SEPTEMBER 25TH | 2017

GEORGIA CENTER FOR CONTINUING EDUCATION

INSTITUTE OF BIOINFORMATICS BI-ANNUAL SYMPOSIUM

PARSING THE MICROBIOME

Georgia Center for Continuing Education

1197 South Lumpkin Street | Athens, GA 30602

2017 Institute of Bioinformatics Bi-Annual Symposium

Parsing the Microbiome

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2017 Institute of Bioinformatics Bi-Annual Symposium

Parsing the Microbiome

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Agenda

SEPTEMBER 25TH | 2017
GEORGIA CENTER FOR CONTINUING EDUCATION
INSTITUTE OF BIOINFORMATICS BI-ANNUAL SYMPOSIUM

PARSING THE MICROBIOME

Agenda	
8:00 - 8:45 a.m.	**REGISTRATION (Conference Registration Desk)** **BREAKFAST sponsored by MYcroarray (entry Masters Hall)** **POSTER SETUP (Pecan Tree Galleria)**
8:45 - 9:00 a.m.	**Welcome and Introductions** Arthur Edison, University of Georgia Pamela Whitten, Provost and Senior Vice President for Academic Affairs, University of Georgia
9:00 - 9:50 a.m.	**Keynote #1 — Daniel Beiting** (introduction by Jessica Kissinger) Title: MicrobiomeDB: A Systems Biology Platform for Integrating, Mining and Analyzing Microbiome Experiments
9:50 - 10:40 a.m.	**Keynote #2 — Daniel Jacobson** (introduction by Jason Wallace) Title: Adventures in Systems Biology for Plants and Humans
10:40 - 11:00 a.m.	**REFRESHMENT BREAK (Pecan Tree Galleria)**
11:00 - 11:50 a.m.	**Keynote #3 — Gipsi Lima Mendez** (introduction by Mary Ann Moran) Title: The Ocean Microbial-Environmental Interactome
11:50 - 12:05 p.m.	**Short talk #1 — Kara Tinker** Title: Exploring the Taxonomic Stability of the Cockroach Gut Microbiome
12:10 - 1:15 p.m.	**LUNCH (Magnolia Ballroom)**
1:15 - 2:00 p.m.	**Poster Session (Pecan Tree Galleria)**
2:00 - 2:15 p.m.	**Short talk #2 — Allison Williams** Title: The Role of Social Behavior in Shaping Gut Microbial Composition, Diversity and Key Functional Pathways Involved in Metabolism and Pathogenicity in Grant's Gazelle
2:15 - 3:05 p.m.	**Keynote #4 — Peter Karp** (introduction by Art Edison) Title: Metabolic Modeling of the Microbiome
3:05 - 3:25 p.m.	**REFRESHMENT BREAK (Pecan Tree Galleria)**
3:30 - 4:00 p.m.	**Three-minute thesis talks** Brent Nowinski, Grazieli Maboni, Rahil Taujale, Tito D. Peña-Montenegro, Jacquelyn Mary Walejko
4:00 - 4:50 p.m.	**Keynote #5 — Katherine Pollard** (introduction by Mary Ann Moran) Title: Meta-Genotyping Reveals Cryptic Variation in the Human Microbiome
4:50 - 5:15 p.m.	**Symposium Closing — Awards, Closing Remarks** Arthur Edison, University of Georgia Jessica Kissinger, University of Georgia

Workshop Agenda

SEPTEMBER 26TH, 2017
INSTITUTE OF BIOINFORMATICS
BI-ANNUAL SYMPOSIUM
WORKSHOP

PARSING THE MICROBIOME

Workshop Leader: Peter Karp

Location: UGA Science Library| Boyd Graduate Studies

210 D. W. Brooks Drive | Room 382

Workshop Agenda	
8:30 - 9:00 a.m.	**BREAKFAST (Science Library Room 382)**
9:00 - 9:15 a.m.	**Welcome and Introductions** Arthur Edison, University of Georgia Peter Karp, ASI International
9:15 - 10:15 a.m.	**Workshop Session 1**
10:15 - 10:30 a.m.	**REFRESHMENT BREAK (Room 382)**
10:30 - 11:30 a.m.	**Workshop Session 2**
11:30 - 12:30 p.m.	**LUNCH (Room 382)**
12:30 - 2:15 p.m.	**Workshop Session 3**
2:15 - 2:30 p.m.	**Workshop Closing**

Keynote Speakers

MicrobiomeDB: a systems biology platform for integrating, mining and analyzing microbiome experiments

Daniel Beiting
University of Pennsylvania, School of Veterinary Medicine, Philadelphia, Pennsylvania

Dr. Beiting is an immunologist using -omics approaches to interrogate the host pathogen interface. He is the co-developer of MicrobiomeDB (http://microbiomeDB.org) a new database for interrogating microbiome data based on associated sample metadata.

Adventures in Systems Biology for Plants and Humans

Daniel Jacobson
Oak Ridge National Laboratory, Oak Ridge Tennessee

Dr. Jacobson develops new mathematical, statistical, and computation methods to gain new insight into complex biological systems. His work focuses on bioenergy sciences and plant-microbe interfaces, and utilizes high-scale computing with network analysis, linear algebra, Bayesian statistics, and systems analytics to parse a range of -omics datasets (genomics, transcriptomics, proteomics, microbiomics, etc.).

The Ocean Microbial-Environmental Interactome

Gipsi Lima Mendez
Department of Microbiology and Immunology, University of Leuvan, Belgium

Dr. Lima-Mendez (Department of Microbiology and Immunology, University of Leuvan, Belgium) led the bioinformatic analysis of microbial interactions in the Tara Oceans Expedition metagenome, and has worked on human gut microbiomes and the development of bioinformatic tools for viral communities.

Metabolic Modeling of the Microbiome

Peter Karp
Director of the Bioinformatics Research Group within the
Artificial Intelligence Center at SRI International

Dr. Karp has authored 160 publications in bioinformatics and computer science in areas including metabolic pathway bioinformatics, computational genomics, scientific visualization, and scientific databases.

Meta-genotyping reveals cryptic variation on the human microbiome

Katherine Pollard
Gladstone Institutes, University of California San Francisco

Dr. Katherine Pollard develops statistical and computational methods for massive genomic dataset analysis, focusing on questions of genome evolution in organisms ranging from microbes to humans.

Short Talk Abstracts

Kara Tinker

Exploring the taxonomic stability of the cockroach gut microbiome

Kara Tinker and Elizabeth Ottesen

Department of Microbiology, University of Georgia

The hindgut microbiome of the American cockroach exhibits a highly stable core microbiota with minimal response to dietary shifts. This core hindgut microbiome is shared between lab-hosted and wild-caught individuals, although wild-caught specimens exhibited a higher diversity of low-abundance microbes that were lost following extended cultivation under laboratory conditions. This taxonomic stability strongly contrasts with observations of the gut microbiota of mammals, which have been shown to be highly responsive to dietary change. In addition, a comparison with human gut microbiome sequences suggests that the cockroach gut microbiota exhibits low individual-to-individual variation both within and across dietary treatments.

This suggests that American cockroaches have evolved unique mechanisms for establishing and maintaining a diverse and stable core microbiome. As the American cockroach is an opportunistic feeder that can survive even the most extreme conditions, a stable gut microbial community would provide an evolutionary advantage. Current work is focused on determining the origin and extent of this unusual taxonomic stability through a survey of cockroach species from across all seven families in the cockroach order. Preliminary work from eleven different cockroach species across four families demonstrates that each cockroach species hosts a unique gut microbiota. While the exact composition of this gut microbiota appears to be phylogeny-driven, each species surveyed appears to exhibit a core gut microbiota with low variation among individuals within that species. This raises new questions about host-microbiome evolution and suggests that cockroaches may represent an intermediate step in the evolution of the obligate host-microbe symbiosis observed in termites.

Allison E. Williams

The Role of Social Behavior in Shaping Gut Microbial Composition, Diversity and Key Functional Pathways Involved in Metabolism and Pathogenicity in Grant's Gazelle

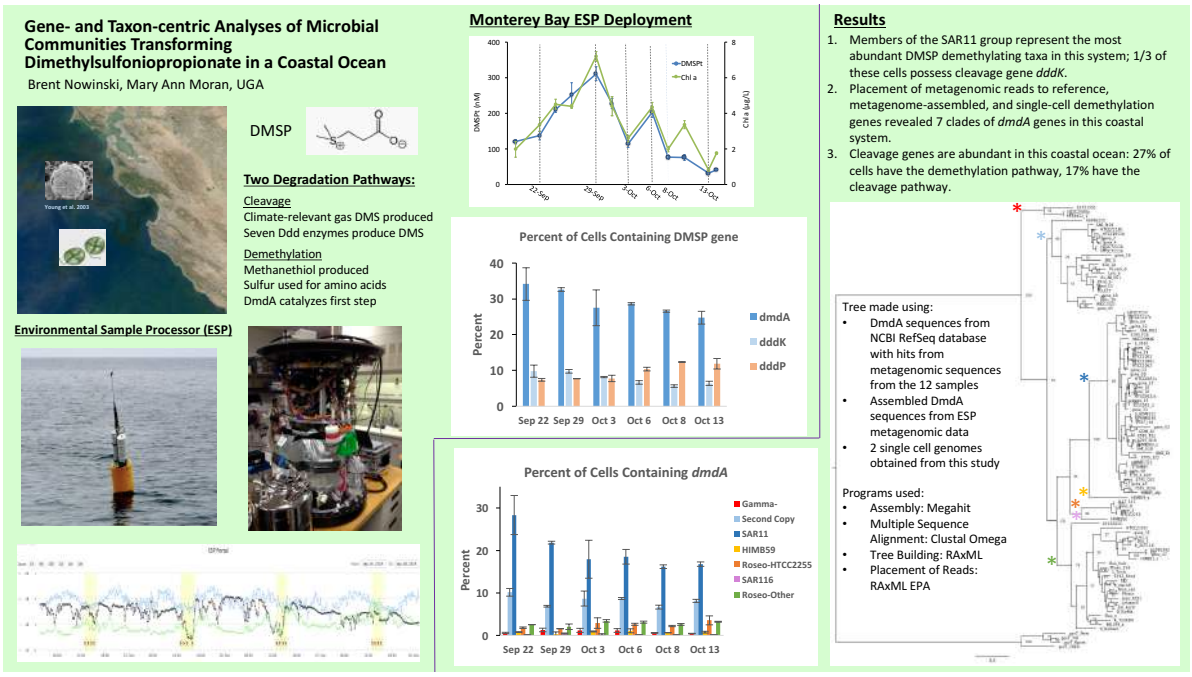
Allison E Williams[1], Se Jin Song[2,3], Rob Knight[3,4], Vanessa Ezenwa[1,5]

[1]Odum School of Ecology, University of Georgia (UGA); [2]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder; [3]Department of Pediatrics, University of California, San Diego; [4]Department of Computer Science and Engineering, University of California, San Diego; [5]Department of Infectious Diseases, College of Veterinary Medicine, UGA

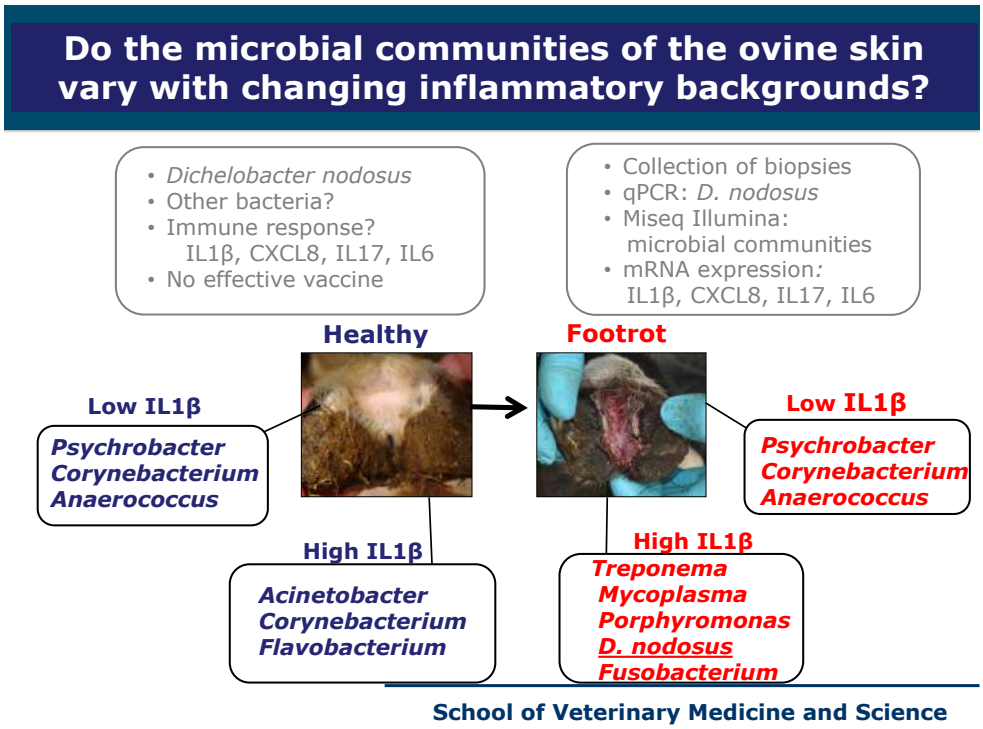
The gut microbiome is made up of trillions of individual microbes and plays a role in health and protection from pathogen infection. Several factors such as diet and genetic variation have been shown to influence gut microbe community membership, and recent work is showing that social behavior also plays an important role in shaping the microbiome. For example, in wild primates close physical interactions between individuals can promote the transmission of gut microbes. One question that arises is how general these patterns are in social species where physical contact is less pronounced. Our study used wild Grant's gazelles (Nanger granti) to examine whether social behavior promotes gut microbe transmission in a species that forms social groups, but has very little physical contact, and whether differences in social behavior result in different functional capacities of the gut microbiome. Using field observations of individual social behavior and gut microbial sequences generated from fecal samples, we tested whether group membership and indices of individual social connectivity could explain gut microbiome structure and diversity. Metabolic and pathogenic functional pathways were also predicted from our gut microbial sequences, and we investigated whether indices of social connectivity could explain differences in functional potential. We predict that group membership would play a role in shaping gut microbiome composition, with individuals from the same social group having more similar gut microbiome communities than those from different social groups. We also predicted that more socially connected individuals would have higher gut microbe diversity than those that are less connected. Finally, we predicted that more socially connected individuals would have enhanced capability for metabolic functions and reduced pathogenic functional pathways than those that are less connected.

3min Thesis Talks

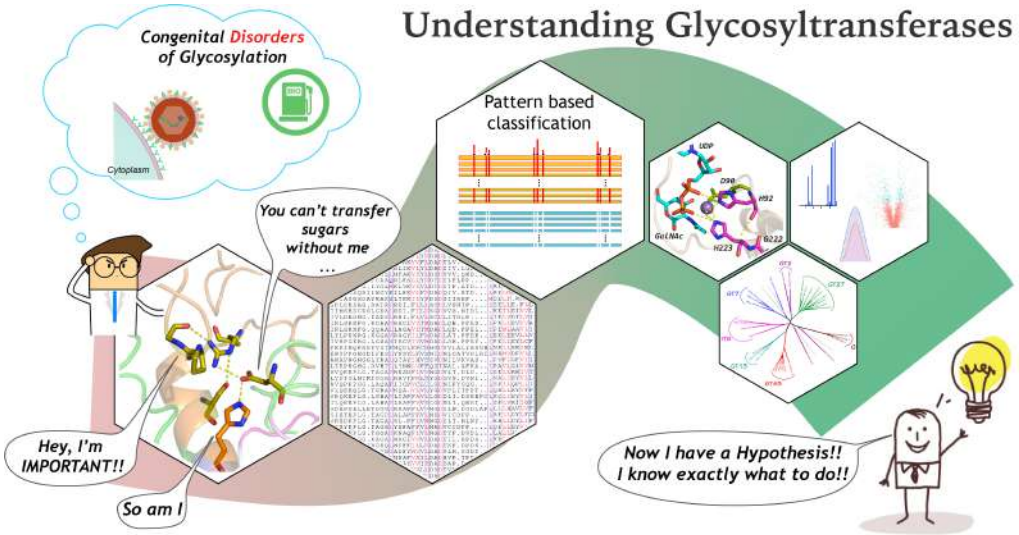
Brent Nowinski



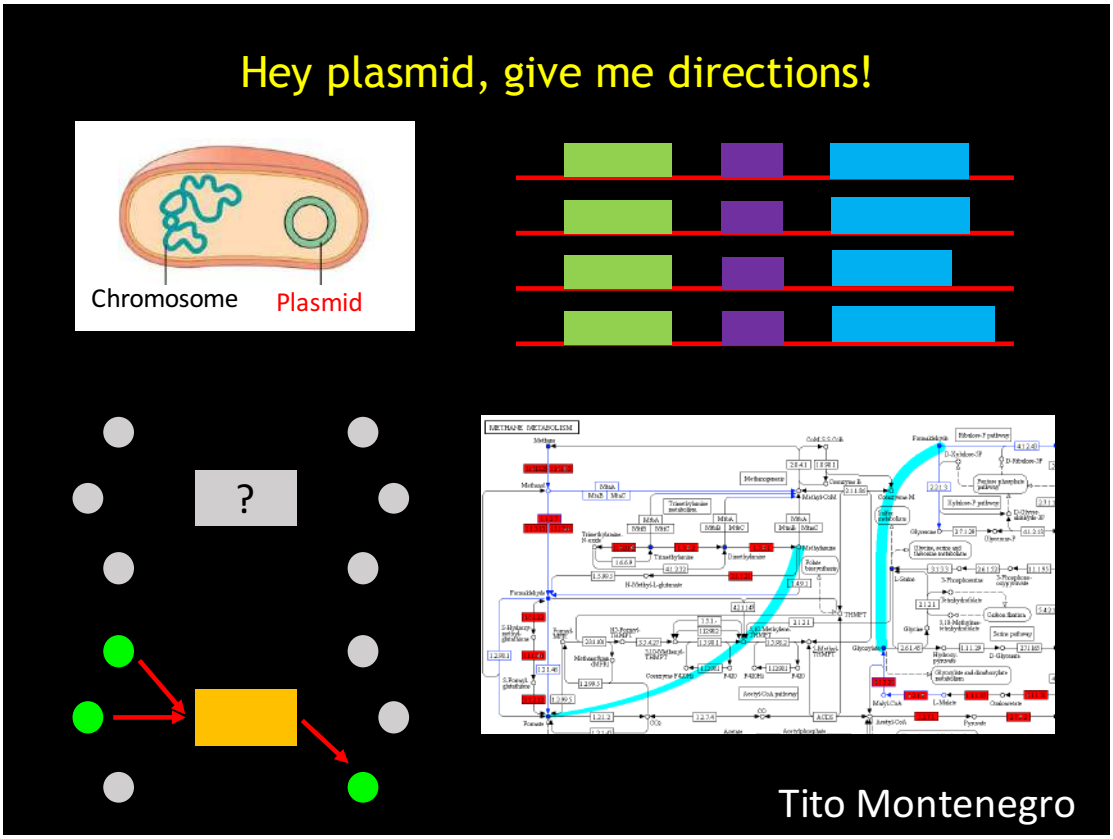
Grazieli Maboni



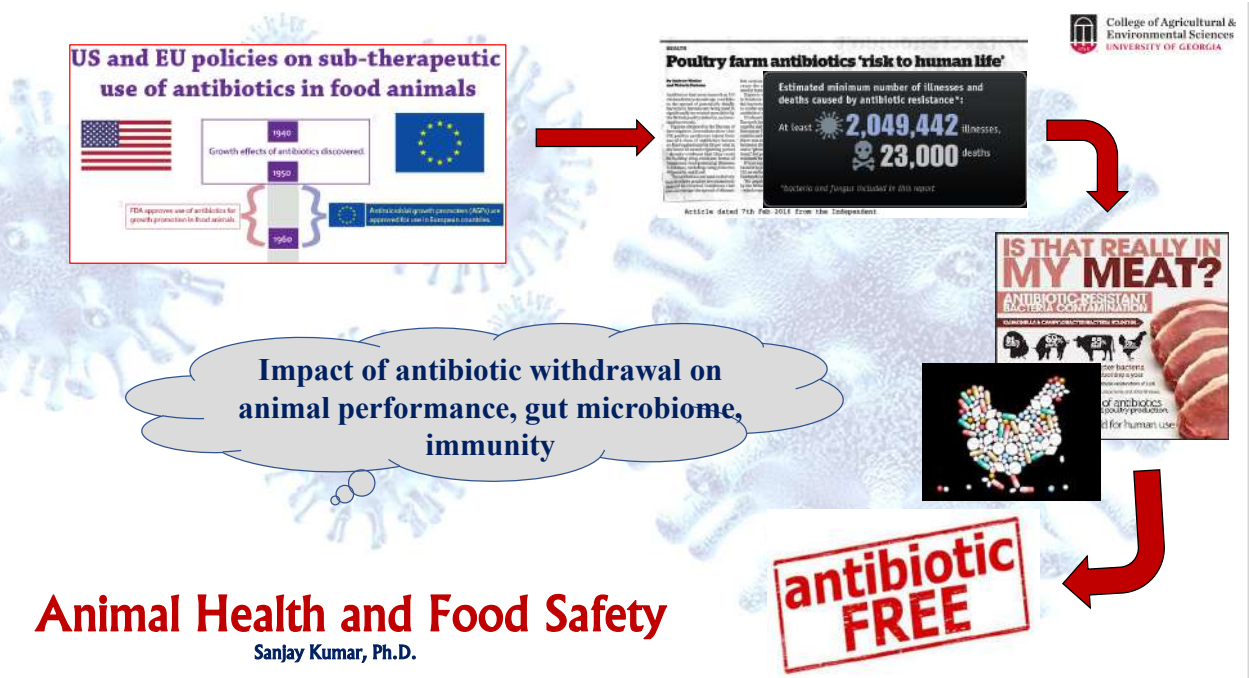
Rahil Taujale



Tito D. Peña-Montenegro



Sanjay Kumar



Jacquelyn Mary Walejko

Slide to be added

Poster Abstracts

1.

Epigenetic age acceleration and microbiome composition in African American women

Anna K. Knight[1], Alicia K. Smith[2], Elizabeth J. Corwin[3], Anne L. Dunlop[3]

1- Genetics and Molecular Biology Program, Emory University, 2- Department of Gynecology and Obstetrics, Emory University, 3- Nell Hodgson Woodruff School of Nursing, Emory University

African American women are more likely to have vaginal microbiomes not dominated by *Lactobacillus* than Caucasian women, potentially increasing their risk for vaginal infections. Such infections have previously been associated with dysregulation of the hypothalamic-pituitary-adrenal axis (HPA), increased stress response, and experiencing stressful life events. We hypothesize that vaginal microbiome composition is associated with age acceleration, a DNA methylation-based metric previously associated with stress, developmental, neuropsychiatric, and behavioral disorders, as well as all cause mortality. To test this hypothesis, we leveraged data from 251 pregnant African American women who provided vaginal microbiome samples for V3-V4 16S rRNA sequencing and blood samples for DNA methylation analysis for at least one timepoint during pregnancy. Microbiome data was processed using the Qiime2 pipeline. Shannon and ChaoI alpha diversity scores were calculated using the R package Phyloseq. DNA methylation was measured on the Human Methylation450 and EPIC BeadChips. DNA methylation data was used to calculate age acceleration. Linear regression models that controlled for cellular heterogeneity were used to evaluate the association between age acceleration and vaginal microbiome composition. A random effects term was included to account for repeated measures on the same subject. The Shannon diversity index was positively associated with age acceleration (p=.03) such that subjects with less even distribution of taxa had a higher age acceleration. This supports our hypothesis that vaginal microbiome composition is associated with age acceleration, and potentially other negative health outcomes. ChaoI diversity was not associated with age acceleration. Future studies will examine the impact of vaginal microbiome diversity on stress and pregnancy complications.

2.

Dimethylsulfoniopropionate Degradation in the Coastal Ocean: Gene- and Taxon-centric Approaches

Brent Nowinski, Christina Preston, Ronald P. Kiene, Christopher Scholin, James Birch, William B. Whitman, Mary Ann Moran

Department of Marine Sciences, Department of Microbiology, University of Georgia; Monterey Bay Aquarium Research Institute; Dauphin Island Sea Lab, University of South Alabama

Dimethylsulfoniopropionate (DMSP) is an abundant organic sulfur compound produced by marine phytoplankton. Marine bacteria transform DMSP via two pathways: the demethylation pathway retains DMSP-derived sulfur in the cell where it can be incorporated into biomass or be oxidized, while the cleavage pathway releases volatile DMS with potential implications for cloud formation. The prominent hypothesis explaining differential regulation of these pathways poses that demethylation is favored when DMSP dominates the organic sulfur pool, while cleavage is favored when other organic sulfur compounds can substitute as the cellular sulfur source. Marine phytoplankton groups differ in their production and release of DMSP and other organic sulfur compounds, so shifts in phytoplankton community composition can alter sulfur source availability. We tracked bacterial DMSP gene diversity and abundance in Monterey Bay surface waters during a 31-day study in which the Environmental Sample Processor (ESP) autonomously filtered and archived the seawater microbial community.

Sequencing of twelve metagenomes during the ESP deployment showed taxa known to carry DMSP genes made up 23-39% of the bacterial community, with SAR11-like cells representing the most abundant DMSP degraders, followed by Roseobacter-, SAR116-, and marine gamma proteobacterium HTCC2080-like cells. Placement of metagenomic reads to reference, assembled, and single-cell genomes revealed seven major clades of DMSP demethylation (dmdA) genes, with SAR11-like cells the source of 56% of placed dmdA reads and having highest abundances in communities with high relative abundances of DMSP producers, including dinoflagellates and haptophytes. Roseobacter-like cells were the source of most of the DMSP cleavage genes, averaging 63% of dddP, dddK, dddQ, and dddD genes, with highest abundances in communities with high relative abundances of small phytoplankton and diatoms. In this coastal system, patterns in DMSP dynamics tracked changes in dominant phytoplankton communities and key bacterial degraders.

3.

A distinct bacterial dysbiosis associated with skin inflammation in ovine footrot

Grazieli Maboni[1,2], Adam Blanchard[1], Sara Frosth[3], Ceri Stewart[1], Richard Emes[1], Sabine Tötemeyer[1]

[1]University of Nottingham, School of Veterinary Medicine and Science, Nottingham, United Kingdom. [2] Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, United States. [3]Department of Biomedical Sciences and Veterinary Public Health, Uppsala, Sweden.

Introduction. Ovine footrot is a bacterial disease caused by *Dichelobacter nodosus* and characterized by the separation of the hoof horn from the underlying skin. Footrot is a major cause of lameness affecting sheep welfare worldwide and causing a significant financial impact. *Fusobacterim necrophorum*, *Treponema* spp. and other bacterial communities are commonly identified in footrot lesions, however, the role of other bacterial communities in the development of footrot as well as inflammatory responses remain unclear.

Aim. This study aimed to investigate whether high load of *D. nodosus* and abundance of bacterial populations are associated with high levels of inflammation in the ovine interdigital skin.

Methodology. Bacterial communities present on the ovine interdigital skin were investigated using the 16S rRNA V3/V4 variable region amplification by Illumina metagenomics sequencing. Ovine mRNA expression levels of Interleukins IL1β , IL6, CXCL8 and IL17 were investigated by RT-qPCR.

Results. This study shows a significant association between the high expression of IL1β and high *D. nodosus* load in footrot samples. Investigation of the microbial population identified distinct bacterial populations in the different disease stages and also depending on the level of inflammation. *Treponema* (34%), *Mycoplasma* (29%) and *Porphyromonas* (15%) were the most abundant genera associated with high levels of inflammation in footrot. In contrast, *Acinetobacter* (25%), *Corynebacteria* (17%) and *Flavobacterium* (17%) were the most abundant genera associated with high levels of inflammation in healthy feet.

Conclusion. This study has shown distinct dysbiosis evident across healthy and footrot samples and the association with inflammation, highlighting the importance of the microbial population in relation to the local inflammatory response within the context of the healthy or footrot affected ovine foot. This demonstrates for the first time the distinct microbial community associated with footrot and high cytokine expression.

4.

Species diversity of root-feeding beetle associated blue-stain fungi in loblolly pine stands

Megan Buland[1], Brittany F. Barnes[1], Kier D. Klepzig[2], Kamal J.K. Gandhi[1], and Caterina Villari[1]

[1]D.B. Warnell School of Forestry, University of Georgia, Athens, GA. [2]Joseph W. Jones Ecological Research Center, Ichauway, Georgia

Loblolly pine (*Pinus taeda* L.), the most commercially important tree species in the southeastern United States, is experiencing relatively higher levels of mortality around the Fall-line and Upper coastal plain regions in Alabama and Georgia. Root-feeding beetles (e.g., *Hylastes, Pachylobius* and *Hylobius* spp.) and their Ophiostomatoid fungal obligates (e.g., *Leptographium* and *Grosmannia* species), also known as blue-stain fungi, have been associated with areas of dieback; however, the actual roles of these beetles, and in particular of their fungal obligates in loblolly pine mortality is yet to be established. Our research objectives are as follows: 1) to assess the diversity and composition of the Ophiostomatoid fungal complex associated with these root-feeding taxa in loblolly pine stands; and 2) to analyze whether pathogen pressure and species composition varies in stands with various management practices and environmental conditions. Root-feeding beetles will be collected in loblolly pine stands with differing management histories, such as controlled burning, and across multiple seasons. At first, a subset of beetles will be used to isolate and identify associated blue-stain fungi based on morphological and molecular features. Then, DNA will be extracted directly from the adult beetles and species-specific PCR primers will be developed to determine the incidence of each fungal species. Results from this study will assist in better understanding if Ophiostomatoid fungi have a role in the dieback of loblolly pine, and their implications for sustainable forest management practices in the southeastern U.S. forests.

5.

Co-administration of the *Campylobacter jejuni* N-glycan based vaccine with probiotics improves vaccine performance in broiler chickens

H. Nothaft[1^], M.E. Perez-Muñoz[2#], G.J. Gouveia[3,4#], C. G. Panagos [4], R.M. Duar[2], J.J. Wanford[5], L. Lango-Scholey[5], V. Srithayakumar[2,6], G.S. Plastow[2,6], C. Coros[7], C.D. Bayliss[5], A. S. Edison[3,4], J. Walter[1,2] and C.M. Szymanski[1,4,8,^]

[1]Department of Biological Sciences, University of Alberta, Edmonton, Canada.[2]Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Canada.[3]Departments of Genetics and Biochemistry and Molecular Biology, University of Georgia, Athens, USA.[4]Complex Carbohydrate Research Center, University of Georgia, Athens, USA.[5]Department of Genetics and Genome Biology, University of Leicester, Leicester, UK.[6]Livestock Gentec, Edmonton, Canada.[7]Delta Genomics, Edmonton, Canada. [8]Department of Microbiology, University of Georgia, Athens, USA.[#]Both authors contributed equally

Source attribution studies report that consumption of contaminated poultry is the primary source for acquiring human campylobacteriosis. Oral administration of an engineered *Escherichia coli* strain expressing the *Campylobacter jejuni* N-glycan reduces bacterial colonization in specific-pathogen-free leghorn chickens, but only a fraction of birds respond to vaccination. Optimizing the vaccine for commercial broiler chickens has great potential to prevent pathogen entry into the food chain. Here, we tested the same vaccination approach in broilers and observed similar efficacy in pathogen load reduction, stimulation of host IgY response, lack of *C. jejuni* resistance development, uniformity in microbial gut composition, and bimodal response to treatment. Gut microbiota comparisons of leghorn and broiler vaccine responders identified one member of the *Clostridiales* XIVa cluster, *Anaerosporobacter mobilis* that was significantly more abundant in both groups. Broilers vaccinated with *A. mobilis* or *Lactobacillus reuteri*, a commonly used probiotic, demonstrated increased vaccine efficacy, antibody response, and weight gain over the study timeframe. To investigate whether the responder / non-responder effect was due to selection of a *C. jejuni* 'super colonizer mutant' with altered phase-variable genes, we analysed all polyG-containing loci of the input strain compared to non-responder colony isolates and found no evidence of phase state selection. However, untargeted NMR-based metabolomics identified a potential biomarker negatively correlated with *C. jejuni* colonization levels possibly linked to the increased microbial diversity in this subgroup. The comprehensive methods used to examine the vaccine response bimodality provide several opportunities to improve the *C. jejuni* vaccine and the efficacy of any vaccination strategy. This work is now an accepted manuscript and soon to be published on the American Society for Microbiology Journal.

6.

An evolutionary systems approach to an integrative study of the proteome and metabolome of Glycosyltransferases in C. elegans

Rahil Taujale1,2, Olatomiwa Bifarin2, Natarajan Kannan1, Arthur S. Edison1,2

1Institute of Bioinformatics, University of Georgia, Athens, GA, 2Complex Carbohydrate Research Center, University of Georgia, Athens, GA

Glycosyltransferases (GTs) are a broad class of proteins involved in the transfer of a glycosyl group from a donor molecule to an acceptor. As such, they are involved in a wide range of biological functions through their roles in glycosylation modifications, synthesis of cellular receptors, biosynthesis of polysaccharides, glycolipids and glycoproteins. In C. elegans, GTs have been implicated to play roles in development, cuticle formation, detoxification, signaling and other pathways. However, only around 20% of the more than 260 GTs have been characterized. The large expansion of some of the GT families, presence of unique sugars and pathways in worms and the unknown specific roles of GTs in the implicated pathways pose specific questions and challenges that require a deeper understanding of the functional units of these GTs in C. elegans.

We used a Bayesian statistical approach to align and classify more than 250,000 GT sequences across all taxonomic groups into functional categories based on the patterns of conservation and variation in large multiple sequence alignments. We use patterns unique to each GT family as a conceptual starting point for investigating their sequence-structure-function relationships. Implementing these methods, we can further pinpoint contrasting, similar and co-evolved features that differentiate, associate and functionally relate the GT families respectively.

We have generated a phylogenetic classification based on the conserved features and mapped phenotypic associations for these families based on literature. Using the co-conserved features as a starting point, we further investigate structural data to pinpoint targets for mutational and metabolomic studies. An initial metabolomic analysis by others in the lab of select GT mutants in C. elegans is revealing specific features that are statistically different, suggesting family specific phenotypic changes.

7.

Hierarchical classifiers in Transcription Reveal Responses of Extreme Microbial Communities to Oil and Dispersant Exposure.

Tito D. Peña-Montenegro1,2, Sara Kleindienst1, Andrew E. Allen3, Jonathan Arnold2 and Samantha B. Joye1

1Department of Marine Sciences, The University of Georgia, 2Institute of Bioinformatics, The University of Georgia, 3J Craig Venter Institute

Chemical dispersants are used commonly in response to oil spills. However, the impacts of dispersants on microbial community composition and activity are poorly understood. We simulated the deep water conditions that prevailed following the 2010 BP/Deepwater Horizon oil spill and evaluated the response of the microbial community to elevated levels of oil, chemical dispersants and dispersed oil using metatranscriptomics. Clustering factors were compared across levels of a hierarchical annotation system to increase the power of prediction and contrast in our datasets. We found significant dispersant-driven changes in terms of diversity and abundance of microbial composition and activity, shifting the dynamics from one dominated by oil biodegradation in oil-only treatments to those dominated by dispersant biodegradation in dispersant-amended treatments. These results have important implications for understanding the impacts of chemical dispersants on the ability of microbial communities to efficiently degrade oil in the environment.

8.

K-shuff: A Novel Algorithm for Characterizing Structural and Compositional Diversity in Gene Libraries.

Kamlesh Jangid (1), Ming-Hung Kao (2), Aishwarya Lahamge (3), Mark A. Williams (4), Stephen L. Rathbun (2), William B. Whitman (1)

Departments of Microbiology (1) and Epidemiology and Biostatistics (2), University of Georgia; Savitribai Phule Pune University, Pune, India (3); College of Agriculture and Life Sciences, Virginia Polytechnic and State University (4)

K-shuff is a new algorithm for comparing the similarity of gene sequence libraries, providing measures of the structural and compositional diversity as well as the significance of the differences between these measures. Inspired by Ripley's K-function for spatial point pattern analysis, the Intra K-function or IKF measures the structural diversity, including both the richness and overall similarity of the sequences, within a library. The Cross K-function or CKF measures the compositional diversity between gene libraries, reflecting both the number of OTUs shared as well as the overall similarity in OTUs. A Monte Carlo testing procedure then enables statistical evaluation of both the structural and compositional diversity between gene libraries. For 16S rRNA gene libraries from complex bacterial communities such as those found in seawater, salt marsh sediments, and soils, K-shuff yields reproducible estimates of structural and compositional diversity with libraries greater than 50 sequences. Similarly, for pyrosequencing libraries generated from a glacial retreat chronosequence and Illumina® libraries generated from US homes, K-shuff required >300 and 100 sequences per sample, respectively. Power analyses demonstrated that K-shuff is sensitive to small differences in Sanger or Illumina® libraries. This extra sensitivity of K-shuff enabled examination of compositional differences at much deeper taxonomic levels, such as within abundant OTUs. This is especially useful when comparing communities that are compositionally very similar but functionally different. K-shuff will therefore prove beneficial for conventional microbiome analysis as well as specific hypothesis testing. See: Jangid et al. (2016) K-shuff: A Novel Algorithm for Characterizing Structural and Compositional Diversity in Gene Libraries. PLoS One. PMID: 27911946 DOI: 10.1371/journal.pone.0167634

9.

NMR based study of the metabolite flux through the fast loop of the Surface Ocean Carbon Cycle

Charalampos G. Panagos, Khan Hekmatyar, Christa B. Smith, Frank Ferrer-Gonzalez, Zhang, Sicong, Mary Ann Moran, Arthur S. Edison

Complex Carbohydrate Research Center, The University of Georgia, Department of Marine Sciences, Marine Sciences Building, University of Georgia

The study of the diatom-bacteria model system is of paramount importance in order to understand the cycle of the CO2 absorbed by the oceans. Almost a quarter of Earth's primary production is cycled within days to weeks of fixation by the billion marine bacteria living in each litre of surface seawater. However, surprisingly little is known about the metabolites that sustain their growth. In this study, we present the real-time flux measurements of the transport and process of important carbon currencies (pyruvate, acetic acid and DHPS) by the bacteria of the diatom-bacteria model. Dynamic Nuclear Polarisation (DNP) is employed to follow the metabolic fate of these molecules, when injected to bacteria. Bacteria grown in variable conditions and the effect on their metabolism, were also investigated.

In addition to the metabolic fate of known carbon currencies, we present the initial steps towards the untargeted metabolomic analysis of the diatom-bacteria system. The main goal of this analysis is to identify novel carbon cycle metabolites, as well as highlight differences between the endometabolomic and exometabolomic footprint of diatoms grown with and without their synergistic bacteria. Challenges common with this kind of analysis include the large number of metabolites present in the system, as well as the high salinity of the samples, which impedes further analysis (NMR, MS). Chromatographic, chemical and spectroscopic solutions for desalting, fractionating and analysing the metabolites of the system are described.

10.

Effect of Antibiotic Withdrawal in Feed on Chicken Gut Microbial Dynamics, Immunity, Growth Performance

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Development of antibiotic resistance in foodborne pathogens, Salmonella spp. and Campylobacter, is a public health concern. Public demand to reduce the use of sub-therapeutic antibiotic growth promoters (AGP) in poultry feeding has resulted in greater adoption of antibiotic-free poultry production systems. There is a need to understand the effects of AGP removal from poultry feed on gut microbiota and its impact on prevalence of foodborne pathogens. The effect of antibiotic withdrawal from poultry feed on gut microbial community, host performance and immunity, and prevalence of Salmonella and Campylobacter was evaluated. Birds were raised on three phase diets (starter [d0-22], grower [d23-35] and finisher [d36-42]) with and without bacitracin dimethyl salicyclate (BMD). At early growth stage, bird performance was improved ($P \leq 0.05$) with BMD treatment, whereas performance was better ($P \leq 0.05$) in control group (no BMD in the feed) at the time of commercial processing. Acetate and butyrate production was affected ($P \leq 0.05$) by age, whereas propionate production was affected ($P \leq 0.05$) by both the treatment and age. The bacterial communities in the cecum were more diverse ($P \leq 0.001$) and rich compared to the ileal communities, and they shifted in parallel to one another as the chicks matured. Differences in diversity and species richness were not observed ($P > 0.05$) between the BMD-fed and control groups. Comparing all ages and diets, the composition of cecal and ileal bacterial communities was different ($P \leq 0.001$). However, both the communities remained unchanged in response to antibiotic withdrawal. Inclusion of BMD in the feed did not affect the bacterial phyla. However, predictable shift in the ileal and cecal bacterial population at lower taxonomic level was observed in control vs BMD-fed group. Cytokines gene expression (IL-10, IL-4, IFN-γ, beta-defensin, and TLR-4) was affected ($P \leq 0.05$) in the BMD-fed group at early stages of growth. The prevalence of foodborne pathogens, Campylobacter spp. and Salmonella spp. showed higher abundance in the ilea of BMD-fed chicks compared to control group. Overall, this study provided insight of the impact of AGP supplementation in the feed on gut microbial modulations, bird performance, host immunity and pathogen prevalence. This information can assist in designing alternative strategies to replace antibiotics in modern poultry production and for food safety.

11.

Systems biology of circadian clock synchronization in Neurospora crassa.

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The circadian clock is a widely conserved emergent property driving daily rhythms across multiple levels of biological organization, with oscillations emanating from core transcription-translation feedback loops which then exert control over downstream pathways. In turn, multiple external factors may influence the clock and allow for re-synchronization with external cues, or compensation for changes in temperature and nutrient availability.

We have recently found that transcriptional clock/oscillator phases in single cells of the clock model Neurospora crassa synchronize over time, but only if they are in the same microenvironment. We hypothesize an extracellular chemical communication mechanism to explain synchronization.

To explore the nature of this mechanism and the underlying gene-metabolite network, we are using a combination of untargeted NMR metabolomics, clock activity-guided fractionation, and genetic knockout mutants to screen for extracellular molecules and genetic components that may allow clocks to interact. Identifying pathways at the intersection of these approaches will further enable targeted MS experiments and transcript analyses. Preliminary NMR data on nonpolar exometabolites has already revealed circadian features, suggesting interactions of this sort occur in Neurospora. This experiment is being repeated with more replicates, clock mutants, and broader chemical diversity.

Our approach will also allow us to explore broader properties of the N. crassa extracellular metabolome in the future. Moreover, this work offers fundamental insights about the control and multilevel properties of a pervasive systems behavior in a model organism with biotechnological relevance.

12.

Composition of the gut microbiota influences infection severity and pregnancy outcome in *Plasmodium chabaudi*-infected pregnant mice

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Placental malaria, a severe clinical manifestation of *Plasmodium falciparum* infection, is a major cause of pregnancy loss, neonatal mortality, and severe maternal illness. Mouse models for malaria infection during pregnancy are vital to understanding the mechanisms underlying these outcomes. Outbred Swiss Webster mice infected with *P. chabaudi chabaudi* AS in early gestation carry their pregnancies to term, allowing us to explore the immunological balance between parasite clearance and pregnancy success. The composition of the gut microbiota may alter this balance. As described by Villarino *et al.*, C57BL/6 mice sourced from different vendors display microbiota-dependent differences in *Plasmodium* infection severity resulting in a susceptible or resistant phenotype. Similarly, pregnant Swiss Webster mice with susceptible gut microbes develop higher parasite burdens than mice with resistant gut microbes, although both cohorts produce live pups at term. Despite the lower parasite burdens observed in resistant mice, these dams tend to produce fewer viable pups per litter, suggesting a trade-off between accelerated parasite clearance and pregnancy success. This cannot be attributed to elevated levels of systemic TNF or IFN-γ, which have previously been associated with the clearance of blood-stage malaria infection and pregnancy loss in *P.c. chabaudi* AS-infected pregnant C57BL/6 mice, as the levels of these cytokines do not differ significantly between Swiss Webster mice possessing susceptibility-or resistance-conferring gut microbes.

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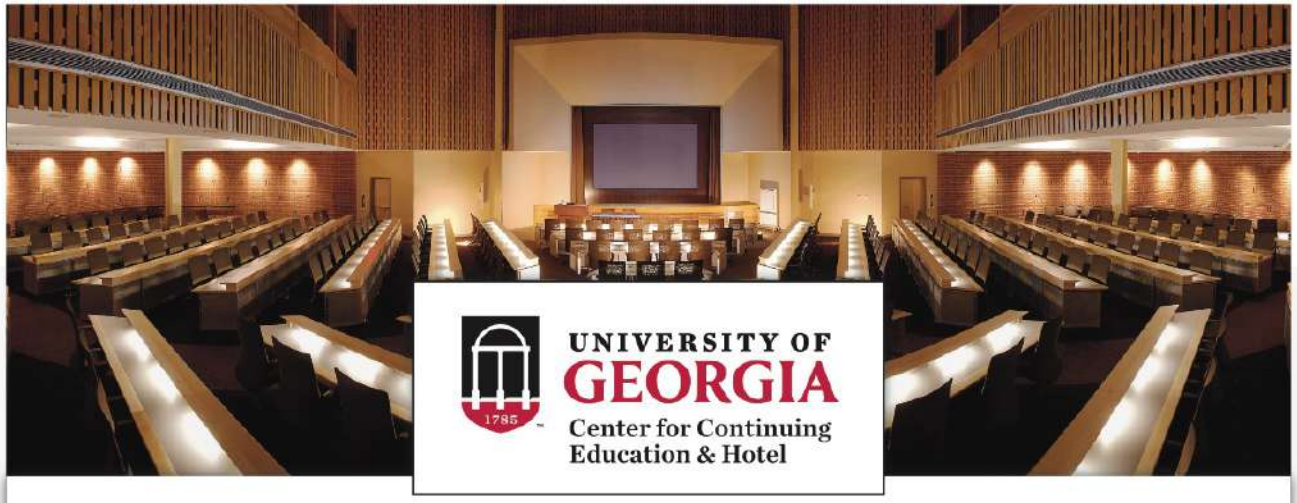

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