observing decreased abundance and diversity of phage isolated from privet-dominated soil, due primarily to loss of bacterial diversity in these plots.

Amanda Haile
Department of Biology, Western Carolina University

Identifying ABC Multidrug Efflux Pump Genes in the Pathogenic Microorganism, *Elizabethkingia meningoseptica*

Elizabethkingia meningoseptica is a gram-negative bacillus implicated in neonatal meningitis, pneumonia, and sepsis. *E. meningoseptica* infections are common in hospitals and complicated by its multidrug resistance against many antibiotics used in intensive care settings. Multidrug resistance is facilitated through efflux pumps, horizontal evolution, modification of the target-binding site, or through the acquisition of genes encoding enzymes responsible for degrading the antibiotic agent before it takes effect. ABC multidrug efflux pumps use ATP to expel antibiotics by pushing it out against the concentration gradient. The purpose of this project is to identify ABC multidrug efflux pumps within *E. meningoseptica* using comparative genomics. The *E. meningoseptica* draft sequence was examined for the presence of homologous genes to 13 known ABC multidrug efflux pump genes from other bacterial species. Potential homologs were analyzed using databases such as BLAST, Concise Microbial BLAST, Pfam, TIGRFAM, and KEGG. Understanding this mechanism for drug resistance in *E. meningoseptica* will allow us to better understand how and which antibiotics are pumped out of the organism. This information can be translated into more effective treatments in patients infected by this pathogenic organism.

Monesha Harris
Department of Physics and Chemistry, Western Carolina University

Determination of Nutraceuticals, Aroma, and Flavor Compounds in Kombucha

This research project consists of determining compounds of interest in finished kombucha and raw materials, including: flavor components, aroma compounds, polyphenols, ionic compounds, and metals. Sample treatment assays and instrument methods have been developed for numerous types of compounds. The following analytical instruments are being employed: Ion Chromatography (IC), Flame Atomic Absorption Spectroscopy (FAAS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), High Pressure Liquid Chromatography (HPLC), and Gas Chromatography Mass Spectrometry (GCMS).

Mariah James (Mariah James & Heather B. Coan)
Department of Biology, Western Carolina University

Effects of Keratin on Melanoma Cancer Cells

Cancer cells accumulate mutations that result in uncontrolled cell growth (proliferation). Previous studies have shown that human hair keratin is able to attenuate cancer cell proliferation. In this study, we used a novel form of processed liquid keratin that is highly purified to test its effect on melanoma (skin cancer) cell proliferation. To this end, we used two melanoma cell lines: a high metastatic potential cell line (A375) and a low metastatic potential cell line (A375.S2) to measure the effects of keratin on proliferation. We exposed both cell lines to different keratin concentrations (0.1mg/ml,

0.01mg/ml, 0.001mg/ml) for 24 hours and subsequently determined the resulting cell numbers (measured using a WST-1 cell proliferation assay). We determined that cell number decreased upon exposure to 0.1mg/ml keratin in both cell types (A375 p=0.00003; A375.S2 p=0.0075) compared to an untreated control (containing media alone without supplemented keratin). However, the lower concentrations of keratin had no effect on cell number. This suggests that cancer cell proliferation decreases in response to keratin exposure. Further study is needed to determine the exact role of keratin in reducing cancer cell growth.

Charley Kelly
Department of Geosciences and Natural Resources, Western Carolina University

Invasive Species and the Soil Microbiome II: Assessment of Soil Properties, Nitrogen Mineralization and Microbial Biomass

Introduction of non-native (invasive) plant species in North American have caused a change in the biodiversity of many forest ecosystem across the country. *Ligustrum sinense* (Chinese privet) is an aggressive, invasive woody shrub which has successfully colonized riparian forests in the Southeastern United States. In this study, the impact of privet on soil function and fertility are being assessed by comparing soil properties (pH, carbon and nitrogen content), microbial nitrogen mineralization rates, and microbial biomass, in privet-dominated forest soils versus soils beneath native riparian vegetation. Privet may alter decomposition rates and nutrient availability within a soil relative to native vegetation. We hypothesize that privet increases the rate of N mineralization, microbial biomass, and the amount of available N. An increase in N mineralization may lead to decreased competitive ability of native plants, as the soils in this region are naturally limited by N and native plants may not effectively compete with privet in an environment of high N availability. We report the soil functional characteristics above from each treatment before and after removal of the privet from our sample plots and compare our results to changes in the soil microbiome determined in Part I of this study.

Jessica Moore
Department of Biology, Western Carolina University

Genomic Annotation and Analysis of the Draft Sequence of the Pathogenic Microorganism, *Elizabethkingia meningoseptica*

Elizabethkingia meningoseptica is a gram negative, rod shaped bacillus that can cause meningitis-like symptoms in immunocompromised individuals. *Elizabethkingia* is a genus within the Flavobacteriaceae family of Bacteroides, consisting of three species: *E. meningoseptica*, *E. anophelis* and *E. miricola*. *Elizabethkingia meningoseptica* exhibits multiple drug-resistance as does *E. anophelis*, which is found in the gut of the malaria vector, *Anopholes gambiae*. A draft genome sequence of *E. meningoseptica* was published in July 2013 as a joint effort by the National Institute of Technology and Evaluation in Tokyo, Japan and Oklahoma State University. The Oklahoma State University draft genome sequence has the designation *Elizabethkingia meningoseptica* ATCC 13253 [Taxon ID 1216967], with 115 contigs that cover a total 3,796,928 base pairs and 3370 protein sequences. This genomic sequence was minimally analyzed when submitted to NCBI, with very little manual annotation. The purpose of this project is to manually annotate the genome of *E. meningoseptica* ATCC 13253, and to gain insight into molecular basis for pathogenesis of this and related species. We have taken a comparative genomics approach in which syntenic blocks are identified and analyzed between the *E. meningoseptica* ATCC 13253, and the draft genome sequences of 4 closely related organisms; *E. meningoseptica* 502, *E. meningoseptica* KuYH, *E. anophelis* Ag1 and *E. miricola*. Studying conserved blocks of genes between the various strains and species of *Elizabethkingia* allows for more consistent gene annotation, identification of functional operons, as well as locating key differences between the species, such as chromosome rearrangements, gene duplications and pseudogenes.

Christian Munoz-Pineda
Department of Biology, University of North Carolina at Asheville

Characterization of Genetic Diversity in Demes of Purple Pitcher Plants (*Sarracenia purpurea* L.) from Western North Carolina

Purple pitcher plant (*Sarracenia purpurea* L.) is a carnivorous species that is widespread throughout the coastal plain of eastern North America. In western North Carolina, *S. purpurea* var. montana (mountain purple pitcher plant) exhibits a much more limited distribution and is found only in isolated montane bogs and fens, which serve as ecosystem islands. Population sizes in mountain bogs are fairly small, ranging from as few as 2 up to approximately 300 individuals. In addition, many bogs are spatially isolated, with *S. purpurea* var. montana located up to 14 km from the next known population. We worked to characterize the genetic structure of eight *S. purpurea* var. montana demes in Western North Carolina using microsatellite loci. DNA was extracted from leaf tissue using Qiagen kits, and 5 microsatellite primer sets were used to amplify polymorphic regions of the genome. PCR products were run through agarose gel electrophoresis to visualize and quantify band sizes. Data from this project will help the United States Fish and Wildlife Service determine *S. purpurea* var. montana's suitability as a candidate species for conservation and preservation programs.

Mike Mutchler
Department of Biology, Appalachian State University

Genetic Screen for G Protein Signaling Components Involved in Nociception in *Drosophila melanogaster*

Chronic pain is a widespread issue in the United States, with over 100 million people claiming to suffer every year. This leads to billions of dollars lost in productivity and spent on treatment and care. There is a need for a more extensive understanding of how pain is perceived and also how it is transduced by sensory neurons. Elucidating the cellular and molecular mechanisms involved in transduction of noxious stimuli could lead to more effective and cost efficient treatment methods. Previous studies have indicated that G proteins play crucial roles in nociception and long-term sensitization to pain; however, the mechanisms are not completely understood. *Drosophila melanogaster* is a powerful model organism to manipulate genes involved in nociception in an attempt to expose different genes' impacts on the transduction of noxious stimuli. *Drosophila* larvae respond to noxious stimuli with well-documented response behavior referred to as nocifensive escape locomotion (NEL), which is marked by rolling along the longitudinal body axis. Using RNA interference to knock down functional transcripts of mRNAs specifically in the nociceptor neurons, we are performing a screen of G protein signaling genes potentially involved in nociception by identifying G protein signaling genes that produce an NEL defect when knocked down. The candidates identified in this initial screen will point to necessary G protein signaling pathways in our *Drosophila* nociception model. Future directions of study will have a focus on the cellular and molecular mechanisms of how the candidates function. This will include analyses of neural morphology and electrical activity.

Paul Rice
Department of Chemistry and Physics, Western Carolina University

Selective Reduction of Diesters using Lithium Borohydride

The control of organic chemical reactions is essential to the efficient production of molecules for pharmaceutical products (drugs). One such type of chemical reaction is the reduction of certain parts of a molecule, otherwise known as functional groups. To be most efficient these reduction reactions need to be as selective as possible. Our research involves the selective reduction of a specific type of functional group known as an ester. Two common chemicals known to reduce ester functional groups are sodium borohydride (a weak reducing agent) and lithium aluminum hydride (a strong reducing agent). Our research involves a selective reduction of a molecule containing two ester functional groups. Using lithium borohydride (which is a medium strength reducing agent) we are able to selectively reduce one ester functional group in the presence of another. We found that, in certain situations, once one ester functional group is reduced, the other ester remains unchanged. In this poster

we will report our results for these reduction reactions of molecules that contain two or more ester functional groups.

Jamie Rowell
Department of Mathematics and Computer Science, Western Carolina University

Statistical Analysis of Fermentation Rate in Hard Cider Brewing

In beer brewing, it is widely known that trace elements, particularly zinc, are required by yeast in order to grow and ferment. The general consensus among large-scale brewers and homebrewers alike is that the most sensitive and time-consuming step of beer production is the fermentation process. Problems encountered during fermentation can lead not only to prolonged fermentation time, but also to the deterioration of beer quality. These problems can often be contributed to a lack of necessary trace elements needed for the fermentation of yeast. While the wort provides some trace elements for the yeast, zinc is generally not available in the required amount in the wort. To prevent such problems, brewers may supplement wort by adding additional zinc salts during wort boiling or by adding yeast to the wort that has been previously treated with zinc. Several studies have been published on the optimal zinc concentration to increase the rate of fermentation, but few have studied this process in quantities that are practical to the average homebrewer. Furthermore, the specific gravity used in determining the rate of fermentation may not only be a function of time and wort content, but also process characteristics outside of the fermenter. In this study, we explore different methods of increasing fermentation rate and present various statistical models that describe specific gravity as a function of time and additional variables. These additional variables include both preplanned experimental factors and nuisance variables that may affect the final product.

Marietta Shattelroe
Department of Biology, University of North Carolina at Asheville

Determining Genetic Variation Among Western North Carolina Ginseng (*Panax quinquefolius* L.) Populations

American ginseng (*Panax quinquefolius*) is a medicinal herb that has been used for centuries in North America, and more recently in Asia. Ginsenosides, the biologically active compounds, are used to treat conditions such as diabetes and cancer. Market demand for wild-grown plants has reduced population sizes to dangerously low levels, resulting in *P. quinquefolius* being listed on Appendix II of CITES. Loss of individuals has been correlated with reduced genetic variation within and among populations in some regions, but this relationship has not been well characterized in western North Carolina. We used 7 microsatellite loci to analyze DNA from 7 western North Carolina populations under different harvesting pressures. Initial results showed that 1 of 4 of the populations had a high number of private alleles. Heterozygosity was also high among this population. AMOVA showed that 79% of detected variation was among individuals within populations, suggesting historic or recent gene flow. These data will be correlated with collection regime and ginsensoside quantity and quality to better inform management strategies of wild populations.

Rebecca Truitt (Rebecca J. Truitt[1], Brittany E. Harlow[2], Michael D. Flythe[2], Robert W. Bryant[3], and Langdon J. Martin[1])
Department of Chemistry, Warren Wilson College[1]; USDA ARS, Forage-Animal Production Research Unit (Lexington, KY)[2]; Asheville Flavor Innovations LLC[3]

Anaerobic Growth of Equine GI Bacteria at Warren Wilson College

To investigate the effects of anti-microbial alpha and beta hop acids (humulones and lupulones, respectively) on animal gut microbiomes at Warren Wilson College, an anaerobic technique developed by previous researchers was adapted using a disposable plastic glove bag and non-invasive fecal collection methods. A time-dependent decrease in pH of bacterial suspensions was observed using goat rumen fluid, and a similar method is under development for use with equine fecal collections from

two Belgian draft workhorses at the college farm. An increased understanding of the microbiome, particularly methanogenic archaea in the rumen or cecum of livestock, has the potential to be especially beneficial for Warren Wilson College, which has a farm with horses, cows, pigs and sheep. These methods could be useful in evaluating the feed supplement potential of recycled hop acid-rich yeast slurry generated at nearby microbreweries. This preliminary work will be used for methods validation of the anaerobic technique at Warren Wilson to lay the foundation for continuing research in this field.

Laol Vang
Department of Biology, Western Carolina University

Identifying Antibiotic Resistance Genes from the Major Facilitator Efflux Pump Superfamily in the Pathogenic Microorganism, *Elizabethkingia meningoseptica*

Elizabethkingia meningoseptica is a gram negative bacillus known to cause potentially fatal meningitis in immuno-compromised individuals, and spreads rapidly within hospital settings. Treatment of *E. meningoseptica* infections are complicated by the multidrug resistance of this organism. There are three known mechanisms that bacteria use to resist antibiotics: 1. Acquire the ability to degrade the antibiotic, 2. Change the structure of the cellular target site for a particular antibiotic, or somehow limit the access of the antibiotic to its target site, 3. Move antibiotic molecules out of the cell via pumps in the cell membrane. Major facilitator (MF) pumps represent one of the four major types of proton driven drug efflux pumps associated with antibiotic resistance. The purpose of this project is to use comparative genomics to identify MF family drug efflux pumps within *E. meningosepetica*. The *E. meningoseptica* draft genome sequence was examined for the presence of homologous genes to over 20 known MF efflux pump genes from other bacterial species. Potential homologs were analyzed using databases such as BLAST, Concise Microbial BLAST, Pfam, TIGRFAM and KEGG. By understanding the mechanism of drug resistance of this pathogenic microorganism, we will further our knowledge of the role of drug resistance in infection, and may lead to possible new treatment options.

Yeng Vang (Yeng Vang, Juan Bautista, Brandon Roark, Elissa Nelson, Jennifer Wyderko, & Robert T. Youker)
Department of Biology, Western Carolina University

Investigation of the Effects of Oleuropein, an Antioxidant Found in Olive Leaves, on the Biosynthetic Folding of the NBD1 Domain of CFTR

The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a polytopic protein that traffics to the apical surface of epithelial cells where it transports chloride. The folding and synthesis of CFTR in the endoplasmic reticulum (ER) is inefficient, and up to 80% of wild type protein may be degraded. A common mutation in CFTR is the deletion of a single amino acid (▫F508) that enhances misfolding, causes complete degradation of the protein, and is a major cause of Cystic Fibrosis (CF). The ▫F508 mutation resides in the first nucleotide-binding domain (NBD1) of CFTR and disrupts the folding pathway of CFTR leading to misfolding and aggregation. A major effort in the CF research community is the search for small molecule drugs that can aid in CFTR folding but unfortunately only a handful have been identified. Oleuropein, an antioxidant found in olive leaves, has recently been shown to prevent the aggregation of the protein Tau. We have measured the in vitro aggregation rate of ▫F508-NBD1 in the absence and presence of oleuropein. Our preliminary results suggest that concentrations up to 10 uM of oleuropein partially prevent the aggregation of ▫F508-NBD1. Ongoing studies include testing additional concentrations of oleuropein in our aggregation assay and measuring the effects of oleuropein on the processing of CFTR in living cells.

Molecules in the Mountains Organizing Committee

Timothy Driscoll	Department of Biology	Western Carolina University
Kelly Grisedale	Forensic Science Program and Department of Biology	Western Carolina University
Jon Lawrie	Western Office	North Carolina Biotechnology Center
Seán O'Connell	Department of Biology	Western Carolina University
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