Science in the Mountains

Entrepreneurial and Biotechnology Opportunities in North Carolina

Program

April 18–19, 2011

Enka Campus of Asheville-Buncombe Technical Community College, Asheville, NC

Promoting regional opportunities for collaboration in research, development and commercialization for higher education institutions, industry and governmental agencies

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The Western Regional Office of the North Carolina Biotechnology Center and the Conference Planning Committee express sincere appreciation to our sponsors for both financial and in-kind support that has made Science in the Mountains 2011 *possible. Their support is critically important to the growth of biosciences education, research and commercialization across Western North Carolina and the state.*

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Science in the Mountains 2011

In addition to its natural beauty, Western North Carolina hails great universities and outstanding research. It's this research that holds the key to growing a vibrant life sciences cluster; however the advancement of research can only be accomplished through interaction and collaboration.

That is what this conference is all about.

Science in the Mountains *brings together the region's faculty and students to share in their research and explore opportunities for collaborations. Leaders and experts from industry and governmental agencies also participate in the conference, providing their expertise and to help promote a culture of entrepreneurism, intellectual property protection and commercialization.*

These interactions enhance the growth of high quality research in our region, research that is getting more and more competitive for federal and state funding. Working together rather than individually will maintain Western North Carolina's competitive advantage.

We hope you enjoy yourself at Science in the Mountains 2011.

Event Planning Committee Members

Steven M. Casey, Vice President, Statewide Development
North Carolina Biotechnology Center

Paul V. Phibbs, Ph.D., Emeritus Professor of Microbiology & Immunology
East Carolina University

Jonathan M. Lawrie, Ph.D., Assistant Professor, Center for Entrepreneurship and Innovation
Western Carolina University

Kathy D. Wright, Regional Coordinator, Western Office
North Carolina Biotechnology Center

Science in the Mountains 2011 Agenda

Monday, April 18, 2011

8:00 Continental Breakfast and Networking

8:30 *Steven Casey*, North Carolina Biotechnology Center
Welcome

8:45 *Terry Bellamy*, Mayor of Asheville
Opening Remarks

9:00 *Cathy Innes*, UNC at Chapel Hill
Thinking Beyond Publishing Your Research — Might You Need a Patent?

9:30 *Michelle McMullen-Tack*, McDonnell Boehnen Hulbert & Berghoff, LLP, Chicago
Intellectual Property Basics for the Faculty Researcher: What Every Researcher Should Know about IP

10:00 *Hutton Kearney*, Fullerton Genetics Center Laboratory, Mission Health System
Whole Genome Approaches to Genetic Diagnoses; Past, Present, and Future Perspectives

10:30 *Julie King*, North Carolina Biotechnology Center
Funding Opportunities for Early Stage Companies

11:00 **Poster Session and Networking**

12:00 pm Lunch and Speaker: *Mark Wilson*, Western Carolina University
Exploring the Newest Generation of DNA Sequencing Techniques for Application in Forensic Science

1:30 **Poster Session and Networking**

2:30 *Briles Johnson*, North Carolina Department of Commerce
BLNC: North Carolina's Business Resources

3:00 *Betsy Wilson*, UNC Asheville
Analysis of Microbial Diversity in Soil from Great Smoky Mountains National Park

3:30 *Ted Meigs*, UNC Asheville
Dissection of G{alpha}12-Mediated Signaling Pathways through Mutational Analysis of Target Protein Interactions

4:00 **Daily Student Poster Awards and Networking**

4:30 Keynote Address: *Louise Temple*, James Madison University
Addicted to Students: Seventeen Years of Doing Science with Undergraduates

Tuesday, April 19, 2011

8:00 Continental Breakfast and Networking

8:30 *Mary Lou Surgi*, Blue Ridge Food Venture
Tour: Natural Products Manufacturing Facility

9:15 *Joe-Ann McCoy*, Bentcreek Institute
The Bent Creek Germplasm Repository (BCGR) at the NC Arboretum, a Tool for Collaborative Bio-Based Research

9:45 *Jack Summers*, Western Carolina University
Inhibition of CuZnSOD by Low Molecular Weight Natural Products

10:15 *Dana Nelson*, US Forest Service, Southern Research Station
Accelerating Forest Restoration through Biotechnology

10:45 *Ron Paulus*, President & CEO, Mission Health System
The Pending Economic Environment for Health Services, Devices and Biotechnology: What Will it Take to Win?

11:00 **Poster Session and Networking**

12:00 pm Lunch and Speaker: *Bob McMahan*, Western Carolina University
Biotechnology and Entrepreneurism

1:30 **Poster Session and Networking**

2:30 *Jennifer Burris*, Appalachian State Univ.
Macromolecular Spectroscopy and Bioanalysis at Appalachian State University

3:00 *Ted Zerucha*, Appalachian State University
Identification of cis-Regulatory Elements Capable of Directing Cell-Specific Gene Expression

3:30 *Tom Ranney*, Mountain Horticulture Research Station
The New Age of Bioenergy Crops: Research and Development of Fuelstocks for North Carolina

4:00 **Daily Student Awards**
Leadership in Science and Entrepreneurship Award
John W. Bardo, Chancellor, Western Carolina University.
Presented by Norris Tolson, President & CEO, North Carolina Biotechnology Center

4:30 *Steven Casey*, NC Biotechnology Center
Closing Remarks

Platform Presenters

Thinking Beyond Publishing Your Research — Might You Need a Patent?

Catherine Innes, Office of Technology Development, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-4105.
Cathy_innes@unc.edu

Moving good ideas from the lab to the marketplace often requires securing patent rights around an idea so that there is a valuable asset to license to others or use to form a company. This session will talk about how we do this at UNC and the role of patents and licenses to commercialize your ideas and form valuable industrial collaborations.

Intellectual Property (IP) Basics for the Faculty Researcher: What Every Researcher Should Know about IP

Michelle McMullen-Tack, Ph.D., J.D., McDonnell Boehnen Hulbert & Berghoff, LLP, Chicago

In general, faculty and academic researchers are aware that securing intellectual property (i.e., patent) rights is important for potential commercialization of their scientific research. However, many researchers are not confident as to when they have a protectable technology or when they should call their technology transfer office. I will help you understand the basics of intellectual property, including patentability. I will provide an overview of the patent process, outline key dates to remember, tips to help protect your developing technology, and common pitfalls to avoid. Additionally, I will explain the Bayh-Dole Act, its role in facilitating commercialization of University technologies and why participating in the IP process benefits your research, your institution, and your community.

"Whole Genome Approaches to Genetic Diagnoses: Past, Present, and Future Perspectives"

Hutton M. Kearney, Ph.D., FACMG, Cytogenetics Director, Fullerton Genetics Center, Mission Health System

The field of genetics has witnessed an explosive growth of knowledge and technological advancement over the last few decades. With the completion of the Human Genome Project, the identification of gene mutations responsible for human disease is rapidly expanding. Additionally, the diagnostic tools that are available allow for interrogation of the genome with unprecedented breadth and resolution. In this presentation, we will review new technologies, diagnostic implications, and ethical considerations for whole-genome approaches to genetic diagnoses.

Finding Funding Gold

Julie L. King, North Carolina Biotechnology Center

Do you know how to research and identify grant funding opportunities? Do you understand the diversity of the grant funding community? Searching for and finding the right grant opportunity to "fit" with a project idea or program can be an overwhelming task. Small organizations and early stage companies are especially challenged with how to begin their search for funders and where to locate opportunities for federal, state, private, and foundation grants. In this session, the basic tools for how to get started in the search for "funding gold" along with funding tricks and tips, funding websites and list serves, and the latest funding opportunities will be shared.

Exploring the Newest Generation of DNA Sequencing Techniques for Applications in Forensic Science.

Mark R. Wilson, Ph.D., Western Carolina University

Challenging forensic DNA samples extracted from, for instance, bones and hair can be degraded and/or contain very little DNA. Other low-level DNA samples, including those arising from "touch DNA", also present a challenge. Commonly used short tandem repeat markers (STRs), are autosomal markers inherited from both parents. When the amount of extracted DNA is very low, STRs markers exhibit stochastic sampling effects, which can result in allele drop-out, and in rare cases, allele drop-in. Therefore, significant challenges remain in the use of DNA analysis on these kinds of human biological samples. Mitochondrial DNA (mtDNA) is present in higher copy number and hence has some advantages for low-level DNA samples. In recent years, due to its increased sensitivity, mitochondrial DNA (mtDNA) has become an important tool for the analysis of these samples. Human mtDNA from hair, bones and other tissues has been used successfully in many criminal investigations, but population variation found in the coding region of the mitochondrial genome has not yet been fully explored in a forensic context.

In order to expand the utility of forensic mtDNA analysis, it is necessary to develop methods to efficiently and reliably obtain high quality DNA sequence information from the entire mtDNA genome. Newly emerging DNA analysis techniques, including whole genome amplification and high-throughput DNA sequencing methods such as pyrosequencing, now make this goal obtainable.

North Carolina's Business Resources

Briles Johnson, Manager, Business Link North Carolina, North Carolina Department of Commerce

Business Link North Carolina or 'BLNC' provides services, answers questions and solves problems for businesses across the state. BLNC is a collaboration of state-wide, state funded business resource providers. The services provided by BLNC include one-on-one consultations with our business counselors via our toll free number and via the website, *www.blnc.gov*.

The following is a sample of questions commonly answered:

- Where do I begin to start a business?
- How do I find training and education for my employees?
- What permits and licenses will my business need?
- How do I gain access to capital?
- My business model is changing, how can I tap into market research?
- How do I set up for government contracting?
- Are there entrepreneurial resources?

BLNC's resource partner group is a collaboration of state-wide, state-funded business resource providers. Our partners provide a vast array of counseling, programs, seminars and resources to help businesses and entrepreneurs start, grow and succeed.

Analysis of Microbial Diversity in Soil from Great Smoky Mountains National Park

Betsy Wilson, Ph.D., Department of Biology, UNC Asheville

Microbes in soil samples collected from the Purchase Knob area of Great Smoky Mountains National Park were analyzed by isolating total DNA and amplifying small subunit ribosomal RNA genes by polymerase chain reaction. Amplification products were incubated with cloning vectors, and the cloning reaction products were transformed into competent Escherichia coli cells. Transformants were identified as antibiotic-resistant colonies on LB agar plates. Cloned small subunit ribosomal RNA genes were sequenced, and sequences were analyzed by alignment with microbe sequences in GenBank and the Ribosomal Database Project. This analysis demonstrates that most numerous microbial phyla are Acidobacteria and Proteobacteria.

Dissection of G{alpha}12-Mediated Signaling Pathways through Mutational Analysis of Target Protein Interactions

Thomas E. Meigs, Ph.D., Associate Professor of Biology, UNC Asheville

Heterotrimeric G proteins transduce signals from a wide variety of cell surface receptors to the cell interior, leading to numerous cellular changes and responses. The alpha subunits of heterotrimeric G proteins are grouped into 4 subfamilies in mammals, including the G12 subfamily which consists of the closely related proteins G{alpha}12 and G{alpha}13. G{alpha}12 has been implicated in several pathways that frequently become aberrantly regulated in cancer cells, including proliferation, migration, cytoskeletal rearrangements, apoptosis, and cell-cell adhesion. However, efforts to study G{alpha}12 function have been complicated by the discovery of nearly 20 different proteins that serve as downstream binding partners. We have undertaken a comprehensive protein interaction screen, in which various target proteins of G{alpha}12 are immobilized, purified, and assessed for binding to a series of cassette mutants of activated G{alpha}12. This approach has provided structural details of G{alpha}12 interaction with several targets, including the kidney disease-related protein polycystin-1, the tumor suppressor p120-catenin, and guanine nucleotide exchange factors that stimulate activity of the oncoprotein RhoA. In addition, several G{alpha}12 mutants have provided us valuable molecular tools for investigating the role of these specific protein interactions in the signaling mechanisms that become dysfunctional in polycystic kidney disease, as well as the aberrations in cell-cell adhesion that contribute to cancer progression.

Addicted to Students: Seventeen Years of Doing Science with Undergraduates

Louise Temple, Ph.D., James Madison University, Department of Integrated Science & Technology

Working with undergraduate students in science education has some very unique features that both enrich the research experience and provide challenges to the creative process. One has to focus primarily on the training of new scientists, preparing them for careers or post-graduate education, while still managing to contribute to our understanding of the natural world with new knowledge. Obtaining funding and publishing findings are required to maintain credibility as a scientist and to set an example for students. At the same time, the training process is quite intense, and other aspects of mentoring are unique to the undergraduates compared to graduate students. Managing such a program and nurturing undergraduates is the focus of this seminar, in which examples will illustrate several principles.

Inhibition of CuZnSOD by low MW natural products.

Jack S. Summers, [1] *Benjamin Hickman,* [1] *Megan E. Arrington,* [1] *Michele R. Yost,* [1] *Brandon Wilson, C. Wilson,* [1] *Jeffrey D. Schmitt.* [2]
[1]Western Carolina University and [2]Wake Forest University

We are working to develop inhibitors of superoxide dismutase (SOD) enzymes which could be used as anti-cancer therapeutics. We use NMR based methods to measure the effects of low molecular weight compounds on SOD active site accessibility. We discovered that flavonols (a class of low molecular weight natural products) inhibit bovine CuZnSOD. We have characterized the effects of inhibitor structure, pH, and concentration on inhibition. The results of these studies and our plans for future work in the area will be discussed.

Accelerating Forest Restoration through Biotechnology

C.D. Nelson, Ph.D., (U.S. Forest Service), *T.L. Kubisiak, B. Olukolu, M Staton, E. Fang, A. Barakat, S. Ficklin, A.G. Abbott, J.E. Carlson, S.A. Merkle, W. Powell, C.J. Nairn, C. Maynard, F.V. Hebard, S. Anagnostakis, J Creighton, J James, S Jeffords*

Increasingly exotic, invasive pests are threatening forest ecosystem health as globalization continues to move organisms around the world. Several existing and emerging threats are well known in the eastern U.S.including chestnut blight, Dutch elm disease, emerald ash borer and hemlock woolly adelgid. A new initiative is developing and utilizing biotechnological tools to respond to these threats in timely and environmentally responsible ways. The Forest Health Initiative (FHI) is integrating genomics and genetics to identify the genes required for effective pest resistance and develop efficient tools for transferring these genes to locally adapted, genetically diverse breeding lines for use in species conservation and restoration. As a test case the FHI is mapping and sequencing the Chinese and American chestnut genomes in an effort to identify the genes providing resistance to chestnut blight (caused by Cryphonectria parasitica) and other limiting pests such as the ink disease pathogen (Phytophthora cinnamomi) and the chestnut gall wasp (Dryocosmus kuriphilus). Disease screening based on clonal testing is being developed to improve the efficiency and effectiveness of mapping resistance genes and genetic transformation is being utilized to transfer desired non-native genes to the native breeding lines. This work is building on many decades of breeding work conducted by USDA, the Connecticut Agricultural Experiment Station, and The American Chestnut Foundation, a couple decades of tissue culture and transformation research at the State University of New York and the University of Georgia, and an ongoing NSF Plant Genome project. In addition to the biological sciences work, the FHI is engaging the environmental and local communities in dialogue about the technologies being developed and their potential applications. The overall approach of the FHI will be summarized as well as research results focusing on the genomics of disease resistance and candidate gene discovery.

The Bent Creek Germplasm Repository (BCGR) at The North Carolina Arboretum, A Tool for Collaborative Bio-Based Research

Joe-Ann H. McCoy, Ph.D., Bent Creek Germplasm Repository, The North Carolina Arboretum, 100 Frederick Law Olmsted Way, Asheville, NC 28806-9315 828-665-2492 ext. 268, *jmccoy@ncarboretum.org*

The Bent Creek Germplasm Repository (BCGR) is a cooperative effort by public and private organizations to both preserve the genetic diversity of native North American medicinal plants by the long-term storage of germplasm and related data and collaborate with researchers to provide high quality research materials for study. This presentation will summarize how researchers can collaborate to utilize genebanks for bio-based research. Both national and state germplasm collections are optimal choices for a wide variety of research ranging from chemical analysis of metabolites of interest for new product development and drug discovery, to endophyte isolation, phylogenetic studies, and cultivar breeding. Examples of current research projects will be discussed. Illustrations of field collection methods, laboratory processing, seed and control-pollinated cage propagation, endophyte isolation, and facilities utilized for seed cleaning, testing and storage will be included.

Macromolecular Spectroscopy and Bioanalysis at Appalachian State University

Jennifer L. Burris, Ph.D., Assistant Professor, Department of Physics and Astronomy, Appalachian State University

Our multidisciplinary research group, focused on biophotonics research, currently includes six faculty and ten students from the departments of Biology, Chemistry, Computer Sciences, and Physics & Astronomy. We recently received an Institutional Development Grant from the North Carolina Biotechnology Center. With this grant we have been able to enhance the Raman spectroscopy laboratory for use with organic materials, to include fluorescence and absorption measurements, and to add equipment capable of detecting biomechanical, structural and molecular (gene and protein) alterations in biological materials. Our vision is to create a multidisciplinary imaging program. Our group has various projects which include profiling expression patterns of collagen in the birth canal for predicting timing of birth, studying the structure of membrane proteins, and developing a non-invasive spectroscopic assay to monitor changes in cells during normal and disease states.

Identification of cis-Regulatory Elements Capable of Directing Cell-Specific Gene Expression

Ted Zerucha, Ph.D., Appalachian State University, Department of Biology

Researchers are increasingly becoming interested in identifying cis-regulatory elements that are able to direct cell-specific gene expression. These elements have a vast potential for applications such as targeting cell-specific expression for gene therapy treatments, for the development of "smart drugs" as well as for directing transgene expression for basic science applications. We are using a novel comparative genomics approach to identify the regulatory elements responsible for controlling expression of the Meis family of homeobox-containing genes. Homologues of the Meis genes (originally named for myeloid ecotropic leukemia virus integration site because a disruption of the first member of this gene family discovered was found to lead to Leukemia) have been identified in all animals studied and have been found to be expressed in similar patterns during the embryonic development of those animals. By comparing the sequences of non-coding DNA regions associated with orthologous Meis genes in different species we have been able to identify several putative regulatory elements that are very well-conserved amongst all species. We are currently working on the identification, isolation and characterization of these and other elements using zebrafish as a model system.

The New Age of Bioenergy Crops: Research and Development of Fuelstocks for North Carolina

Thomas G. Ranney, Ph.D., *Darren Touchell, Ron Gehl, Irene Palmer*, and *Stephanie Haines*. Mountain Horticultural Crops Research & Extension Center, North Carolina State University, Fletcher, NC

North Carolina's future energy supply will undoubtedly include a diversity of bioenergy crops. To be successful, it's essential that these crops 1) maximize net energy production per unit area, 2) can be grown on marginal land with minimal inputs, 3) are profitable throughout the value chain, and 4) are sustainable with minimal environmental impacts. Perennial grasses including cold hardy sugarcanes (Saccharum spp.), miscanthus (Miscanthus spp.), and giant reed (Arundo donax) are some of the crops currently under evaluation. Researchers at NC State University including Drs. Thomas Ranney and Darren Touchell (Horticultural Science), and Dr. Ron Gehl (Soil Science) are taking an interdisciplinary approach to tailoring these crops to North Carolina. State-wide evaluation trials are underway to evaluate regional adaptability, biomass yields, fertilizer responses, and sustainable production systems. Breeding efforts are focusing on the development of new high-yielding, non-invasive cultivars with regional adaptability and improved cold hardiness.

Poster Abstracts

Monday Session

Underlined names identify primary student authors of posters being judged for awards.

001: Actaea racemosa (black cohosh) Hydroethanolic Extract Alters LPS-Induced Expression of Pro- and Anti-Inflammatory Factors in the Cervix of Ovariectomized Mice

Nicole Diggins, Joe-Ann McCoy[1], Guichuan Hou, Chris Okunji[2], Subrina Jesmin[3], and *Chishimba N. Mowa.* Dept of Biology, Appalachian State University, Boone, NC; [1]Bent Creek Germplasm Repository, The North Carolina Arboretum, Asheville, NC; [2]Gaia Herbs Inc., Brevard, NC; [3]Division of Gene Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan

Cervical remodeling (CR) and parturition are essentially inflammatory-like responses, implying that normal and abnormal factors that either induce or diminish inflammatory conditions, could potentially lead to obstetrical complications, such as prolonged labor (diminished inflammation) or preterm labor (heightened inflammation). For instance, infectious diseases, such as vaginal bacterial and periodontal (gum) diseases, which increase levels of inflammatory factors, are also associated with preterm labor. Plant extracts with proven anti-inflammatory activity, such as Actaea racemosa L. (black cohosh)(Fam. Ranunculaceae) could potentially be used to attenuate preterm labor in high-risk women. Here, we test the effectiveness of the hydroethanolic extracts of A. racemosa [0.1, 0.3 and 3mg/mouse twice a day for 2 days, IP (3mg, for PO)] to attenuate LPS-induced expression of pro- (IL-6, TNFα) and anti- (IL-10) inflammatory factors in the cervix using ovariectomized mice, real-time PCR and Western blot analysis. Our preliminary data show that A racemosa attenuates LPS-induced expression of IL-6 in the cervices of mice (x1 fold, > x4 fold for 0.1mg and 0.3/3mg IP, respectively, compared to positive control) in a dose-dependent manner; whereas IL-10 increased at 0.1mg (x1 fold), and decreased at subsequent doses (0.3/3mg, IP, >x1 fold), respectively. We conclude that expression of LPS-induced pro-inflammatory factors is blocked by *A. racemosa* (IP), whereas the extent of anti-inflammatory factor expression is inversely proportional to the degree that *A racemosa* attenuates pro-inflammatory factors in the cervix of ovariectomized mice.

002: VEGF Induces Vascular Leakage, Edema and Cervical Epithelial Growth in Mice Cervix

Siobhan Donnelly, Jordan Estes, Subrina Jesmin1, Scott Rhyne, and *Chishimba Nathan Mowa.* Dept of Biology, Appalachian State University, Boone, NC; [1]Research Institute, National Center for Global Health and Medicine (NCGM), Tokyo, Japan

Cervical remodeling (CR) is characterized by pronounced vascular alterations and cellular growth, such as increase in vascular leakage, white blood cell (WBC) tissue infiltration and cervical epithelial growth. However, factors that underlie these changes are not completely known. Here, we build on our previous data and investigate in depth the specific effects of vascular endothelial growth factor (VEGF), the best characterized angiogenic factor, on vascular leakage, edema, and cervical epithelial growth. Ovariectomized mice were treated with recombinant VEGF protein to determine the optimal dosage (vehicle, 50ng, 200ng and 400ng/mouse twice daily for 4 days, IP and intra-vaginal alternately) and cervical tissues were harvested and analyzed using scanning electron microscope (SEM), immunohistochemistry (Anti-BrdU) and tissue hydration techniques. Mice used for cervical epithelial growth studies were also administered BrdU. VEGF was found to induce pronounced cervical epithelial growth and WBC infiltration and moderately increased edema in a dose-dependent manner. These results demonstrate that VEGF is a potent regulatory factor of multiple processes of CR.

003: Compact Imaging Systems for Real Time Imaging of Biopsy Samples

Gregory Kintz, VP of Engineering, Laser Biopsy Inc., 1465 Sandhill Road, Suite 1006, Candler, NC 28715

Laser Biopsy Inc., LBI, is developing a unique cost-effective medical imaging system to provide pathologists with instant two-dimensional (2D) virtual sections of tissue samples in a digital format, to enable real-time diagnosis of endoscopic, needle, and open biopsies, either directly in the operating room or via secure networks.

Most cancer diagnoses are made by biopsying suspicious lesions under some form of image guidance, followed by histopathological analysis of the fixed and stained tissue. However, with more effective screening strategies, disease is being diagnosed earlier, and in such cases, cancer is often not clearly distinguishable by imaging. Under these conditions, the surgeon operates relatively "blind" during the biopsy procedure, removing a certain number of random biopsies from "representative sites" within the organ, without the benefit of any real-time feedback to target the areas of the organ with cancer.

In order to address this major gap in current state-of-the-art cancer care, Laser Biopsy Inc. is developing a compact low-cost Multiphoton microscopy (MPM) system to enable rapid nondestructive analysis of tissue samples within a minute of obtaining a biopsy specimen, without the need for any fixation chemicals, dye stains, or microtome sectioning. Multiphoton-based imaging technology allows visualization of tissue, providing information both about tissue architecture and cellular morphology. It is our overarching hypothesis that multiphoton histopathology from tissue biopsies can provide real time feedback to surgeons, thus improving the accuracy of targeted biopsies and improving patient diagnosis and treatment.

The LBI instrument (Argus™) will produce 2D virtual sections of the tissue in less than a minute directly in the operating room or endoscopy suite. The digital image files can be viewed locally or remotely over secure networks (telepathology) for diagnosis by a pathologist. The benefits are: better patient outcome with improved quality of diagnosis (a much larger number of virtual sections with 3D reconstruction), faster turnaround time, reduced cost per section, and intrinsic archival digital records.

The potential for using multiphoton microscopy has been examined by a number of researchers. Researchers at Weill Cornell Medical College and Cornell University, Ithaca, have previously reported the utilization of MPM imaging to observe tissue architecture and cell morphology in fresh, unstained tissue samples, as well as preliminary correlations of the MPM images with "gold standard" [formalin-fixed, hematoxylin and eosin (H&E) stained] histological sections.

LBI has developed a design model for a low cost, robust multiphoton imaging system with an optimized field of view that is 25 times larger than that of a typical objective without sacrificing resolution. This allows the rapid imaging of an entire endoscopic biopsy specimen without the need for "tiling" many separate images together. The progress of our current Phase I SBIR research will be reported.

004: Compact Optical Imaging Systems

Gregory Kintz, Director of Optical Engineering, Profusa, 1046 Columbine Road, Asheville NC 28803

Mission and Approach: PROFUSA is revolutionizing continuous monitoring of body chemistries through highly miniaturized, long-lasting, accurate, tissue-integrating biosensors. Our approach to creating better sensors diverges from traditional engineering methodologies. PROFUSA is focused on understanding and directing the body's response to implanted sensors. Our ultra-small sensors are well tolerated by the body because they integrate with the surrounding tissue.

Goal: PROFUSA's short-term goal is to develop a self-calibrating, implantable continuous glucose monitor with a minimum of 90 days operational life (compared to currently available 2-7 day sensors). The long-term goal is to create self-calibrating sensors that control an insulin pump for at least 12 months.

Technology: PROFUSA's sensor employs a novel hydrogel, which integrates into skin tissue. The hydrogel's precisely engineered microarchitecture encourages capillary growth within the sensor itself, thereby providing exquisite access to real-time blood glucose and other body chemistries. **Patient Interface:** The syringe-injectable sensor has no electronics and contains fluorescent glucose-sensing molecules, which are monitored transdermally by an external thin-film patch. Blood glucose data is transmitted from the exterior BandAid-like patch via radio frequency to a smart phone for patient viewing and onto web-based e-system for physician viewing.

Impact: PROFUSA's continuous glucose sensor will motivate and enable diabetic patients to more tightly control their glucose levels without fear of severe glucose lows, leading to significant reduction of diabetes complications. Our mission is to provide accurate, continuous data that integrates with existing insulin pumps to complete the vision of an artificial pancreas.

PROFUSA Asheville Facility: PROFUSA's Asheville Facility is developing the BandAid-like optical sensor patch under the direction of Gregory Kintz, Director of Optical Engineering. A test bed using laser sources and spectrometers monitor fluorescence from sensor implants using tissue phantoms. Prototype patch sensors are under construction to support PROFUSA's research. PROFUSA has worked with the RAPID Center at WCU. PROFUSA is currently writing grants with Clemson University.

006: Development of a Shelf Stable Rainbow Trout Product

Charles Hudson, Sunburst Trout Company, Canton, NC 28716

Justification: Conversion of usable protein into a marketable product is an important goal for food producers and food processors. At Sunburst Trout Company useable, though under-utilized, high quality rainbow trout protein is generated as a by-product of the pin boning process.

Objectives: The objectives of this product are to turn usable trout protein into a marketable product that minimizes storage time of raw material, appeal to Sunburst's well established natural foods clientele, establish the company's first shelf stable product, and increase revenue.

Methods: Product ideation began for value added products derived from underutilized trout protein. A jerky product was conceived. The Jerky needed to be produced mainly using existing equipment. The trout protein is passed through a 1/8" grinding plate to produce the forcemeat. The Jerky was initially formed using a hamburger patty machine; it is now extruded using a LEM power jerky cannon. Smoking and drying are performed in the existing AFOS Smoker. The flavor profile was developed to include a spicy note, garlic, black pepper, and a slight sweetness. A locally brewed Tamari is also used for flavoring. It is wheat and artificial preservative free. Smoke is added for 2.5 hours of total cook/dry time. Internal temperature is controlled and reaches a max of 1800F. The remaining time the internal temperature is maintained between 1550F -1650F. Temperature is recorded using a continuous time and temperature monitoring device. Air flow is adjusted manually. The jerky is cut and portioned into 2 ounce vacuum pouches and vacuum sealed. The water activity for each lot is tested using a Decagon Devices Pawkit water activity meter and complies with our HACCP plan.

Results: Use of the grinder with a 1/8" plate produces a uniform forcemeat, which enables more consistent drying. Use of an extruder resulted in a product shape that was more acceptable to the consumer. Addition of sugar to the product brought the water activity to a safe level, while maintaining the desired texture. The use of the locally brewed soy sauce resulted in a "clean label" for our natural foods customers. It also had the benefit of making the flavor more complex. Usable trout protein is quickly turned into a marketable product resulting in increased revenue. Significance to the Culinology® Field: The methods, ingredients, and HACCP plan used in Sunburst's Trout Jerky can be adapted to other fish species, to produce a revenue generating product from under-utilized usable protein.

009: Hormones in Artificial Media Incite Variable Responses in Tobacco Callus and Regenerated Plantlets

Tiffany Noel Dial, Velinda Locklear Woriax, and *Debbie Hanmer* . Department of Biology, The University of North Carolina-Pembroke, Pembroke, NC

Growth of plants that are non-transformed genetically is enhanced by certain plant hormones in artificial media. Auxins are used to promote initiation of adventitious roots while cytokinins are added in culture for shoot formation. Two different media, Tobacco Root and Shoot (TRS, containing the auxin, Indole-3-acetic acid, and the cytokinin, Kinetin) and Woody Plant Media (WPM, lacking any hormones) were used to establish growth from tobacco callus or regenerated plantlets. Results indicate that shoots develop on pieces of callus grown on TRS media but not on WPM, regardless of the size of the callus pieces. However, when tobacco plantlets are applied to media, adventitious roots form in both media. When tobacco plantlets are applied to TRS, plant shoot growth occurs both above and below the surface of the media. Growth of shoots on plantlets placed upon WPM occurs only above the surface of the media.

010: An RNA Interference Screen for Virulence Factors in the Basidiomycete, Cryptococcus n-eoformans

[1]*Indrani Bose* and [2]*Tamara Doering*. [1]Western Carolina University, Department of Biology, Cullowhee NC 28723; [2]Washington University, Department of Molecular Microbiology, St. Louis MO 63110

Cryptococcus *neoformans* is a basidiomycetous yeast that is found ubiquitously in nature. It has gained prominence in recent years because it is one of a handful of fungi that can proliferate and cause systemic infections in mammalian hosts. It is an opportunistic pathogen that is the causative agent of cryptococcosis, sometimes leading to a fatal meningoencephalitis in immunocompromised patients. In order to develop systemically, the fungus has to successfully proliferate at the high temperatures of the host organism. This ability to grow at the high body temperature of warm-blooded species is critical for its ability to cause disease, and the genes responsible for this necessary for its virulence. In order to identify genes required by this organism for growth at high temperature, we devised an RNA interference (RNAi) screen to silence genes at random. We have created an RNAi library of genomic DNA inserts, approximately 2Kb in size. These inserts are cloned in a telomeric, ADE2 marked vector, in between two GAL7 promoters present in opposite orientation. In the presence of galactose in the medium, each insert is transcribed from the two promoters leading to the formation of double-stranded RNA (dsRNA) in the cell. This activates the RNAi pathway, silencing genes corresponding in sequence to that of the cloned insert. We have tested the vector for activation of the RNAi pathway by silencing known genes with well-studied phenotypes, such as the LAC1 and URA5 genes. Silencing of the LAC1 gene renders the strain unable to produce melanin, while silencing of the URA5 gene allows the cells to grow on 5-FOA. Once the method was validated, this strategy was used for forward genetics to identify genes whose function is required for viability at 37˚C. The screen has identified essential genes such as RHO1 (encoding a small GTPase) and LPC1 (required for sphingolipid biosynthesis), and non-essential genes such as CHS5 (a chitin synthase gene) and others. This is a fast and easy way to study the phenotypes of a wide range of genes without altering the genomic make-up of the cells.

011: Identification of Microorganisms Cultured from Hemlock Associated Soil in Great Smoky Mountains National Park

Danny Lammers and *Betsy Wilson*. Biology Department, UNC-Asheville, One University Heights, Zeis Hall 313, Asheville, NC 28804

Soil proves to be a highly abundant source of life for various strains of bacteria. Due to an estimated 1X10E9 bacterial cells per gram of soil, numerous phylogenetic groups of naturally occurring bacteria are believed to be present worldwide. While many of these have been identified and cultured, it is hypothesized that a vast amount of soil bacteria has yet to be cultured, studied, and identified due to the nearly infinite possibilities of required growth conditions. If cultured, these newly discovered bacteria could be subjected to future study in order to determine their biological roles in their respected ecosystems. This study sought to not only grow rare, previously uncultured soil bacteria, but also obtain pure samples of each culture and determine their ecological role in the soil. Hemlock associated soil samples were obtained from Great Smoky Mountains National Park and brought back to the lab for cultivation. The bacteria samples found in the soil were allotted a ten week incubation period under aerobic conditions on nutrient poor medium plates at room temperature to ensure proper growth time for rare, late forming organisms. DNA was isolated from pure cultures and polymerase chain reactions were used to amplify the small subunit ribosomal RNA genes. Analysis of the sequences of these genes was used to identify the soil isolates.

013: Fermentation Science at ASU: Brewing Up Employable Graduates and Regional Economic Development through a New Academic Program

Seth Cohen[1], *Brett Taubman*[2] and *Shea Tuberty*[3]. [1]ASU Viticulture Program, [2]Dept of Chemistry, [3]Dept of Biology, Appalachian State University, Boone, NC

Appalachian State University has developed a new Fermentation Science Program in the College of Arts and Sciences. The academic program will provide students an opportunity to acquire fundamental scientific knowledge and management skills necessary to advance sustainable growth industries including production of beer, wine, spirits, bio-fuels, and solvents as well as fermented meats, dairy, and alternative non-alcoholic food products. Students will benefit from an innovative, hands-on approach to learning production principles, business management, and integrating sustainable energy platforms into modern practice. The southeast region of the U.S. will benefit from an applied fermentation science degree program and an expanded employee base prepared to elevate the changing agricultural economy. Economic support will come through developing new and sustainable products and processes that foster regional economic development through value-addition, agri-business, tourism, entrepreneurship, and sustainable small business planning and management. The intended outcome is to engage and retain students in applied scientific and agricultural disciplines through completion of a baccalaureate degree program. Graduates will be a diverse group comprising applied scientists and business students with wide-ranging areas of interest from foods and fuels to pharmaceuticals. Through integrated business experience, internships, and collaborative research projects, students will acquire hands-on skills and relationships requisite for successful employment or new business development. We anticipate results in terms of student enrollment, successful student-industry collaborations, and producing qualified graduates to support growth industries in the U.S.

014: Prokaryotic Diversity from Soils in Eastern Hemlock Forests

Sean O'Connell, Emily York Salter, and *Carter Dillow.* Department of Biology, Western Carolina University, Cullowhee, NC

Eastern Hemlock (Tsuga canadensis) is a keystone tree species in Eastern North America that is under attack from an exotic pest, Hemlock Woolly Adelgid (*Adelges tsugae*). Vast stretches of forest have been decimated by this aphid: our research is focused on discovering microbial partners of hemlock before they disappear. Samples have been collected from hemlock roots and bulk soils and a variety of culture-based, molecular cloning, DGGE, and T-RFLP methods have been employed to ascertain the microflora unique to these trees. This study was divided into three parts: 1) culturable bacteria were obtained on low nutrient media and compared to clones obtained from the same rhizosphere and bulk soils, 2) archaea were detected via PCR of the 16S rRNA gene, and 3) T-RFLP was used to screen samples for amoA from bacteria and archaea from hemlock roots. Soil isolates were dominated by Firmicutes with Paenibacillus being the most common genus while soil clones were dominated by Acidobacteria with Group 1 being most numerous. Hemlock rhizosphere isolates were co-dominated by Actinobacteria and Alphaproteobacteria, with Bradyrhizobium abundant, and rhizosphere clones were dominated by Acidobacteria with Group 1 most numerous, but different species than in the bulk soils. Both Euryarchaea and Crenarchaea were found and the orders Thermoprotei and Thermoplasmata were represented for each phylum, respectively. RDP Classifier parameters for all sequences showed low matches (12-48% confidence) to known archaeal genera. T-RFLP of amoA genes revealed archaea to be a member of the rhizosphere community, with averages of 47.7 to 52.3 peaks per tree sampled within different forest types. Bacterial amoA genes could not be amplified from most of the same samples and when they could, they were less numerous (average of 7 peaks). Acidobacteria, presumptive nitrogen-fixing species (e.g., Bradyrhizobium), and archaeal ammonia oxidizers appear to be important components of hemlock ecology. Understanding interactions between bacteria, archaea, and hemlock could aid in successfully reforesting areas and restoring benefits to numerous animals dependent on this tree.

015: Increased Expression of Nuclear Beta-Catenin may Contribute to Metastasis in Canine Skin Cancers

Joshua Corbin and *Kari Loomis*, Department of Natural Sciences, Mars Hill College, Mars Hill, NC 28754

The Wnt/beta-catenin signaling pathway (also known as the canonical Wnt signaling pathway) plays a critical role in the development and homeostasis of many organisms. Two cell types which are greatly influenced by Wnt signaling control are melanoblasts and melanocytes. There are approximately seventy genes under the control of canonical Wnt signaling. Many of the Wnt-regulated genes promote cell proliferation, differentiation and motility. Beta-catenin is a signal transducer of the pathway and binds to transcription factors that promote the expression of the Wnt genes. Under normal circumstances, beta-catenin is broken down when it enters the cytoplasm before it reaches the nucleus; however under the influence of Wnt signaling, or in the presence of certain gene mutations involved in the pathway, beta-catenin is stabilized in the cytoplasm and is translocated to the nucleus where it promotes transcription of Wnt genes, often times leading to cell proliferation and motility. Because of the impact beta-catenin has on transcription, the overexpression of cytoplasmic and nuclear beta-catenin is often identified in many cancer types, one of which is melanomas. Research has shown that the overexpression of cytoplasmic and nuclear beta-catenin can be detected immunohistochemically in canine cutaneous melanomas (CCM) (Han et. al. 2010). In this study formalin-fixed canine skin tumors were embedded in paraffin wax. The tissues are to be stained immunohistochemically for the presence of beta-catenin; the sections will also undergo H&E staining in order to classify tumor types and identify cells undergoing apoptosis. Because beta-catenin, when translocated to the nucleus, is involved in melanocyte proliferation and migration, we hypothesize that the percentage of cells over-expressing beta-catenin in the cytoplasm and nucleus increases in metastasizing cells, which are located in areas of elevated inflammation in canine skin tumors. Furthermore, we speculate that such regions of inflamed tissue will have limited beta-catenin expression in the plasma membrane of cells and fewer cells undergoing apoptosis.

016: Radiation Induces Transition of Alveolar Type II Epithelial Cell to Myofibroblasts: in vitro and in vivo Studies

Devipriya Nagarajan and *Weiling Zhao*. Department of Radiation oncology, Wake forest University School of Medicine, Winston-Salem, NC

Lung cancer continues to be the most common fatal cancer in both men and women in USA, accounting for 31% and 26% of all cancer deaths, respectively. Radiotherapy is a mainstay of the treatment of locally advanced lung cancer, but has often resulted in normal tissue complications including pneumonitis and fibrosis. Lung fibrosis is characterized by excessive matrix deposition produced by myofibroblasts and the origin of myofibroblasts has become a subject for intense investigation. Recent studies suggest that epithelial cells can undergo transdifferentiation into myofibroblasts, through a process termed "epithelial–mesenchymal transition" (EMT), and plays an important role in tissue injuries leading to organ fibrosis, EMT is characterized by a shift from epithelial to bipolar cell morphology with increased expression of mesenchymal markers, and decreased expression of epithelial markers. To the best of our knowledge, there are no reports in understanding the effects of radiation on EMT in normal lung cells or tissues and hence the present study was designed to determine whether radiation could induce EMT in vivo and in vitro. To test our hypothesis, FVB/N mice were exposed to thoracic radiation with a single dose of 12 Gy and lung tissues were harvested at 14 wks postirradiation. Western blot analysis revealed an increase in the expression of vimentin and α-SMA (mesenchymal markers) with a progressive decrease in the expression E-cadherin and aquaporin in the irradiated lungs. In addition, immunofluorescence analysis showed the expression of α-SMA protein in alveolar type II epithelial cells, suggesting that alveolar type II cells of lung have undergone EMT following radiation. To demonstrate the potential mechanism(s) associated with this change, rat alveolar type II lung RLE-6TN epithelial cells were irradiated with a single dose of 8Gy and cell lysates were collected at various time intervals ranging from 15 min to 96 hrs. Western blot analysis showed a time-dependent decrease in the protein level of E-cadherin and increase in the acquisition of α-SMA at 24 to 96 h after irradiation, which suggests a phenotypic transformation of the cells to a mesenchymal morphology. Snail

and slug, transcriptional repressors of E-cadherin, were upregulated at 30 min to 7h postirradiation. MAPK/ERK may involve in activation of snail by blocking GSK-3ß activity, which is required for disassembly of cell adhesion and transformation. Western blot showed that radiation increased the phosphorylation of ERK and also inhibited GSK-3ß activity at 15 min to 3 h postirradiation, which suggests that activated Erk may inhibit GSK-3ß, and subsequently increases gene transcription and protein stability of snail. Altogether, these findings demonstrate the occurrence of EMT in vitro and in vivo after radiation and suggest that EMT may play an important role in the pathogenesis of radiation-induced pulmonary injury. Further mechanistic studies are still under progress.

018: Use of Pteridine and Lipofuscin Fluorescence for Age Determination in Apis mellifera (Honey Bees)

Christina Kotraba, Christopher Coburn, and *Lori Seischab.* Department of Biology, Western Carolina University, Cullowhee NC

In 2006, honey bee colonies began to disappear in large numbers throughout the world. Despite extensive research, no specific cause has yet been identified. To understand the epidemiology of disease transmission both within and between honey bee colonies, a method for determining the age distribution within a colony is needed. Currently, there is no precise method for estimating the age of a honey bee. This is particularly challenging because honey bees are holometabolous insects, which means their body size is fixed at the time of pupal eclosion. We are investigating whether levels of tissue extractable lipofuscins and/or pteridines may be used to accurately predict the honey bee age. These compounds are autofluorescent and have been found to accumulate in tissues over time in a variety of arthropod taxa.

019: Using a Catalytic Beacon to Identify Effective siRNA Target Sites

Susan Clark, Olena Northrup, and *Christopher Coburn.* Department of Biology, Western Carolina University, Cullowhee NC

Small double-stranded RNA molecules can induce sequence-specific post-transcriptional gene silencing in eukaryotic cells. The mechanism by which this occurs is referred to as RNA interference (RNAi). RNAi has become an essential tool for analyzing gene function and is believed to have therapeutic potential for silencing genes associated with human disease. RNAi is triggered by the introduction of double-stranded short interfering RNAs (siRNAs) into the cell. One strand, referred to as the guide strand, is complementary to a region of the target mRNA. The second strand is referred to as the passenger strand. The siRNA assembles with proteins to form an active RNA-induced silencing complex (RISC). The passenger strand is destroyed in the process, leaving the guide strand capable of directing RISC to the target mRNA, which is then cleaved. The primary difficulty in developing a successful strategy for RNAi involves insufficient accessibility of the target site. For an siRNA to be effective, the target site must be accessible for base-pairing with the guide strand. We have developed a catalytic beacon that can be used to experimentally verify the accessibility of putative target sites before undertaking costly and time consuming siRNA trials. The beacon is an oligonucleotide probe that is activated upon hybridization to the target sequence. The procedure can be performed in a 96-well plate, thereby allowing multiple target sites to be tested in a single experiment.

Poster Abstracts

Tuesday Session

Underlined names identify primary student authors of posters being judged for awards.

005: Rheological Characteristics of Aqueous Wax Emulsions Used for the Controlled Release of Pheromones as an Alternative to the Use of Pesticides for Insect Pest Management.

Stephen Ballew and *Cynthia Atterholt*. Department of Chemistry and Physics, Western Carolina University, Cullowhee, NC, 28723

Most pesticides produce some risk of harm to the environment because pesticides are designed to kill or adversely affect living organisms. It is desirable that alternate, safer forms of pest control be developed. One alternative is the controlled release of pest insect sex pheromones to produce a mating disruption effect. Aqueous paraffin wax emulsions have shown much promise as formulations for this controlled release when applied to tree bark or foliage. Soy wax has also recently become of interest in pheromone formulations because it is renewable, biodegradable, commercially available, and is acceptable for organic farming. Emulsions exhibit complex flow behavior which can be studied using rheometry. Rheometry refers to experimental techniques used to determine the fundamental relations between force and deformation in materials. The rheological properties of emulsions are very important for production, storage, and application of these formulations. In this project the flow and viscoelastic properties of aqueous 30% paraffin and soy wax emulsions were investigated using two different common emulsifiers: Span 60 (sorbitan monostearate) and triethanolamine stearate, and a 50%-50% mixture of both. The investigations were carried out in both the rotational and oscillatory modes of a parallel-plate rheometer. The flow curves at three different temperatures (15, 25 and 35 °C) of each emulsion were fitted with the Herschel-Bulkley model with the yield stresses determined using the one tangent method. The resulting equations can predict the flow behavior of the samples at different conditions. The emulsions were also tested using a rotational temperature sweep at a low shear rate from 15 °C to 50 °C to investigate temperature dependent changes. The viscoelastic properties were investigated using oscillatory shear tests and expressed in terms of elastic modulus and loss modulus. This gives information about time-dependant behavior like storage and the elastic character of the formulations which were found to be gel-like. The Span 60 emulsions displayed faux shear-thickening behavior due to droplet subdivision while the other emulsions displayed true shear-thinning behavior. The yield stresses and other flow parameters for the emulsions varied with temperature depending on the formulation in question. All soy wax emulsion showed an increase in viscosity near 50 °C in the temperature sweeps, while the paraffin wax emulsions did not. Every emulsion showed long-term and short-term stability except for the 50%-50% paraffin emulsion with viscoelastic data indicating a short shelf-life with a tendency to separate.

007: Use of Genetic Markers and Chemical Quantification to Identify Populations of Actaea racemosa (Black Cohosh) With Desirable Properties for Breeding a Regional Cultivar

Jason Clement[1], *Patrick Looney*[1], *Kathy Mathews*[2], *Sarah Pate*[2] and *Joe-Ann McCoy*[3].

[1]Department of Chemistry & Physics, Western Carolina University, Cullowhee, NC. [2]Department of Biology, Western Carolina University, Cullowhee, NC. [3]Bent Creek Germplasm Repository, The North Carolina Arboretum, Asheville, NC.

The first year of a collaborative project to characterize genetic, chemical and growth characteristics of the medicinal herb, black cohosh (*Actaea racemosa*), has yielded promising results. Over 60 individual plants from 20 populations collected throughout the species' native range are being propagated in control-pollination regeneration field cages to provide research materials for both genetic and analytical testing. Growth data collected include morphological descriptors, emergence dates, flowering dates, and seed production rates. HPLC quantification of triterpenoid glycosides and other biologically active compounds are being performed to observe both intra- and interpopulation variation throughout the native range of the species. DNA samples were collected from leaves of all individuals and used to develop microsatellite markers for the species. Multiple microsatellite primers are being screened for variability within and among accessions, as well as among Actaea species. Ultimately, we hope to correlate genetic markers with superior phytochemical production and desirable growth traits for the creation of a regional cultivar that can be unambiguously identified. Given the increasing demand for high-quality herbal products, the creation and production of a cultivar with demonstrably higher levels of triterpenoid glycosides could prove advantageous to western NC growers.

008: Genetic and Demographic Diversity of American Ginseng (Panax quinquefolius) in Western North Carolina

Megan Rayfield, Karissa Keen, Jennifer Rhode Ward, Jonathan Horton, and *H. David Clarke.* University of North Carolina at Asheville, Biology Department, [1] University Heights, Asheville, NC 28804

Wild American ginseng (Panax quinquefolius), which grows across the Eastern United States, has been harvested and exported to Asia for use as a stimulant in Eastern medicinal preparations. Because its biologically active compounds, ginsenosides, are most concentrated in roots, collection for export is fatal to ginseng plants, impacting their demography and survival. Aggressive harvesting and non-compliance with harvesting guidelines has caused P. quinquefolius to be listed as a CITES Appendix II species since 1973. Studies examining the genetic diversity at allozyme loci have shown loss of genetic diversity, outbreeding, and genetic structure in unprotected populations. This project uses newly published microsatellite primers to assess genetic relatedness among ginseng individuals in four protected Western North Carolina populations, then correlates those data with plants' demographic variables. Leaves from 160 individuals of known size, age, and reproductive status have been collected, DNA has been extracted, and samples are being PCR-amplified with 12 different primer sets. Preliminary data from 2 primer sets have shown genetic differences both within and among populations. Genetic data are being correlated with analyses of ginsenoside content, with the long-term goal of attributing patterns of ginsenoside production to specific genotypes. This would assist horticulturists in developing and propagating ginseng plants with high quantities of ginsenosides or specific ginsenoside profiles, allowing the development of a commercially-viable local ginseng industry.

012: Design and Construction of a Raman Spectroscopy System

Colin Curtis, Robb Young, and *Jennifer Burris.* Dept of Physics and Astronomy, 231 CAP Building, 525 Rivers Street, Appalachian State University, Boone, NC, 28607

Raman spectroscopy is a technique for detecting and analyzing the portion of light, reflected from objects, which is inelastically scattered. Although Raman scattered light makes up about one ten-millionth of total scattered light, it is valuable because Raman spectra are unique to various materials and offer insight as to specific molecular and physical properties. Thanks to an Institutional Development Grant from the North Carolina Biotechnology Center, our lab is outfitted with a combination spectrometer/CCD, a diode laser, and the additional optical components necessary to provide us the ability to use Raman Spectroscopy techniques to investigate biological materials. Future uses of this system will include determining the Raman spectrum of collagen and assessing the amounts of collagen in sample tissues.

017: A Laboratory Evaluation and Comparison of The Release Rates of Oriental Fruit Moth Pheromone from Soy, Paraffin, and Microcrystalline Wax

Afton Harris and *Cynthia Atterholt.* 4216 Little Savannah Rd. Apt. 56/Cullowhee, NC 28723

Insect pheromones can be used to control insect pests by mating disruption, as part of an Integrated Pest Management program. Insect pheromones are species specific, nontoxic, and can be released to the environment at low concentrations to interfere with insect communication. This controlled release of insect pheromones affects insect pest populations by interfering with the male insects' ability to locate female insects for mating. Pheromones have been used in some agricultural crops for pest management. This research has involved measurements of Oriental fruit moth pheromone release rates under controlled conditions in the laboratory from paraffin, microcrystalline and soy wax solids. The types of wax affected the release rate, causing microcrystalline to have the highest release rate, and paraffin the lowest release rate.

020: Detecting the Protein Expression of Drug Transporters within Caenorhabditis elegans

Ellie E. McCabe and *Jennifer Perry Cecile.* A.R. Smith Department of Chemistry, Appalachian State University, Boone, North Carolina 28608

Organic anion transporters (OATs) are membrane transport proteins that eliminate negatively charged xenobiotics in brain, kidney and liver cells of mammals. In this research, we use a nematode, the Caenorhabditis elegans (C. elegans), as a model for mammalian OATs. The C. elegans OAT is believed to be expressed in the intestinal membrane and shows homology to mammalian OATs. We have designed an antibody using amino acids from the C-terminal end of the C. elegans OAT in order to quantify protein expression levels and determine location of expression. In addition we have developed a protocol to obtain protein samples from whole nematodes. The presence of additional transporters such as p-glycoprotein (P-gp) and the multi-drug resistant protein (MRP) within the C. elegan model is also investigated. Protein expression of these transporters has been assessed in three different strains of C. elegans (N2, NL-130, NL-152) which contain various transporter knockouts. Future research includes using protein expression levels to compare signaling and regulation pathways between the mammalian and C. elegans OATs.

021: Organic Anion Transporter Structure and Function in Caenorhabditis elegans

Drew A. Bridges, Alicia E. Woock, Stephen M. Policke, Amber L. Harold, and *Jennifer Perry Cecile.* A.R. Smith Department of Chemistry, Appalachian State University, Boone, NC 28608

Organic anion transporters (OATs), members of the major facilitator superfamily, promiscuously excrete negatively charged xenobiotic and antiviral drugs in the kidney and liver of mammals. Investigation in this project focuses on a single mammalian OAT homologue present in *Caenorhabditis elegans* (*C. elegans*). A computational structure of the *C. elegans* OAT has been analyzed for the theoretical active site and compared to the active site of mammalian OATs. In addition, a stable fluorescence uptake assay with anionic fluorescein is utilized to verify interaction of mammalian OAT substrates and inhibitors with the *C. elegans* OAT. Use of three strains of the C. elegans has been essential in order to test inhibitors that vary in affecting not only the OATs but also the efflux transporters. Information gained from these experiments and the computational structure will be used to resolve the binding region of the *C.elegans* OAT and relate the protein structure to function.

022: Lipid Composition Changes to Organic Anion Protein Expression

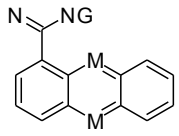
Lauren Kloeppinger, Ming W. Fan, and *Jennifer Perry Cecile.* A.R. Smith Department of Chemistry, Appalachian State University, Boone, NC 28607

The organic anion transporters (OATs) transport negatively charged drugs and xenobiotics across cellular membranes and are important components of drug secretion in liver and kidney. Recent work has shown that the scaffolding protein caveloin is important in the function of isoforms 1 and 3 (OAT1 and OAT3). In this investigation we examine multiple fractions of the cellular membrane and how lipid composition affects protein expression. Samples from homogenized rat kidney tissue separated into insoluble and soluble membrane fractions or cytosolic components are examined. Gel electrophoresis and Western blotting techniques are utilized to detect proteins primarily associated with insoluble lipid raft regions (such as caveolin and flotillin) as well as OAT3. Protein expression of rOAT3 shifts from detection at lower molecular weight to higher molecular weight in insoluble membrane fractions. Redistribution of proteins from insoluble to soluble fractions was also observed when the samples were treated with potassium iodide, a cytoskeletal disruptor. These changes indicate possible protein interaction between caveolin and OAT in insoluble membrane regions which may lead to regulation OAT function.

023: Antimicrobial Agent from a Pseudomonas sp. Strain from the Great Smoky Mountains National Park

Jason A. Clement,[1] *Rachel M. Bleich,*[1] *Ben Jeuck,* and [2] *Sean O'Connell*[2]. [1] Department of Chemistry and Physics, [2] Department of Biology, Western Carolina University, Cullowhee NC, 28723

The southern Appalachian range is considered the most biodiverse temperate region in the world. The microbial biodiversity of old-growth forests in the Great Smoky Mountains National Park (GSMNP) represents a unique opportunity for the discovery of sources of new antimicrobial agents. Furthermore, the use of alternative culturing techniques allows for the isolation of bacterial strains that may not be available using typical culturing methods. In our collaborative project, the use of alternative culturing methods has been applied to soil samples from old-growth hemlock stands in the GSMNP. This has afforded several strains of bacteria that produce antibacterial substances. We have cultured one of these strains, of genus Pseudomonas, in 10 liter fermentation batches. Bioassay-guided fractionation of a chloroform extract of the broth supernatant has afforded the known antimicrobial compound phenazine-1-carboxylic acid (1). These results demonstrate a successful application of alternative culturing strategies to growing an isolated bacterial strain for the purpose of producing antimicrobial agents.



024: Antitumor Compounds from Aralia racemosa

Jason A. Clement,[1] *Matthew J. Flood,*[1] *Timothy J. Willis,*[1] *Ryan M. Kelly,*[2] and *Jeffrey D. Schmitt*[2,3]. [1] Department of Chemistry and Physics, Western Carolina University, Cullowhee NC, 28723; [2] Bent Creek Institute, North Carolina Arboretum, Asheville NC, 28806; [3] Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27157

The southern Appalachians are home to an extraordinary variety of plant species many of which have been used medicinally by local populations. The vast majority of these species have not been studied for their antitumor activity, constituting a significant bioexploration opportunity. During a targeted screening program for identifying plants indigenous to Western North Carolina with potential antitumor activity, initial screening against the MCF-7 breast tumor cell line identified an extract of *Aralia racemosa* as having cytotoxic activity. Bioassay-guided fractionation afforded three diterpenoids and two acetylenic lipids with moderate antitumor activity. The structures of these compounds were determined by NMR, IR, and LC-MS spectroscopic methods.

025: Antitumor Oplopane Sesquiterpenoids from Arnoglossum atriplicifolium

Jason A. Clement,[1] *Rachel M. Bleich,*[1] *Hailey E. Campbell,*[1] *Kristin Naylor,*[1] *Matthew J. Flood,*[1] *Ryan M. Kelly,*[2] and *Jeffrey D. Schmitt*[2,3]. [1] Department of Chemistry and Physics, Western Carolina University, Cullowhee NC, 28723; [2] Bent Creek Institute, North Carolina Arboretum, Asheville NC, 28806; [3] Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27157

Western North Carolina is home to one of the most diverse collections of botanical species in the temperate world. A targeted screening program for identifying plants indigenous to Western North Carolina with potential antitumor activity was launched, and an extract of *Arnoglossum atriplicifolium* was identified in an initial screening against the MCF-7 breast tumor cell line (whole plant) as having cytotoxic activity. Following bioassay-guided fractionation of the crude extract, three oplopane sesquiterpenoids, including one novel compound, were isolated from the plant. Their structures were characterized by 1D and 2D NMR and LC-MS methods.

026: Phytochemical Investigation of Eupatorium serotinum (Late Boneset)

Jason A. Clement, and *Timothy J. Willis*. Department of Chemistry and Physics, Western Carolina University, Cullowhee NC, 28723

Western North Carolina possesses a rich biodiversity, with many plant species that have not been fully characterized chemically. The perennial plant Eupatorium serotinum (late boneset), has a folk usage as an anti-inflammatory agent, yet very little has been reported regarding the chemistry of this plant. We will present our results for the phytochemical investigation of this plant.

028: Molecular Confirmation of Novel Morphological Characters to Identify Two Medically Important Mosquito (Diptera: Culicidae) Species That Were Previously Indistinguishable as Adult Females

Charlie Sither[1], *Brittannia Bintz*[2], *Virginia Hopkins*[2], *Mark Wilson*[2], *Bruce Harrison*[3], and *Brian Byrd*[1]. [1]Environmental Health Science Program, Western Carolina University; [2]Forensic Science Program, Western Carolina University; [3]Public Health Pest Management, N.C. Dept. of Environment and Natural Resources

Aedes atlanticus and Ae. tormentor are well known as nuisance mosquitoes and potential vectors of Eastern Equine Encephalitis and West Nile viruses. Until recently, these two species were long considered morphologically indistinguishable as adult females. Here we report investigations of the rDNA second internal transcribed spacer (ITS2) as a molecular target to distinguish these species. The ITS2 was PCR amplified from reared larval specimens of both species using conserved primers complementary to the rDNA 5.8S and 28S regions. Nucleotide sequences were subsequently obtained from both species by direct sequencing or TOPO-TA cloning. Sequence analyses demonstrated useful heterogeneity that distinguishes the two species. Our molecular work has validated novel morphological characters that may also be used to separate the two species. The results of additional on-going validation studies and progress towards a restriction endonuclease assay to distinguish the two species will be discussed.

Guest Speaker Biographies

Michelle L. McMullen-Tack is a partner of McDonnell Boehnen Hulbert & Berghoff LLP (MBHB), with more than 10 years of experience in intellectual property law. Her practice comprises patent procurement, patent enforcement, and interference practice, with a special emphasis in diagnostics, pharmaceuticals, peptide therapeutics, vaccines, metabolomics, stem cell, and regenerative medical technologies. Dr. McMullen-Tack's counseling experience includes rendering opinions on patent validity, patent infringement, and freedom-to-operate. She represents pharmaceutical and biotechnology companies, both large and small, as well as university clients and start-ups. Her litigation experience has encompassed a range of topics, from pharmaceuticals to business methods. Dr. McMullen-Tack's scientific research and publications were in the field of molecular endocrinology and developmental biology. Her scientific work included the production and characterization of transgenic animal models of human disease. Dr. McMullen-Tack received a BS in Microbiology from the University of Illinois at Urbana-Champaign, a PhD in Biochemistry, Molecular Biology, and Cellular Biology from Northwestern University, and a JD from DePaul University College of Law. Contact information: MBHB, 300 South Wacker Drive, Chicago, IL 60606; Phone 312.913.3344; *mcmullen-tack@mbhb.com*.

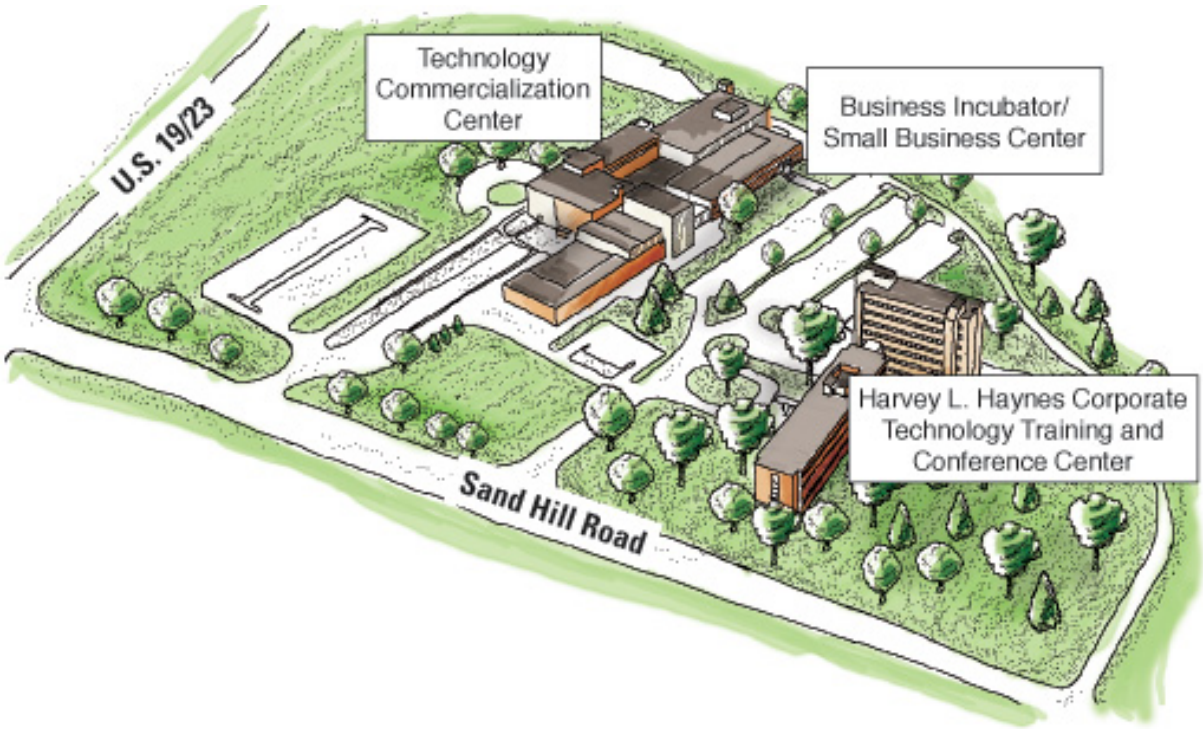
C. Dana Nelson is a supervisory research geneticist and Project Leader with the U.S. Forest Service, Southern Research Station's, Southern Institute of Forest Genetics located near Gulfport, Mississippi. He has a PhD and MS in forest genetics from the University of Minnesota and Oklahoma State University, respectively, and a BS in forestry from Iowa State University. Dr. Nelson has worked in Forest Service R&D for 16 years and in private industry for 7 years and is currently an adjunct faculty member at three universities — Florida, Texas A&M, and Mississippi State. His research interests include quantitative and molecular genetics of pest resistance and methods in marker assisted selection and breeding. Contact information: USDA Forest Service, Southern Research Station, 23332 Success Road, Saucier MS 39574-9344; Phone (228) 832-2747 x201; *dananelson@fs.fed.us*

Louise M. Temple, Professor of Integrated Science and Technology, James Madison University, received the PhD degree in Microbiology and Immunology from the Medical College of Virginia, Virginia Commonwealth University (VCU). Following postdoctoral research experiences in microbial molecular biology and immunotoxicology, Dr. Temple served 10 years on the biology faculty at Drew University, Madison, NJ. There she developed an externally supported research program on the biology and pathogenic properties of Bordetella avium, a bacterial pathogen of turkeys. For the last seven years, Dr. Temple has been a member of the James Madison University faculty, where she is currently developing B. avium as a heterologous antigen delivery system to vaccinate against diseases of poultry. Recently, her interests have expanded to include the discovery and genomics of bacteriophages, in particular as a means of capturing the interest of younger students. Dr. Temple has mentored and advised over 100 undergraduates in independent, individual or team research projects and she has been an active member of the Council for Undergraduate Research for more than ten years. Her research and educational projects and programs have been supported by grant funding from NIH, USDA, US Poultry & Egg, National Science Foundation, and Howard Hughes Medical Institute. Contact information: Dept of Integrated Science & Technology, MSC 4415, 701 Carrier Drive, James Madison Univ, Harrisonburg, VA 22807; Phone (540) 568-4415; *templelm@jmu.edu*.

Campus Map and Directions

Asheville-Buncombe County Technical College
Enka Campus

1459 Sand Hill Rd.
Candler, NC 28715
(828) 254-1921



Driving directions from I-40 west bound or east bound:

- Take Exit 44
- At the traffic light at the end of the exit ramp, turn right (south on 19/23)
- Go to the fourth traffic light and turn left on Sand Hill Road
- Go to the second entrance on the left and turn into the campus
- The Haynes Conference Center will be on your right and the Incubator will be on your left
- You may park on the left or in the lot straight ahead of you.

North Carolina
Biotechnology Center

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