Molecules in the Mountains

2012 Meeting

Western Carolina University

North Carolina Biotechnology Center

12 April 2012

Schedule		
All talks are in the University Center Theater and Posters, Lunch, and Breaks are in Illusions (down the hall)		
8:00	Registration Poster and talk set-up Coffee break	
9:00	Seán O'Connell	Welcome & Introduction of Mark Lord, Associate Provost
Session 1: Seán O'Connell, Chair		
9:15	Plenary Speaker: Laura Georgi	New tools for a monumental task: application of biotechnology to restoration of the American chestnut
10:15	Ben Tanner	Organic biomarker compounds record Holocene environmental change at Panthertown Bog, N.C.
10:35	Sarah Pate	Development and characterization of microsatellites markers for *Actaea racemosa* L. (black cohosh, Ranunculaceae)
11:00	Coffee break & Poster viewing	
Session: 2: Cindy Atterholt, Chair		
11:30	Nadja Cech	Goldenseal (*Hydrastis candensis*) against methicillin-resistant *Staphylococcus aureus* (MRSA): Layers of synergy
11:50	Mario Figueroa	Fungal blood: Mycology and natural products chemistry of endophytes from medicinal herbs
12:10	Indi Bose	An RNA interference for virulence factors in *Cryptococcus neoformans*
12:30	Lunch	
Session 3: Mark Wilson, Chair		
1:30	Plenary Speaker: Bruce Budowle	Forensic genetics and molecular methods for assessing biodiversity: A look at the past, present, and future

Schedule		
2:30	Poster Session	
Session 4: Pattie Foley, Chair		
3:30	Pattie Foley	Biodiversity inventories and forest fragmentation: Can mosquitoes suck out the data for us?
3:50	Brittania Bintz	Development of a novel human mitochondrial DNA (mtDNA) amplification method for use with Illumina® next-generation sequencing instrumentation
4:10	Groves Dixon	Quantification of minor variants in human mitochondrial DNA amplicon libraries using 454 pyrosequencing
Postscript		
4:30	Seán O'Connell	Concluding remarks
4:45	Adjournment	Optional post meeting get together

1.1) Organic Biomarker Compounds Record Holocene Environmental Change at Panthertown Bog, N.C.

Benjamin R. Tanner
Department of Geosciences and Natural Resources, Western Carolina University

Panthertown Bog is a peat-accumulating wetland in the Southern Appalachian region of North Carolina that is located at an elevation of greater than 1100m. A series of 4 organic-rich cores have now been recovered from the site using a Dutch auger and these cores have been radiocarbon dated. Organic deposition began in the early to mid Holocene at each of the core sites (</= 7150 +/- 50 radiocarbon years before present). An additional vibracore sample from the site shows alluvium below the organic material and the age of this unit remains unknown. Organic carbon percentages and C/N ratios are similar down-core at the different core sites suggesting a similar site history at each location. Sediment grain size data and organic biomarker data (using n-alkanes) have been collected as well and I interpret these records in the context of environmental change. This research will ultimately help to fill a long-standing gap in the paleoenvironmental record of North America since peat accumulating wetlands are rare in the Southern Appalachians. The research also demonstrates how organic biomarker compounds can be used in this type of wetland setting to study past environmental change.

1.2) Development and characterization of microsatellites markers for *Actaea racemosa* L. (black cohosh, Ranunculaceae)

Sarah J. Pate, Jason A. Clement, Joe-Ann H. McCoy, Stacey L. Lance and Katherine G. Mathews
Department of Biology and Department of Chemistry & Physics, Western Carolina University, and the North Carolina Arboretum

Actaea racemosa (black cohosh) is one of the top ten selling herbal drugs internationally and is used to treat menopausal symptoms. It is distributed throughout the Appalachian range in the U.S. and Canada and west into the Ozarks. Due to extensive wild harvesting of plant rhizomes, the sustainability of this species in the wild is of concern. Development of a regional cultivar could provide an alternative to wild harvesting and allow for selection of desirable traits. In order to begin this process, we developed eight polymorphic microsatellite markers in *A. racemosa* to analyze population genetic structure, provide a genetic context for studies of phytochemical variation, and identify potential outlier loci that may be informative. We screened 75 individuals from 16 original collection localities that are maintained in a common garden at Bent Creek Germplasm Repository in Asheville, NC. All loci used were polymorphic with at least three alleles per locus, and exhibited high levels of heterozygosity. These results are typical of long-lived, outcrossing perennials. Genetic structure analyses also demonstrated high admixture among populations and little geographic structuring, indicating widespread gene flow. Accessions showing peak growth and high phytochemical production may be selected for cultivar development. Most of our microsatellite loci cross-amplified in the related southeastern U.S. species *A. pachypoda*, *A. podocarpa* and *A. rubra*, indicating the broader utility of these markers in the genus. We also identified multiple single nucleotide polymorphisms in the internal transcribed spacer (ITS) region of nrDNA among *A. racemosa*, other eastern U.S. *Actaea* species, and Asian *Actaea* species, which could be used to identify common adulterants in medicinal preparations of black cohosh.

2.1) Goldenseal (*Hydrastis candensis*) against Methicillin-Resistant *Staphylococcus aureus* (MRSA): Layers of Synergy

Nadja B. Cech
Department of Chemistry and Biochemistry, University of North Carolina Greensboro

In the US alone, the drug resistant pathogen methicillin-resistant *Staphylococcus aureus* (MRSA) kills more people each year than does HIV/AIDS, and there is a pressing need to devise new strategies to combat bacterial infections. One such strategy is the use of multi-component treatments that target the pathogen via different mechanisms. Our recently published results show that the medicinal plant goldenseal (*Hydrastis canadensis*) has promise as such a treatment. *H. canadensis* has long been known to contain the antimicrobial alkaloid berberine, but we have recently identified several flavonoids that synergistically enhance the antimicrobial activity of berberine. These flavonoids act as efflux pump inhibitors, causing the alkaloids to build up inside bacterial cells. As such, extracts from *H. canadensis* are more effective against MRSA than are its individual constituents in isolation. Perhaps even more interesting, we have recently demonstrated that the synergistic activity of goldenseal extends beyond its antimicrobial activity. *H. canadensis* possesses unidentified constituents that act against bacteria via an entirely different mechanism, quorum quenching. These constituents inhibit toxin production by the bacteria, reducing its virulence. Collectively, our data show that a botanical extract can target a pathogen (MRSA) with multiple diverse constituents acting through varied biological pathways. We are currently involved in several projects to capitalize on these findings, which include investigations of the role of endophytes in the activity of *H. canadensis* (with PI Nicholas Oberlies), and a collaborative SBIR project (with PI Randy Beavers) to develop fully-characterized *H. canadensis* extracts for use in clinical trials.

2.2) Fungal Blood: Mycology and Natural Products Chemistry of Endophytes from Medicinal Herbs

Mario Figueroa, N.H. Oberlies, N.B. Cech, H. Raja, & S. Faeth
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro

For over a decade, the Oberlies Research Group has been studying the chemistry of the extract 'silymarin,' which is produced from milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)] and is used traditionally for hepatoprotective properties. Recently, we initiated studies on the endophytic fungi of medicinal herbs, i.e. fungi that live asymptomatically within the tissues, in order to probe how the chemistry of such fungi may influence the chemistry (and perhaps, biological activity) of the medicinal herb. A prime example emerged with an endophytic *Penicillium* sp. (termed 'G85'), which was isolated from surface sterilized stems of milk thistle into axenic culture. Pure cultures of this organism produced a red exudate (also known as a 'guttation') on Potato Dextrose Agar emended with antibiotics. Chemical analysis of the guttate, as well as the organic extract of a solid phase culture of this fungus, using a combination of UPLC-HRMS and NMR techniques, revealed the presence of a series of structurally related anthraquinone derivatives. Four known compounds, emodin, emodic acid, ω-hydroxyemodin, and isorhodoptilometrin, along with four new derivatives were isolated. Ongoing studies are expanding this research into other endophytic fungi from milk thistle, as well as endophytes from a suite of other medicinally relevant plants.

2.3) An RNA interference for virulence factors in *Cryptococcus neoformans*

Indrani Bose & Tamara Doering
Department of Biology, Western Carolina University

Cryptococcus neoformans is a basidiomycetous yeast found ubiquitously in nature. It is an opportunistic pathogen that causes cryptococcosis, sometimes leading to a fatal meningoencephalitis in immunocompromised patients. It is one of only a handful of fungi that can proliferate and cause systemic infections in mammalian hosts. The ability to grow at the high body temperature of warm-blooded hosts is critical for its ability to cause disease, and the genes responsible necessary for its virulence. To identify genes required 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, 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. 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.

4.1) Biodiversity Inventories and Forest Fragmentation: Can Mosquitoes Suck Out the Data for Us?

Patricia A. Foley, Ron Davis, & Brian Byrd
Forensic Science Program, Department of Geosciences and Natural Resources, and Environmental Health Program, Western Carolina University

Forest fragmentation dramatically alters the structure and function of natural ecosystems affecting physical conditions and reducing the quantity and quality of habitat for native species. Fragmentation is a leading threat to wildlife populations and biodiversity worldwide, but can also impact human health by increasing transmission of vector-borne infectious diseases such as La Crosse encephalitis which is highly endemic in this region. As interior forest conditions are reduced, the abundance of edge-adapted wildlife species increases and mosquito feeding will shift accordingly. Because edge species are much more tolerant and abundant around human habitation, the potential for transmission of diseases like La Crosse encephalitis increases. Despite extensive housing development in this region, little is actually known about how development impacts wildlife diversity and community dynamics. This is due to the elusive nature of wildlife species and the logistic challenges associated with invasive traditional wildlife sampling methods. This study, proposes the novel idea of using blood-engorged mosquitoes and bloodmeal DNA analysis as a non-invasive tool for sampling wildlife species composition within fragmented and non-fragmented forest habitats in Jackson County, NC.

4.2) Development of a Novel Human Mitochondrial DNA (mtDNA) Amplification Method for use with Illumina® Next-Generation Sequencing Instrumentation

Brittania J. Bintz, B.C. Smith, E.S. Burnside, H. Stawski, G.B. Dixon, & M.R. Wilson
Department of Chemistry & Physics and Forensic Science Program, Western Carolina University

Challenging forensic DNA samples including bones and hair often contain DNA that is degraded and/or is present in very low concentrations. Mitochondrial DNA (mtDNA) analysis is often utilized on these sample types. Studies employing newly emerging DNA sequencing technologies have been designed to interrogate targets down to the single molecule level. While these technologies are capable of producing large quantities of usable sequencing data, they are laborious and peripheral instrumentation can be costly. For example, typical library preparation for the Illumina® GAIIx platform includes DNA fragmentation (often using an expensive Covaris® mechanical DNA shearing instrument), end repair and addition of adenine overhang, adapter ligation and DNA size selection and purification. We have developed a novel method, using forensically relevant sample types, for human mtDNA amplicon generation for single-read DNA sequencing on the Illumina® GAIIx. This method includes traditional PCR amplification of target DNA with Roche FastStart™ high-fidelity enzyme blend with 3’ → 5’ exonuclease proofreading ability. A high-fidelity enzyme was chosen in order to reduce misincorporation of bases during amplification, which may have an impact on NGS sequence data downstream. Adapters and multiplexing indices are included on the 5’ end of the mtDNA hypervariable (HV) region-specific primers, and are incorporated into the amplicon during PCR. Multiplexing indices are typically sequenced separately from the target amplicons and require a Paired-End Module fluidics system for delivery of a multiplexing index specific primer. We have designed our amplification primers with the multiplexing indices directly 5’ of the target specific primer sequence. The resulting sequences can be parsed by index using a custom bioinformatics software that contains an index parsing algorithm. We have shown that this amplification strategy produces higher concentrations of amplicons than the current strategy used in forensic

laboratories. Further, we have shown that these amplicons can also be sequenced using dideoxy terminator methods, without any apparent hindrance from the extended primer sequences. Thus, this method enables forensic laboratories to adopt one mtDNA amplification protocol for multiple downstream sequencing analyses. Additionally, this library preparation proves to be more efficient, and more cost effective than methods recommended by Illumina®. We have recently started developing sample preparation methods for whole mtDNA analysis. Two novel primer sets were designed to anneal to highly conserved regions of the mtDNA genome. These primer sets amplify overlapping long targets of 8.9 and 10.7 kb in length. Amplification methods include the use of TaKaRa™ LA Taq high-fidelity enzyme, which enables amplification of targets up to 20 kb in length. We have identified two new commercially available kits for enzymatic fragmentation and tagging of our amplicons for rapid NGS library preparation including the NEBNext® dsDNA fragmentase and the Illumina® Nextera™ Tagmentation kit. Preliminary data shows our whole mtDNA genome sample preparation approach to be successful.

4.3) Quantification of minor variants in human mitochondrial DNA amplicon libraries using 454 pyrosequencing

Groves Dixon & M.R. Wilson
Forensic Science Program, Western Carolina University

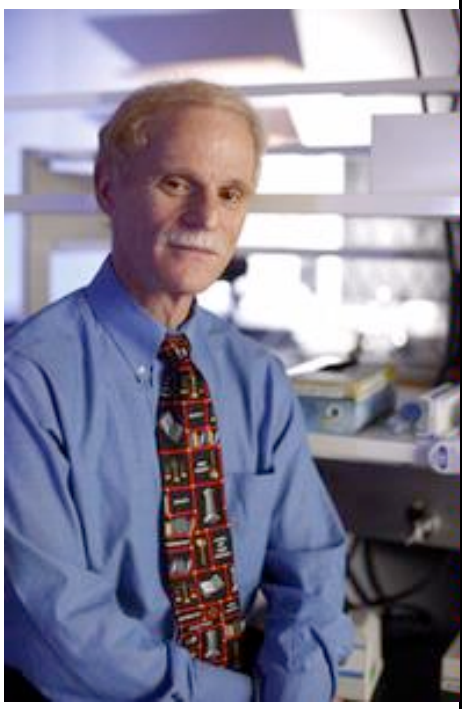
We extracted DNA from hair, blood and buccal samples from twenty individuals. From these, we generated modified amplicon libraries for use on the Roche GS Junior. Reference sequences for mtDNA hypervariable (HV) regions were obtained for each donor with Sanger sequencing using these modified libraries. Mixtures of mtDNA HV amplicons were prepared in ratios of 95% / 5%, 98% / 2%, and 99% / 1%. We sequenced these mixtures using 454 pyrosequencing technologies to assess the ability of the Roche GS Junior instrument to accurately detect minor variants of mixed mtDNA. We showed that modified amplicon libraries for an MPS platform can be sequenced using traditional Sanger sequencing. This protocol allows for selective use of a sequencing method based on the quality of the sample. Straightforward exclusions can be interpreted directly from Sanger sequence data, however in cases where Sanger sequence data provides insufficient resolution for confident interpretation, the analyst can return to the same original amplified library for MPS. Using the Roche GS Junior, we were able to detect low level variants at mixture ratios of 99% / 1%. Thus, we demonstrated the capability of the Roche GS Junior to detect low-level variants. Additionally, we optimized a protocol that allows seamless inclusion of the technology into forensic crime laboratories using current mtDNA testing methodology.

Dr. Laura Georgi received a Ph.D. in Plant Pathology in 1986 from Cornell University, where she studied virus transmission by plant-parasitic nematodes. From 1987 to 1991, she was a post-doctoral fellow at the University of Missouri-Columbia. As a recipient of an Individual National Research Service Award, she investigated the genetic control of arrested development in the nematode *Caenorhabditis elegans*, cloning and sequencing a gene in the signaling pathway and investigating its tissue-specific expression by transforming nematodes with promoter-reporter gene constructs. Between 1992 and 2009 she was employed by Clemson University, where her research focus shifted from nematodes to plants, in particular peach. She contributed to the development of the genomic tools for modern genetic analysis of the Rosaceae and Fagaceae culminating in the sequencing of the peach genome, notably constructing bacterial artificial chromosome libraries for peach and European plum (Rosaceae) and Chinese chestnut (Fagaceae). From 2009 to 2011 she worked at Rutgers University mapping fruit-rot resistance in cranberry and investigating a gene involved in the biosynthesis of cranberry's red anthocyanin pigments. Last fall she accepted an invitation from the American Chestnut Foundation to resume research on chestnut as a part of the Forest Health Initiative project applying biotechnology to the development of disease-resistant American chestnuts for forest restoration. A career bench scientist, her name appears on 22 original research papers, 10 as first author, and two reviews.

New tools for a monumental task: application of biotechnology to restoration of the American chestnut

Dr. Laura L. Georgi
The American Chestnut Foundation

Abstract: The American chestnut, *Castanea dentata*, formerly dominated the Appalachian forests. It was a very large, fast-growing tree useful for timber and for nuts enjoyed by wildlife as well as man and domesticated livestock. Around the beginning of the twentieth century, the trees started to die of a blight. They were infected by fungus, *Cryphonectria parasitica*, unintentionally introduced from Asia. Within five decades, this introduced pathogen spread throughout the tree's natural range, reducing it from a dominant canopy tree to an insignificant understory sprout that rarely grows large enough to reproduce. Over the years, many have labored to restore the species to health, using new tools as they became available. These include conventional breeding: crossing blight-susceptible American chestnuts with Asian chestnut species, which show resistance to the fungus, followed by repeated crosses to American trees and selecting for blight resistance at each generation, to produce trees that are genetically predominantly American but with blight resistance from the Asian species. Another toolset is a collection of viruses first discovered several decades ago that infect the blight fungus and reduce its pathogenicity. More recently still, biotechnological tools have made whole genome sequences accessible, and advances in genetic engineering provide a means to exploit the flood of sequence information. I shall review the status of the various approaches and discuss how each assists the others and contributes to the restoration of the American chestnut as a forest tree species.



Dr. Bruce Budowle received a Ph.D. in Genetics in 1979 from Virginia Polytechnic Institute and State University. From 1979-1982, Dr. Budowle was a postdoctoral fellow at the University of Alabama at Birmingham. Working under a National Cancer Institute fellowship, he carried out research predominately on genetic risk factors for such diseases as insulin dependent diabetes mellitus, melanoma, and acute lymphocytic leukemia. From 1983-2009, Dr. Budowle was employed at the FBI Laboratory Division and carried out research, development, and validation of methods for forensic biological analyses. Dr. Budowle has worked on laying some of the foundations for the current statistical analyses in forensic biology and defining the parameters of relevant population groups. He has published more than 485 articles, made more than 550 presentations, and testified in well over 200 criminal cases in the areas of molecular biology, population genetics, statistics, quality assurance, and forensic biology. He has been a chair and member of the Scientific Working Group on DNA Methods, Chair of the DNA Commission of the ISFG, and a member of the DNA Advisory Board. He was one of the architects of the CODIS National DNA database, which maintains DNA profiles from convicted felons, from evidence in unsolved cases, and from missing persons.

Some of Dr. Budowle's efforts over the last decade are in counter terrorism, primarily in identification of victims from mass disasters and in efforts involving microbial forensics and bioterrorism. Dr. Budowle was an advisor to New York State in the effort to identify the victims from the WTC attack. In the area of microbial forensics, Dr. Budowle has been the chair of the Scientific Working Group on Microbial Genetics and Forensics, whose mission was to set QA guidelines, develop criteria for biologic and user databases, set criteria for a National Repository, and develop forensic genomic applications. In 2009 Dr. Budowle became Executive Director of the Institute of Applied Genetics and Professor in the Department of Forensic and Investigative Genetics at the University of North Texas Health Science Center at Fort Worth, Texas. His current efforts focus on the areas of human forensic identification, microbial forensics, and emerging infectious disease.

Forensic genetics and molecular methods for assessing biodiversity: A look at the past, present, and future

Dr. Bruce Budowle
University of North Texas Health Science Center

Poster presentations

1	Caleb Beck	Primer design for mosquito blood meal project
2	Beth Budden	Characterization of putative acetate kinase in the pathogenic yeast, *Cryptococcus neoformans*
3	Jason Clement	Variation of triterpenoid saponin concentrations in Black Cohosh (*Actaea racemosa* L.) from across its native range
4	Groves Dixon	Clonal dynamics and decline of trembling aspen *Populus tremuloides* (Michx.) in the Kaibab National Forest, Arizona
5	Jordan Estes	Echinacea down regulates LPS-induced expression of pro-inflammatory factors in the cervix
6	Kendall Fuller	Comparison of bacterial communities in living and dead Eastern Hemlock (*Tsuga canadensis*)
7	Thomas S.K. Gilbert	Mapping regions of heat shock protein-90 necessary for Interaction with G{alpha}12
8	Niki Justice	Microbe hunting in Great Smoky Mountains National Park and the search for *Pseudomonas aeruginosa*
9	Ashley Lefler	A comprehensive assessment of the mixture analysis and deconvolution applications of popular human STR mixture analysis software packages
10	Ron Michaelis	Genetic influences on aggression and territory selection In Song Sparrows
11	Jessica Moore	Characterization of the *mdm1* gene in zebrafish
12	Bao-Tran Nguyen	Echinacea down-regulates expression of pro-inflammatory factors in the cervix of preterm labor mice model
13	Takako Ohashi	Characterization of vascular endothelial growth factor and receptors in the cervix of non-pregnant women
14	Carol Petricevic	Differing stratification requirements between garlic mustard populations found along the invasion route
15	Megan Rayfield	Genetic diversity in five wild populations of American ginseng (*Panax quinquefolius* L.) in Western North Carolina
16	David Russell	Evaluation of Qiagen's Investigator® Quantiplex HYres Quantification Kit
17	John Schwabe	Proteomics analysis of cervical remodeling during early and late pregnancy in mice
18	Ryan Simmons	Investigating the role of CnMtw1 in kinetochore function through the use of conditional mutants

19	Brandon Smith	Low-level variant detection in mitochondrial DNA using the Illumina® GA IIx next-generation sequencing (NGS) platform
20	Robert Stanley	Expression profile of vascular endothelial growth factor [VEGF] and its receptors in the postpartum cervix of mice
21	Hilde Stawski	Preparing sequencing libraries of human mitochondrial DNA using Illumina™ Nextera™ and NEBNext® dsDNA Fragmentase® technology for massively parallel sequencing
22	Kathy Turnbull	Analysis of some *Cryptococcus* isolates from India

Abstracts (posters)

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1. Primer Design for Mosquito Blood Meal Project

Caleb Beck & Patricia A. Foley
Department of Biology and Forensic Science Program, Western Carolina University

Can a single set of primers amplify a conserved region in the mitochondrial DNA of various taxa? My research attempted to answer this question by extracting DNA from tissue samples, amplifying that DNA with a specific set of primers and sequencing the amplicons to verify the results. This will be foundational work for a larger study which involves identifying blood meals from mosquitoes. It will test the efficiency of the primers to amplify DNA of different taxa, determine optimal amplification and sequencing conditions and establish a procedure for the identification of a blood meal.

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2. Characterization of putative acetate kinase in the pathogenic yeast, *Cryptococcus neoformans*

Beth A. Budden and I. Bose
Department of Biology, Western Carolina University

The spherical, encapsulated basidiomycetous yeast, *Cryptococcus neoformans*, is an environmental opportunistic pathogen that has become a leading cause of mortality secondary to HIV/AIDS, particularly in sub-Saharan Africa [Park et al. 2009]. Few chemotherapeutic agents are currently available to treat cryptococcosis, and growing concerns over resistance to some of these medications have emphasized the need for alternative treatments. By obtaining a thorough understanding of the metabolic pathways involved in the survival and pathogenicity of this organism it is hoped that pathways and/or proteins unique to the organism can be used as potential targets for chemotherapeutic agents [Casadevall and Perfect 1998]. A homolog of an enzyme previously thought to exist only in bacteria, acetate kinase, has been identified in certain lower eukaryotes including *C. neoformans*. To date, an acetate kinase homolog has not been found in higher eukaryotic organisms. To get a better understanding of acetate kinase's role in *C. neoformans*, the *ACK1* gene was deleted, and the *ack1Δ* strains are being studied for phenotypic effect and function. In addition to understanding its role in physiologic processes of the cell, an *ACK1-mCherry* construct has been made to tag the enzyme with the red fluorescent protein, mCherry, to study its subcellular localization. This tag will also allow purification of the endogenous protein for enzyme assays as well as for identification of possible interacting proteins.

Casadevall, A., Perfect, J.R. *Cryptococcus* neoformans. (1998) Cryptococcus neoformans. Washington, DC. American Society for Microbiology.

Park, Benjamin J; Wannemuehler, Kathleen A; Marston, Barbara J; Govender, Nelesh; Pappas, Peter G; Chiller, Tom M. (2009). *AIDS*. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. **23**(4) p 525-530.

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3. Variation of Triterpenoid Saponin Concentrations in Black Cohosh (*Actaea racemosa* L.) from across its Native Range

Jason A. Clement, P.M. Looney, K.G. Mathews, & J.H. McCoy
Department of Chemistry & Physics and Department of Biology, Western Carolina University, and the North Carolina Arboretum

Black cohosh (*Actaea racemosa* L.) is a medicinal herb used for the treatment of menopausal symptoms. The natural supply of this herb is in danger due to overharvesting of the wild source material. Thus, it is desirable to develop a cultivar of the plant with superior properties that may be grown in western North Carolina. Much of the interest in black cohosh has focused on the putative biological activity of triterpenoid saponins found in the plant, such as actein. We have recently completed a study of a black cohosh collection at the Bent Creek Germplasm Repository at the North Carolina Arboretum. The plants represent collections from several states across the native range of the plant, all currently growing at the Arboretum. We have found significant differences in saponin concentrations between plants from different states. This data will assist in the selection of plants for beginning a breeding program.

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4. Clonal dynamics and decline of trembling aspen *Populus tremuloides* (Michx.) in the Kaibab National Forest, Arizona

Groves Dixon & Laura DeWald
Department of Biology, Western Carolina University

Sudden aspen decline is distinguished from the typical gradual successional decline by rapid reduction of aspen coverage associated with high rates of crown dieback and stem mortality incurred from a combination of environmental stress factors (Worrall 2008). Adding to the complexity of sudden aspen decline is that single genets (genetically distinct individuals) that can comprise entire groves of ramets (physiologically distinct stems) might have varied degrees of resilience or susceptibility to different stresses. To improve our understanding of aspen clonal dynamics as it might relate to aspen decline, leaf and cambial tissues were collected from 15 sites within aspen groves in the Kaibab National Forest, AZ. Five microsatellite loci were used to determine the number of distinct genets and this information was used to infer the relative frequency of sexual reproduction at these sites. Preliminary results indicate the presence of 19 distinct genets among the 15 sites. This presentation will discuss the relationship(s) between the molecular data with previously collected data on the health (degree of crown dieback and percent stem mortality) and environmental conditions (elevation, slope, and aspect) of each site. Evidence for disparate health responses between genets that share similar environmental conditions will also be discussed.

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5. Echinacea down regulates LPS-induced expression of pro-inflammatory factors in the cervix

Jordan A. Estes, SM Donnelly, RL Stanley, & CN Mowa
Department of Biology, Appalachian State University

Inflammation-induced preterm labor accounts for 40-50% of preterm labor. Current therapies although effective may be harmful to the fetus and/or the mother. Thus, there is need to explore other remedies that are not only effective but safe to both the fetus and the mother. Echinacea has been effectively and safely used to treat infection-related illnesses for many years, and its anti-inflammatory activities are well documented. Here we test its effectiveness in attenuating expression of classical pro-inflammatory factors in the cervix. Using cervices from non-pregnant ovariectomized mice treated in ex vivo [RPMI 1640 media supplemented with 10% Fetal Bovine Serum (FBS)], we examined Echinacea's ability to down-regulate lipopolysaccharide (LPS)-induced expression of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) in a dose dependent manner [low

(0.01 mg/well), medium (0.1 mg/well), and high (1 mg/well)]. Tissues were evaluated using real time-PCR, histology and confocal immunofluorescence. Gene expression studies revealed that the highest dose of Echinacea (1 mg/well) showed the most dramatic decrease in IL-6, while histological analysis using Hematoxylin and Eosin (H&E), as well as confocal immunofluorescence of TNFα confirmed the results of the gene expression studies. We conclude that Echinacea could potentially be used in modulating inflammation-induced cervical remodeling in preterm labor.

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6. Comparison of bacterial communities in living and dead Eastern Hemlock (*Tsuga canadensis*)

Kendall L. Fuller & S.P. O'Connell
Department of Biology, Western Carolina University

The purpose of this study was to identify bacterial species associated with the rhizosphere of living and dead hemlocks (those that have been likely killed by adelgid infestation). Samples were collected from Albright Grove, Great Smoky Mountain National Park (GSMNP), in early February 2011 from the soil attached to hemlock roots from six trees, consisting of paired live/dead trees that were found side by side. Species richness and evenness within the samples were evaluated. Variation based on time of year and over time was also assessed for hemlock rhizospheres. Some of the bacteria detected in this work were unidentified at the phylum level and are likely new taxa to science. Overall, *Acidobacteria* was the dominant phylum making up 63% of all samples, followed by *Proteobacteria* at 23%, other phyla were represented at levels ≤6%. Results indicated significant differences in the composition at the phylum level of Live 2011 and Dead 2011 samples. Other significant differences were found at lower levels of classification (e.g., genus and species) between all six comparisons of the four sample sets. The association of microbial communities with living hemlocks is important. If hemlocks cease to exist in the GSMNP because of the infestation of the HWA, then unidentified microorganisms that may be plant specific to hemlock may become extinct as well. If reforestation efforts were ever to take place for Eastern Hemlock in GSMNP, microbial communities associated with healthy trees could be vital in the success of this effort.

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7. Mapping Regions of Heat Shock Protein-90 Necessary for Interaction with G{alpha}12

Thomas S.K. Gilbert & T.E. Meigs
Department of Biology, University of North Carolina Asheville

The heterotrimeric GTP-binding protein G{alpha}12 plays an important role in cellular signaling by mediating a range of cellular responses that include cytoskeletal rearrangements, cell growth, and migration. Numerous studies have identified G{alpha}12 as a potent transforming oncoprotein when overexpressed or mutationally activated, and recent reports have implicated this protein in metastatic invasion. The chaperone protein Heat shock protein-90 (Hsp90) has also been identified as a potential oncoprotein as well as a target for G{alpha}12 binding. Previous work in our lab has revealed several key amino acids of G{alpha}12 necessary for Hsp90 binding. Our current set of experiments identifies regions of Hsp90 necessary for interaction with G{alpha}12. Co-precipitation assays measuring G{alpha}12 binding to the fruit fly homolog of Hsp90, termed Hsp83, suggested three evolutionarily conserved regions of human Hsp90 as potential G{alpha}12 binding sites. These regions were deleted from the coding sequence of Hsp90, and a series of protein-protein interaction assays were performed to assess whether each of the three regions individually were involved in G{alpha}12 binding. Removal of a specific group of C-terminal amino acids significantly reduced Hsp90 binding to G{alpha}12. Experiments are in progress to examine the effects of C-terminally modified Hsp90 on G{alpha}12 signaling function in cultured cells.

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8. Microbe hunting in Great Smoky Mountains National Park and the search for *Pseudomonas aeruginosa*

Sarah "Niki" Justice & SP O'Connell
Department of Biology, Western Carolina University

The data presented here reflect the results of the 16S rDNA surveys from 10 years of sampling in Great Smoky Mountains National Park, representing 288 bacterial sequences. In addition, a focused effort has been undertaken to isolate *Pseudomonas aeruginosa*. Five phyla, 12 classes, 15 orders, 32 families, and 66 genera have been detected while another 24 sequences have been found to have no close relatives to described bacteria. Water samples have produced 27 genera not observed from soil while soil diversity has shown 25 unique genera. Seventeen genera have occurred in both soil and water. Common genera from water included *Janthinobacterium* and *Rhodoferax* while soil has been dominated by *Streptomyces* and *Burkholderia*. *Bacillus* and *Flavobacterium* have been commonly found in both soil and water. One surprising result is that out of 17 *Pseudomonas* isolates, none were identified as *P. aeruginosa*, and furthermore, directed selective culturing also has not detected this species. Ongoing work is being undertaken to directly detect *P. aeruginosa* in soil and water samples via PCR-based methods. Novel bacterial species have been recovered from GSMNP, including some that may represent new families or higher taxonomic levels. These microbial species may include some with unusual chemical capabilities and metabolic byproducts of interest to science and industry and further work is warranted. It is interesting that a species that has been reported in the literature to be quite numerous in soil and water has thus far escaped our detection.

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9. A Comprehensive Assessment of the Mixture Analysis and Deconvolution Applications of Popular Human STR Mixture Analysis Software Packages

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Recent advancements in technology have enabled forensic DNA analysts to generate and interpret data from samples that wouldn't historically be typable. As a result, a major obstacle being faced today by crime laboratories is the interpretation of STR data from mixed samples. Many complications arise from mixture data that makes accurate and reliable interpretation of these samples difficult and time consuming. Some vendors are including mixture analysis and deconvolution applications within their software programs to ease some of the difficulties associated with STR mixture analysis. Use of such software packages are purported to be of great value to a forensic laboratory. However, before any new or modified software package can be implemented, an extensive validation study should be performed to assess the true accuracy and reliability of the software. This research involves the analysis of complex mixtures utilizing two popular software packages that contain STR mixture analysis and deconvolution applications. The programs have been assessed on their ability to handle interpretational complications typically associated with real-world mixture samples. A comprehensive feasibility outline will be presented that covers the precision, accuracy, and reliability of each program. The two programs that have been reviewed include GeneMarker® HID by SoftGenetics® and GeneMapper® ID-X by Applied Biosystems®.

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10. Genetic Influences On Aggression And Territory Selection In Song Sparrows

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Variations in gene sequences influence the level of expression of multifactorial traits such as aggression. They may also influence an individual's ability to adapt to a specific environment. We have obtained aggression data and DNA samples from 78 song sparrows, some of which live in rural habitats away from people, and some of which live in proximity to human habitats. We are analyzing the sequence of the D4 type dopamine receptor (DRD4) gene, in an effort to determine whether any specific variants in the DRD4 sequence are associated with high or low levels of aggression in our birds. In addi-

tion, we will determine whether any specific DRD4 sequences are found more often in urban versus rural birds.

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11. Characterization of the *mdm1* gene in zebrafish

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The fidelity of DNA replication in combination with the DNA repair and cell cycle machinery functions amazingly well to prevent the accumulation of mutations and chromosomal rearrangements that can contribute to cancer. Despite these mechanisms, genomic changes do occur in cells over time, leading to the activation of the p53 pathway—which drives the cell into apoptosis so it can do no further damage to the organism. Thus the p53 tumor suppressor protein and other interacting gene products are critical for the maintenance of genomic stability. The p53 tumor suppressor protein is primarily regulated by the Mdm2 protein, which functions as an ubiquitin E3 ligase to promote p53 degradation. The *mdm2* gene was originally cloned from the transformed 3T3DM murine cell line along with *mdm1* and *mdm3*. Since *mdm1* appears to be overamplified in transformed cells, we hypothesized that it may play a role in altered growth like the other Mdm proteins; Mdm2, Mdm4 and MdmX. Interestingly, the *mdm1* gene is located within a region of known genomic instability activity in zebrafish. The goal of this project was to characterize the expression of the *mdm1* gene in zebrafish embryos using semi-quantitative RT-PCR, anti-sense morpholino gene knockdowns, RNA rescue, overexpression analysis, whole-mount *in situ* hybridizations and western blots. The results of this work suggest that the primary role of *mdm1* is in the development of the eye and portions of the central nervous system, with no obvious involvement in the regulation of the p53 tumor suppressor gene.

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12. Echinacea down-regulates expression of pro-inflammatory factors in the cervix of preterm labor mice model

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Preterm labor is a prevalent obstetrical problem worldwide. It affects approximately 1 in 8 births in the United States and accounts for 75% of infant mortality. Causally, half of these premature births are known to result from infection, which is associated with inflammation. Echinacea is a well-known anti-bacterial and anti-inflammatory agent that has been used safely for thousands of years. Here, we test whether this medicinal plant extract could be used to modulate infection-induced expression of pro-inflammatory cytokines associated with preterm labor, such as inter-leukin-6 (IL-6) in the cervices of mice. Nine mice (n=3) were divided into 3 treatment groups: a) negative control (vehicle only), b) positive control (lipopolysaccharide; LPS), and c) treatment group (three doses of Echinacea prior to single LPS administration). The cervices were harvested and analyzed using real-time PCR. Results showed that mice pre-treated with Echinacea had levels of inflammation 925-fold lower than those of positive control. We conclude that Echinacea could potentially be used to modulate infection-induced preterm labor.

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13. Characterization of Vascular Endothelial Growth Factor and Receptors in the Cervix of Non-pregnant Women

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Vascular changes such as vasodilation, angiogenesis and permeability are prominent in the cervix during pregnancy. However, the significance of these vascular changes to cervical remodeling is unclear. Our lab has previously characterized the expression of vascular endothelial growth factor (VEGF), its

two main receptors, KDR and Flt-1, and their likely role in cervical remodeling using rodent animal models (Mowa et al., 2004a,b; 2008). More recently, we have begun to characterize the expression profile of these vascular molecules in human cervix in collaboration with Sheffield University Medical School, in order to compare our data in rodents with human, and ultimately understand their potential role in normal and dysfunctional cervical remodeling. Here, we report the basic histology of cervix, presence and expression profile of VEGF and its receptors, KDR and Flt-1, in cervices of non-pregnant women using H&E staining and confocal immunofluorescence, respectively. VEGF was mostly moderately expressed in fibroblast and epithelial cells. Both receptors were also expressed in some cells but only sparsely. We currently are examining the mRNA and protein expression of these molecules using real time PCR and Western blot analysis, respectively. Future studies will examine the expression of these molecules in pregnant women.

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14. Differing stratification requirements between garlic mustard populations found along the invasion route

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Originally from Eurasia, garlic mustard, *Allialaria petiolata* (Brassicaceae), was observed in Long Island, NY in the mid 1800s. The plant has spread throughout eastern North America, from the Great Lakes region south to the Southern Appalachians and adjacent Piedmont. Plants and seeds from populations along garlic mustard's historic invasion route (NY, OH, NC) were compared to test whether this invasive plant adapted to the local environment as it spread south or if it is generally phenotypically plastic. To test germination response, seeds from 5 populations were stratified for 66, 80, or 100 days. After 80 days, germination differed among the populations, but not in relation to latitude: populations with the highest (15%) and lowest germination (3%) were both from NY. Germination also differed among stratification treatments; seeds stratified for 66 days had < 1% germination while those remaining in the cold treatment had 10-11% germination. Germination was highest after 100 days, as was expected.

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15. Genetic Diversity in Five Wild Populations of American ginseng (*Panax quinquefolius* L.) in Western North Carolina

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Wild American ginseng (*Panax quinquefolius* L.), which grows across the Eastern United States, has been harvested and exported to Asia for over two hundred years for use as a stimulant in Eastern medicinal preparations. Because the biologically active compounds, ginsenosides, are most concentrated in the roots, collection for export requires the removal of the entire plant, which has the potential for negative genetic consequences such as a reduction in allelic diversity or an increase in inbreeding. 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 American ginseng individuals in five protected Western North Carolina populations. Leaflets from 158 individuals were collected, total genomic DNA was extracted, and samples were PCR-amplified with 3 different primer sets. Data 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 research aims to bring insight to the level of genetic diversity in wild populations of American ginseng and highlights the possible need to strengthen harvesting guidelines in order to protect the future of this federally protected plant.

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16. Evaluation of Qiagen's Investigator® Quantiplex HYres Quantification Kit

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The Qiagen Investigator® Quantiplex HYres Kit is designed to quantify the total human and male DNA in a sample. As part of a field test I examined the kits ability to quantify male and female contributors in mixed samples, as well as tested the reproducibility and precession of the kit. Results of this field test indicate that the Investigator® Quantiplex Kit is highly reproducible and precise, and has the ability to detect the male component DNA in samples that contain a high concentration of female DNA. The ability to quantify the male and female DNA component individually becomes crucial in forensic DNA analysis in situations where the sample quantity is a limited mixed stain and a decision on the best practice for obtaining a result must be determined.

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17. Proteomics analysis of cervical remodeling during early and late pregnancy in mice

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Parturition is dependent on complex changes in the composition and strength of the cervix via cervical remodeling. Changes in the rate of production and degradation of collagen, as well as changes in its extracellular organization, have been identified in late pregnancy and implicated in the changing integrity of the cervix. Understanding these highly complex changes is central to understanding the processes that control cervical remodeling using techniques that explore the genome-wide expression of proteins. No studies, thus far, have examined the full breadth of the signature proteins in the remodeling cervix during early and late pregnancy compared to baseline, i.e., non-pregnant. The purpose of this study is to profile the patterns of protein expression in the cervices of non-pregnant and pregnant mice to elucidate changes in major biological themes (signature proteins) that may be relevant to the mechanism of cervical remodeling and parturition. Mouse cervices from day 0 day 11, and day 17 of pregnancy were analyzed by genome-wide proteomics analysis and Western blot were performed to verify proteomics data. ANOVA analysis of the proteomics data yielded 73 variably expressed proteins. All of the collagen related proteins were present in their highest concentrations during pregnancy, suggesting their involvement in changes in the ECM preceeding birth. Of note, we saw that biglycan and lumican, proteins that control the organization and spacing of collagen fibrils, are up regulated during late pregnancy. The actions of these SLRP's may play an important role in cervical remodeling. These data are important in that they show significant changes in the protein composition of the cervix during pregnancy that were previously uncharacterized and suggest a complex process of ECM reorganization coupled with a bolstered immune defense and inflammation are responsible for causing natural birth.

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18. Investigating the Role of CnMtw1 in Kinetochore Function through the use of Conditional Mutants

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Cryptococcus neoformans is a basidiomycetous yeast with clinical importance due to its role as an opportunistic pathogen in immunocompromised individuals. Previous experiments in other fungal species have described a protein essential to the structure and function of the yeast kinetochore. The necessity of this protein for kinetochore function was first determined using a *S. pombe* strain carrying a temperature-sensitive mutation in the *Mis12* gene that causes the protein to malfunction above a certain temperature (Goshima et al., 1999), resulting in cells that were unable to complete mitotic segregation, with the dividing nucleus stuck in the neck between budding cells. A later study described the budding yeast homolog *MTW1* (Mis12-like protein) in *S. cerevisiae* using a similar temperature-sensitive mutant and yielded similar results (Goshima and Yanagida, 2000). The goal of this project is to characterize the Mtw1 homolog in *C. neoformans* in order to determine whether it maintains the functional importance to kinetochore structure exhibited by the previously described homologs. Constructs for

conditional mutants such as those used in previous studies have been made, one containing a copper-repressible promoter that decreases the expression of *Mtw1* in the presence of copper and that has already been successfully produced and another that expresses the same functional temperature-sensitive mutation used to study homologs of this protein. These constructs were subsequently transformed into cells via biolistics. Once strains containing these regulatable mutations have been established, cellular responses to restrictive growth conditions will be measured and quantified to determine if Mtw1 plays the same essential role in kinetochore function as has been shown for its homologs.

Goshima G, S Saitoh, M Yanagida. 1999. Proper metaphase spindle length is determined by centromere proteins Mis12 and Mis6 required for faithful chromosome segregation. Genes & Development. 13: 1664 – 1677.

Goshima G, M Yanagida. 2000. Establishing Biorientation Occurs with Precocious Separation of the Sister Kinetochores, but Not the Arms, in the Early Spindle of Budding Yeast. Cell. 100: 619 – 633.

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19. Low-level variant detection in mitochondrial DNA using the Illumina® GA IIx next-generation sequencing (NGS) platform

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Heteroplasmy, a phenomenon in which there are more than one mitochondrial DNA type within an individual, may go unnoticed given the current limits of detection of dideoxy terminator technologies. Using the Illumina® GA IIx we are developing a novel strategy for the sequencing of DNA that can resolve the minor component of a mixed sample down to the 1% level of detection. Our methodology includes the use of PCR primers for mtDNA hypervariable (HV) regions which have been modified to include a 5' adapter and index sequence. The results of this experiment will be a useful guide for labs wanting to develop novel sequencing protocols that optimize throughput using this burgeoning technology.

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20. Expression Profile of Vascular Endothelial Growth Factor [VEGF] and Its Receptors In The Postpartum Cervix of Mice

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Background: The birth canal undergoes pronounced changes prior to term (pre-partum), to enable a timely passage of the fetus, and immediately after birth (post-partum), to heal and repair the "wound" of the post-partum cervix. VEGF is the key regulator of various vascular events and is critical in the wound healing response. Previous results from our lab have characterized the localization, delineated genes related to and effects of VEGF and its corresponding receptors during pre-partum cervical events. The goal of the present study was to examine the expression profile of VEGF and its corresponding receptors in the *post-partum* cervix. Methods: Timed-pregnant (day 10) mice (C57BL6/129SvEv from Charles River) were allowed to undergo normal gestation and labor. Animals (n=3) were then sacrificed postpartum at the following time specific intervals: a) 0hrs, b) 15hrs, and c) 2 days. Cervical tissues were analyzed for presence and quantity of VEGF, and its receptors Flt-1 & KDR using qRT-PCR, Western blot, and immunofluorescence. Results: At 15hrs post-partum the both the gene and protein expression of VEGF and its receptors (Flt-1, and KDR) were found to be elevated compared to both 0hrs and 2days postpartum. Conclusion: VEGF and its receptors Flt-1 & KDR are readily expressed during the first part of post-partum cervical repair. VEGF, Flt-1, and KDR likely play crucial roles in the wound healing response of the post-partum cervix. Studies to elucidate the role VEGF, its receptors, and specific cellular localization in post-partum cervical healing are ongoing.

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21. Preparing Sequencing Libraries of Human Mitochondrial DNA Using Illumina™ Nextera™ and NEBNext® dsDNA Fragmentase® Technology For Massively Parallel Sequencing

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Forensic DNA casework largely relies on the analysis of short tandem repeats from nuclear DNA (nDNA). In some cases, however, nDNA may not be suitable for analysis (i.e. degraded or present in quantities too small to obtain a genotypic profile). In these instances, mitochondrial DNA (mtDNA) is often a good alternative. MtDNA is a circular genome of approximately 16.5 kb, is maternally derived, and is present in 500 – 1000 copies per cell versus two copies of nuclear DNA. The higher copy number allows for a greater probability to recover intact mtDNA in degraded samples. Currently, forensic scientists sequence two hypervariable (HV) regions found in the non-coding control region of the mtGenome1 since sequencing of the entire genome is rather labor-intensive. Additionally, sequencing difficulties of the C-stretch and the identification of heteroplasmy in samples can make base calling difficult when traditional Sanger sequencing methods are used, which can undermine the value of mtDNA in casework. These problems might be addressed by introducing next generation sequencing (NGS) to the crime laboratory. NGS is a high-throughput technique that combines multiple sequencing reactions at a time, giving the ability to sequence whole genomes more rapidly and allows for deeper analysis of the genome for identification of variants. Library preparation is the primary bottleneck in the NGS workflow, since it can be very time consuming. Therefore, the goal of this research is to compare two NGS library preparation methods, Illumina® Nextera™ and New England Biolabs NEBNext® dsDNA Fragmentase®, using buccal cell extracts. Sanger sequencing will be performed to generate whole genome reference sequences for all donors. NGS Sequencing data will be generated using both the Roche GS Junior and the Illumina® GA IIx platforms.

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22. Analysis of some *Cryptococcus* isolates from India

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Cryptococcus is a yeast-like opportunistic fungus, which can infect people with compromised immune systems due to circumstances such as leukemia, AIDS, and organ transplants. Two species within this genus are pathogenic – *C. neoformans*, which is commonly seen throughout the world, and *C. gattii*, which is found mainly in the tropics (Mitchell et al., 1995). Currently, these two species are subdivided into eight different molecular types, VNI-VNIV, and VGI-VGIV (Meyer et al., 2003). This classification is based on restriction fragment length polymorphism (RFLP) analysis and other molecular techniques. To gain an understanding of the epidemiology of *Cryptococcus*, a better analysis of the distribution of the subtypes this fungus is needed. In this project, 50 putative *Cryptococcus* strains from the environment and clinics of Southern India have been collected, and are being studied using physiological tests, such as melanin production and growth at high temperatures, and molecular tests such as the RFLP of two genes, CAP1 and GEF1.

Meyer W, Castaneda A, Jackson S, Huynh M, Castaneda E. 2003. MedlineMolecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg Infect Dis 9:189–95.

Mitchell, T G, and J R Perfect. 1995. Cryptococcosis in the era of AIDS 100 years after the discovery of *Cryptococcus neoformans*. Clin. Microbiol. Rev. 8:515-548.

Molecules in the Mountains Organizing Committee

Cindy Atterholt	Chemistry & Physics	Western Carolina University
Pete Bates	Geosciences & Natural Resources	Western Carolina University
Pattie Foley	Forensic Science	Western Carolina University
Jon Lawrie	Western Office	North Carolina Biotechnology Center
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