NC STATE UNIVERSITY

GENETICS & GENOMICS ACADEMY

Annual Retreat 2022

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1st ANNUAL RETREAT

Friday, August 26th, 2022
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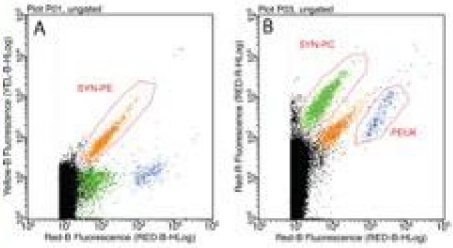

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- Technician-assisted and unassisted sorting options
- Sort to different tube and well plate formats for downstream cultivation or molecular analysis
- Free consultations and group trainings

Contact: Dr. Ryan Paerl, rpaerl@ncsu.edu

Location: 1147 Jordan Hall. Main Campus

https://microfacs.wordpress.ncsu.edu/

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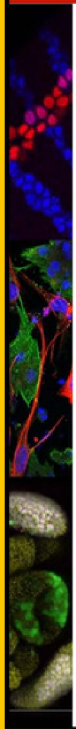
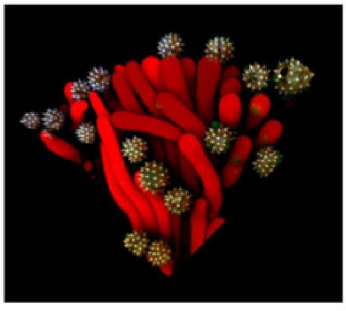


Cellular and Molecular Imaging Facility (CMIF)

New location with state-of-the-art light microscopes in Plant Sciences Building on Centennial Campus
Officially open in Fall of 2022 (available for tests and demonstrations now)

Highlights:

- **Zeiss LSM 980** with Airyscan 2 and Picoquant FLIM/FRET
- **Leica Stellaris 8** confocal microscope with White Light Laser and FALCON imaging
- **Leica LMD7** - laser microdissection, and **Leica CM3050** cryotome
- **Olympus VS200** high throughput slide scanner
- **Leica Thunder for Model Organisms** – high end dissecting microscope with scanning stage, brightfield, fluorescence, deconvolution and optical clearing.



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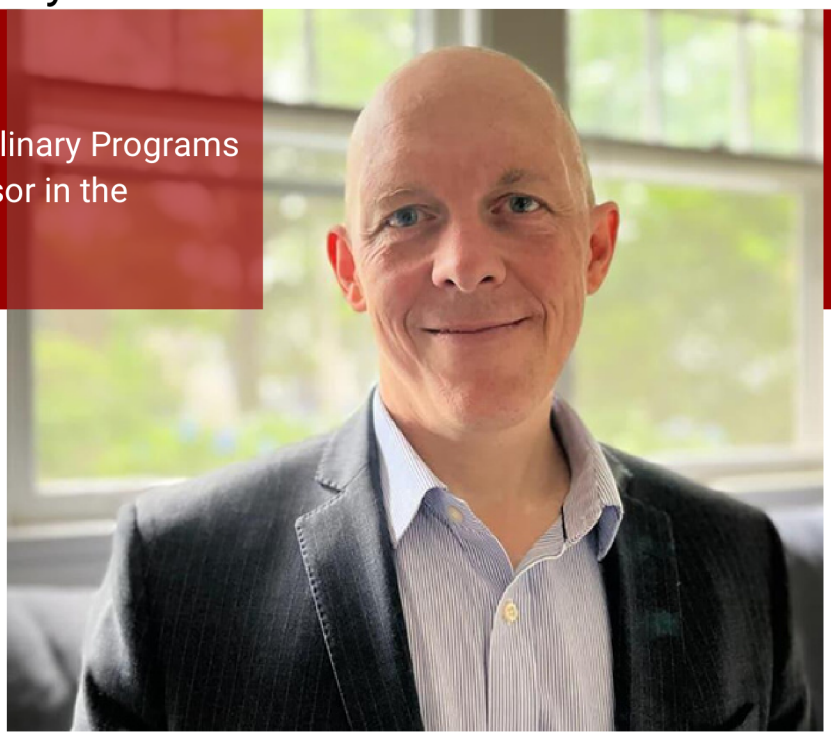
Keynote Speaker
Genetics and Genomics Academy 1st Annual Retreat

Dr. Rob Dunn
North Carolina State University
Senior Vice Provost for University Interdisciplinary Programs
William Neal Reynolds Distinguished Professor in the Department of Applied Ecology



Dr. Dunn is the Senior Vice Provost for University Interdisciplinary Programs at NC State. In the context of this job he is working to lead and launch interdisciplinary programs at NC State and also to make it easier for faculty, postdocs and students who want to work across disciplines to do so in ways that have a great impact on society. Dr. Dunn is an ecologist and evolutionary biologist whose work focuses on the biology of the everyday. He aims to apply general concepts and theory from ecology and evolutionary biology to biological realms that have been relatively ignored. Often, this work is done with large collaborative teams.

One thread of this research has focused on homes. Working with Noah Fierer, for example, and a large team of collaborators, Dr. Dunn wrote the first papers using e-DNA to study the biology of bacteria, fungi and plant materials in houses. With a related team, he then studied the ecology of biofilms on showerheads. He teamed up with public health professionals and clinicians to then study the conditions under which water systems favor the abundance of opportunistic pathogens in showerheads. A separate thread of this research involved collaboration with statisticians to study the ways in which e-DNA could be used in forensic studies (NC State statistics graduate student Neal Grantham was able to show that the fungi on indoor items, for example, could identify the geographic origin of those items within 50 km in the U.S. and at the country level globally). Another thread of this research focused on the human body and its ecology. In this research, Dr. Dunn has partnered with primatologists, archaeologists and public engagement specialists to understand the ways in which bodily associated of skin microbiomes, gut microbiomes, vaginal microbiomes and extended microbiomes (such as those associated with fermentation) have changed over the last five to ten million years of human evolution. A third recent thread has considered the ecology and evolution of fermented foods. The ecology and evolution of the microbes in these foods have been relatively ignored because of, among other things, the focus of food scientists on industrialized food systems (especially in the United States). Dr. Dunn teamed up with cheese microbiologists, bakers, chefs, bioinformaticians, historians, artists, a theologian and many others to conduct the first global study of the ecology and evolution of sourdough bread. In association with this study, Dr. Dunn and his collaborators began broader collaborations with regard to the evolutionary importance of fermented foods to humans. To date, Dr. Dunn has published more than two hundred scientific papers, a similar number of articles for general audiences and seven books.

Event Schedule

Welcome & Updates - Duke Energy Hall ABC

8:30 AM Coffee and light breakfast. Poster and vendor set up.

9:00 AM Opening remarks by Dr. Fred Gould, GGA Director
Welcome from Provost Arden, Dean McGahan, Dean Dole, Dean Meurs
Updates from the GGA Executive Committee

- Introduce New Faculty
- The four major objectives of the GGA
- GG Scholars Program—overview and new student introductions
- Reformulation of Research Interest Groups (RIGs) through interactive activities
- Interface with the PDU
- Seminar Series
- Genetics & Functional Genomics Merger
- Discussion

Coffee Break & Posters - Duke Energy Hall D

10:30 AM Please take a coffee break and visit the posters and vendors around the room. Retreat will reconvene at 11:00 AM.

Keynote Presentation - Duke Energy Hall ABC

11:00 AM **Dr. Rob Dunn**, Senior Vice Provost for University Interdisciplinary Programs and William Neal Reynolds Distinguished Professor in the Department of Applied Ecology, NC State University
The Future of Interdisciplinarity at NC State, from Sourdough Bread to Earth's Wicked Problems

Dr. Dunn's talk will be divided into two parts. First, he will use the story of his research on sourdough bread to show the ways in which interdisciplinary scholarship (and, indeed, in this case transdisciplinary scholarship) can not only transform what is discovered but also what is studied. The talk will show the ways in which the study of sourdough bread is a kind of microcosm for the study of larger (more Wicked) challenges. In the second part of the talk, Dr. Dunn will describe the Office of University Interdisciplinary Programs at NC State. In doing so, he will discuss some of the short and long-term goals of the office and the role that the GGA and other Academies will play in achieving those goals. He will conclude with a series of questions posed to GGA about the sorts of interdisciplinarity that are possible for the Academy but also about the ways in which the Academy fits into an understanding of the scale of the wicked problems that face humanity in the next hundred years. In the end, sourdough ecology is an interesting but insufficient metaphor for the Earth's bigger challenges, challenges that will require even more integrated and radical interdisciplinarity than we have achieved in pondering the pleasures of bread.

Event Schedule

Lunch & Posters - Duke Energy Hall D

12:00 PM Please break for provided lunch and visit posters, as well as the vendor tables. Trainees having lunch with Dr. Dunn should take their lunch to room 4105. Retreat will reconvene at 1:30 PM.

Science Talks: Session 1 - Duke Energy Hall ABC

1:30 PM **Dr. Orlando Arguello-Miranda**, Assistant Professor, Department of Plant and Microbial Biology, CALS
A Pack of Machine Learning Algorithms to Image Complex Microbial Communities

1:45 PM **Dr. Elizabeth Jones**, Postdoctoral Research Scholar in Zanno lab, Department of Biological Sciences and NC Museum of Natural Sciences Paleontology Research Lab, COS
Ancient DNA: The Making of a Celebrity Science

2:00 PM **Dr. Eric Brooks**, Assistant Professor, Department of Molecular Biomedical Sciences, CVM
Cellular control of cranial neural tube closure

2:15 PM **Dr. Terri Long**, Associate Professor, Department of Plant and Microbial Biology, CALS
Iron at the intersection of development and multi-stress resilience

2:30 PM **Dr. Bala Rao**, Professor, Department of Chemical and Biomolecular Engineering and Director of Academic Programs at Biomanufacturing Training and Education Center (BTEC), COE
Stem cell models of early human placental development

2:45 PM **Mark Simmers**, Graduate Student in Cowley lab, Department of Biological Sciences, COS
Placental activation of LncRNA Tuna in Cd induced placental insufficiency

Coffee Break & Posters - Duke Energy Hall D

3:00 PM Please take a coffee break and visit the posters and vendors around the room. Retreat will reconvene at 3:30 PM.

Event Schedule

Science Talks: Session 2 - Duke Energy Hall ABC

3:30 PM **Dr. Joe Gage**, Assistant Professor, Department of Crop and Soil Sciences, CALS
How does genomewide allelic variation for gene expression impact phenotype?

3:45 PM **Dr. Jennifer Kuzma**, Goodnight-NCGSK Foundation Distinguished Professor in the School of Public and International Affairs, CHASS, and co-founder and co-director of the Genetic Engineering and Society (GES) Center
Exploring the Nexus of Science and Values in Oversight of Emerging Biotechnologies

4:00 PM **Dr. Emily Delorean**, NSF Postdoctoral Research Fellow in Dr. Amanda Hulse-Kemp Lab, Department of Crop and Soil Sciences, CALS
Representing true plant genomes: Assembly of highly contiguous haplotype-resolved hybrid chili pepper genomes

4:15 PM **Dr. Javier Lopez Soto**, Assistant Professor, Department of Molecular Biomedical Sciences, CVM
Cell-specific epigenetic control of calcium ion channel splicing and function

4:30 PM **Dr. Jonathan Hall**, Assistant Professor, Department of Biological Sciences, COS
C/EBPβ Determines Cellular Outcomes to Environmental Stressors in Skin Through Regulation of the Type 1 Interferon and DNA Damage Response Networks

GGA Paper of the Year Presentation - Duke Energy Hall ABC

4:45 PM All GGA contest winners announced!

4:50 PM **Fausto Rodriguez Zapata and Dr. Allison Barnes,** Genetics Graduate Student and Postdoctoral Scholar in Rubén Rellán-Álvarez Lab, Department of Molecular and Structural Biochemistry, CALS
An adaptive teosinte mexicana introgression modulates phosphatidylcholine levels and is associated with maize flowering time

Adjourn - Duke Energy Hall ABC

5:05 PM Closing remarks by Dr. Fred Gould
Poster awards announced

Thank you for joining us, we look forward to seeing you next year!
Abstracts for all talks and posters are provided on the following pages.

Abstracts Science Talks

Session 1

Session 1

Dr. Orlando Arguello-Miranda, Assistant Professor, Department of Plant and Microbial Biology, CALS

A Pack of Machine Learning Algorithms to Image Complex Microbial Communities

Artificial intelligence approaches, such as Deep Learning, have successfully accelerated the analysis of microscopy images in diverse areas ranging from cancer biology to agriculture. However, the algorithms used in such approaches are frequently optimized to recognize a specific type of cells or complete a particular image analysis task. Although this methodology performs well for data sets with similar cells, it often fails to tackle complex microscopy images, for instance, when analyzing microbial communities or host-pathogen systems. To overcome this limitation, we have created a deep learning approach to analyze complex microscopy images based on a “pack” of machine learning algorithms that target different aspects and objects in a microscopy image and return a single coherent analysis. As proof of principle, we have used this tool to track single cells in engineered fungal communities and image the entire life cycle of baker’s yeast. We envision our approach will be helpful for the standardized imaging of complex microbial communities and the analysis of host-pathogens or symbiotic interactions, among others.

Dr. Elizabeth Jones, Postdoctoral Research Scholar in Zanno lab, Department of Biological Sciences and NC Museum of Natural Sciences Paleontology Research Lab, COS

Ancient DNA: The Making of a Celebrity Science

Ancient DNA research—the recovery of genetic material from long-dead organisms—is a discipline that developed from science fiction into a reality between the 1980s and today. Drawing on scientific, historical, and archival material, as well as original interviews with more than fifty researchers worldwide, Elizabeth Jones explores the field’s formation and explains its relationship with the media by examining its close connection to de-extinction, the science and technology of resurrecting extinct species. She reveals how the search for DNA from fossils flourished under the influence of intense press and public interest, particularly as this new line of research coincided with the book and movie Jurassic Park. This talk will provide a glimpse into the historical and sociological interplay between science and celebrity in the rise of this new research field and in the making of a celebrity science.

Session 1

Dr. Eric Brooks, Assistant Professor, Department of Molecular Biomedical Sciences, CVM

Cellular control of cranial neural tube closure

Defects in cranial neural tube closure account for approximately one third of observed NTDs. However, the cellular mechanisms controlling cranial closure have remained opaque relative to the better understood dynamics of more posterior regions. During neural fold elevation, the highly convex, outwardly curved cranial neural plate undergoes a dramatic inversion of curvature, driving elevation of the lateral borders of the tissue above the midline. This process is a prerequisite for apposition and final fusion of the neural folds, and defects in this process lead to failures in cranial closure. We show that cranial closure is initiated by the sustained apical constriction of a large population of thousands of cells located lateral to the midline, whereas midline cells remain static. This pattern of constriction stands in contrast to those observed in more posterior regions of the neural plate, where cell shape changes at the midline and/or more restricted lateral positions drive neural fold elevation. Further, loss of Ift122 or Ttc21b, two members of the conserved intraflagellar transport A (IFT-A) complex required for cilia function and sonic hedgehog (Shh) signaling, disrupts patterned cell remodeling in the cranial neural plate, resulting in highly penetrant exencephaly. These mutants exhibit a failure of apical constriction associated with defects in apical actomyosin organization in lateral cells, resulting in a failure of the cranial neural plate to convert from convex to concave. These defects are due to a dysregulated pattern of Shh signaling, as transgenic activation of the hedgehog signaling response throughout the midbrain neuroepithelium recapitulates the exencephaly defects of IFT-A mutants. This anti-constrictive role of Shh signaling is likely to explain a large class of exencephalic mutants known or predicted to impact the spatial regulation of Hh signals within the tissue.

Session 1

Dr. Terri Long, Associate Professor, Department of Plant and Microbial Biology, CALS

Iron at the intersection of development and multi-stress resilience

Iron (Fe) is an essential micronutrient that plays critical roles in central metabolic plant processes such as photosynthesis and respiration. The mechanisms by which plants maintain Fe homeostasis are particularly intriguing. While it is relatively abundant, in most soils Fe is insoluble and therefore of limited bioavailability, however excess Fe accumulation in plants can lead to cellular damage. Thus, plants must extract sufficient Fe from recalcitrant growth environments, while also ensuring that Fe content does not exceed a specific range. Arabidopsis and other dicots have developed mechanisms to sense Fe deficiency in the shoot, which triggers roots to solubilize, reduce and uptake Fe across multiple root cell types before transport to the shoot. We have uncovered several molecular mechanisms that control how plants recognize and respond to iron deficiency stress and found new evidence for how specific cell types within the root are involved in these processes. Considering how critical Fe is for overall plant health, it is no surprise that these mechanisms also impact responses to a range of other abiotic and biotic stress conditions.

Session 1

Dr. Bala Rao, Professor, Department of Chemical and Biomolecular Engineering and Director of Academic Programs at Biomanufacturing Training and Education Center (BTEC), COE

Stem cell models of early human placental development

The outer shell of the blastocyst-stage embryo called the trophectoderm is the precursor to the placenta. Human pregnancy begins with specific interactions between the trophectoderm and the epithelial cell layer of the uterine endometrium. Adhesion of the trophectoderm to the epithelium initiates implantation followed by invasion of the epithelium by the embryo. The trophectoderm then gives rise to the epithelial cytotrophoblast, which forms all the trophoblast cell types of the placenta. Abnormalities in embryo implantation and subsequent trophoblast development are associated with many reproductive diseases including infertility, pregnancy loss, fetal growth restriction, and preeclampsia. Yet, the molecular mechanisms that early human placental development are poorly understood due to ethical and legal constraints on research with human embryos, and significant mechanistic differences in implantation between common animal models and humans. Thus, there is a critical need for in vitro models of human placentation that faithfully mimic human physiology, are ethically sound, and experimentally robust, reproducible and easily accessible. I will discuss the use of stem cell models – specifically those derived from trophoblast cells of the human placenta and human pluripotent stem cells – for constructing in vitro models of early placental development.

Mark Simmers, Graduate Student in Cowley lab, Department of Biological Sciences, COS

Placental activation of LncRNA Tuna in Cd induced placental insufficiency

Cadmium (Cd) is a toxic heavy metal found throughout the environment and one of the top ten toxicants of major health concern identified by the World Health Organization. In utero Cd exposure causes fetal growth restriction, malformation, and spontaneous abortion; however, the mechanisms by which its actions impact birth outcomes are poorly understood. Cd is inefficiently transported to the fetus, rather it accumulates in the placenta, suggesting that these negative outcomes are a consequence of disrupted placental function. To understand the impact of Cd on gene expression within the placenta, we developed a mouse model of Cd-induced fetal growth restriction and performed RNA-seq on control and Cd exposed placentae. The top differentially expressed transcript was the long non-coding RNA Tcl1 Upstream Neuron-Associated (Tuna), which was up-regulated 7-fold in Cd exposed placentae. Tuna is a poorly characterized lncRNA, shown to be critical for neural stem cell differentiation and a contributor to both oncogenic and tumor suppressive mechanisms. However, within the placenta, there is no evidence that Tuna is normally expressed or functional at any developmental stage. To determine the spatial expression of Cd-activated Tuna within the placenta, we used in situ hybridization as well as placental layer specific RNA isolation and analysis. Both methods confirmed the absence of Tuna expression within control samples and determined that Cd-induced Tuna expression is specific to the junctional zone of the placenta. Our primary hypothesis is that the activation of Tuna in the junctional zone of the developing placenta leads to the formation of protein complexes capable of disrupting gene expression, contributing to impaired placental function and fetal growth restriction. To test this hypothesis, we over-expressed Tuna in cultured choriocarcinoma cells and compared gene expression profiles to that of control and Cd exposed cells. We demonstrated significant overlap between the genes activated by Tuna over-expression and genes activated by Cd exposure, with enrichment in pathways relevant to oxidative phosphorylation, mitochondrial dysfunction, and NRF2-mediated oxidative stress response, suggesting that Tuna is a key mediator of Cd-induced gene expression changes. We are currently performing experiments using siRNA knockdown to determine the contribution of Tuna to Cd-induced placental dysfunction to further understand the molecular mechanisms linking Cd exposure to negative birth outcomes.

Abstracts Science Talks

Session 2

Session 2

Dr. Joe Gage, Assistant Professor, Department of Crop and Soil Sciences, CALS

How does genomewide allelic variation for gene expression impact phenotype?

As a new assistant professor, the research focus of the group that I lead is actively developing and evolving. Broadly, we use quantitative genetics, genomics, and high throughput phenotyping tools to study gene regulation and genotype-by-environment interactions in crop plants such as maize. In this talk, I will present several short vignettes on questions that we are thinking about, such as: How does allelic variation affect regulation of gene expression and ultimately, phenotype? What are the genetic mechanisms underpinning genotype-by environment interactions? How do environmental conditions during developmentally important time periods impact final phenotypes? These vignettes will provide a high level overview of the topics our group is pursuing and hopefully help identify mutual interests and opportunities for collaboration.

Dr. Jennifer Kuzma, Goodnight-NCGSK Foundation Distinguished Professor in the School of Public and International Affairs, CHASS, and co-founder and co-director of the Genetic Engineering and Society (GES) Center

Exploring the Nexus of Science and Values in Oversight of Emerging Biotechnologies

Emerging biotechnologies like gene-editing, gene drives, and synthetic biology pose challenges to oversight systems that struggle to keep pace with technological advances and operate in complex societal contexts. Yet oversight is important for ensuring the safety of and fostering confidence in novel biotech products. This talk will explore recent controversies and challenges associated with governing the products of gene-editing and other emerging biotechnologies. Many of the tensions and controversies arise at the nexus of scientific information, the interpretation of uncertainty associated with future impacts, and diverse public values. Models for responsible governance from the social sciences will be examined, as well as data on factors that influence public confidence in emerging technologies, to provide examples of how we can move forward with overseeing emerging biotechnologies. This presentation will serve to spark collective discussion on how best to manage the potential risks of emerging biotechnologies while promoting beneficial applications, encouraging responsible innovation approaches, and respecting diverse public values associated with technology development.

Session 2

Dr. Emily Delorean, NSF Postdoctoral Research Fellow in Dr. Amanda Hulse-Kemp Lab, Department of Crop and Soil Sciences, CALS

Representing true plant genomes: Assembly of highly contiguous haplotype-resolved hybrid chili pepper genomes

As sequencing costs decrease and availability of high fidelity, long read sequencing increases, generating experiment specific de novo genome assemblies becomes feasible. In many crop species, the genome of a hybrid or heterozygous individual is of interest. However, most genome assembly methods result in a single haplotype representation that is not biologically true of either haplotype within a diploid individual. The resulting genome assembly is often fragmented and exhibits a mosaic of the two haplotypes, referred to as haplotype-switching. Useful haplotype level information, such as causal mutations and structural variation, is therefore lost. To overcome this challenge, we have applied the animal genome assembly method called trio-binning to an experimentally interesting hybrid individual of chili pepper (Capsicum annuum). Trio-binning uses short read illumina sequences from the parents of the hybrid plant to sort the long read sequences of the hybrid to the corresponding parental haplotype. We crossed two double haploid chili pepper lines to form an F1 hybrid. We sequenced the parents to 45x depth with Illumina short read paired end sequencing. The F1 hybrid was sequenced with PacBio HIFI sequencing to 60x depth. We used the TrioCanu software to sort long reads into parental bins. We then supplied the binned reads to the assembler software, Hifiasm, and generated two highly contiguous genome assemblies with N50 values of 16.2 Mb and 16.1 Mb. After scaffolding with Bionano optical maps, our N50 values increased to 190.0 Mb and 207.9 Mb. We were able to capture 3.11 Gb and 3.17 Gb of the estimated 3.5 Gb chili pepper genome size in the main assemblies. These resulting assemblies represent the true genome structure of the F1 hybrid as well as the parental genomes.

Dr. Javier Lopez Soto, Assistant Professor, Department of Molecular Biomedical Sciences, CVM

Cell-specific epigenetic control of calcium ion channel splicing and function

Only ~7,100 wild cheetahs remain, illuminating the importance of maintaining captive breeding programs against extinction. Unfortunately, ex situ cheetahs suffer from high rates of gastrointestinal (GI) diseases. GI-diseases are the leading cause of ex situ cheetah death or euthanasia worldwide. Each loss has a significant negative impact on maintaining a self-sustaining population because cheetahs have very low genetic diversity. Interestingly, wild cheetahs rarely suffer from GI-diseases, which may be partially due to stark dietary differences. Recent evidence suggests non-digestible animal fibers such as fur, connective tissue, and skin play an important role in cheetah gut health. Managed cheetahs eat primarily ground, raw lean meat that lacks animal fiber components. I hypothesize that low animal fiber diets fed to managed cheetahs have a negative impact on gut microbiota diversity, which leads to dysbiosis of the GI-tract and increased risk of GI-disease. I will characterize the cheetah gut bacteria from non-invasive fecal sampling across three diet types (low fiber -commercial only, moderate fiber - commercial + carcass supplement, and high fiber - carcass only) and three classifications of GI-health (GI-healthy, moderately GI-unhealthy, and severely GI-unhealthy). I will identify differences in bacterial richness, community composition, and relative abundance across these groups. The three specific objectives of this study are to determine if: 1) fecal microbiota differs across three captive diet types containing varying levels of animal fiber, 2) gut microbiota of captive US cheetahs differ from wild Namibian cheetahs and which captive diet, if any, exhibit associated gut microbiota similar to Namibian cheetahs, 3) microbial composition differs across three classifications of GI-health. These objectives will give us a clearer understanding of how diet may affect cheetah gut microbiota and whether these gut microbiotas may be associated with unhealthy GI in our zoo population. The results from this study will set the groundwork for future zoological studies investigating carnivore GI-health risks based on diet. This is the largest non-domestic felid gut-microbiome study to date. This study will aid in understanding gut microbiome dysbiosis and the link with terminal GI-diseases. Findings will guide management to improve cheetah health and well-beingCell-specific alternative splicing modulates myriad cell functions and is disrupted in disease. The mechanisms governing alternative splicing are known for relatively few genes and typically focus on RNA splicing factors. We find that cell and exon-specific DNA hypomethylation permits CTCF binding, the master regulator of mammalian chromatin structure, which, in turn, controls splicing in a subset of sensory neurons. Our studies provide an exciting path for understanding cell-specific control of functional alternative splicing in normal and disease states, and also for revealing translational targets for chronic pain treatment.

Session 2

Dr. Jonathan Hall, Assistant Professor, Department of Biological Sciences, COS

C/EBPβ Determines Cellular Outcomes to Environmental Stressors in Skin Through Regulation of the Type 1 Interferon and DNA Damage Response Networks

Cellular stress, DNA damage, pathogen insult, and immune mechanisms can activate molecular pathways that result in regulated cell death. Increasing our understanding of the pathways that regulate cell death decisions in response to cellular stress is critical as mis-regulated cell death is linked to cancer, neurodegeneration, and autoimmune diseases. The epidermis is the first line of defense to cutaneous microbes, viruses and environmental insults, and keratinocytes play a critical role in the host innate immune response mediated by type 1 interferons (IFNs). In addition to antimicrobial and antiviral responses this same IFN system also mediates diverse cellular and biological responses such as proliferation, apoptosis, senescence, and the DNA damage response. Recent data from our laboratory suggest that the CCAAT/enhancer-binding protein-β (C/EBPβ) transcription factor is a novel regulator of the keratinocyte type 1 IFN response. We observed the conditional deletion of C/EBPβ in mouse epidermis (CKOβ) resulted in increased expression of IFNβ and a subset of ISGs which included cytosolic pattern recognition receptors (PRRs). Cytosolic PRRs sense viral/pathogen RNA and DNA as well as damaged host cytosolic DNA and RNA to trigger a type 1 IFN response. CKOβ keratinocytes treated with direct activators of cytosolic PRRs or challenged with DNA damage displayed increased apoptosis that was dependent on the interferon β receptor (INFAR). We hypothesize that C/EBPβ negatively regulates the type 1 IFN response in keratinocytes and the loss of C/EBPβ sensitizes keratinocytes to pathogen RNA/DNA or damaged host RNA/DNA to induce cell death.

GGA Paper of the Year[1] Presentation

Fausto Rodriguez Zapata and Dr. Allison Barnes, Genetics Graduate Student and Postdoctoral Scholar in Rubén Rellán-Álvarez Lab, Department of Molecular and Structural Biochemistry, CALS

An adaptive teosinte mexicana introgression modulates phosphatidylcholine levels and is associated with maize flowering time

Despite more than a century of genetic research, our understanding of the genetic basis of maize adaptation to new environments is in its infancy. Recent work in many crops has pointed to the potentially important role for introgression in underpinning adaptation, but clear examples of adaptive loci arising via introgression are lacking. Here, we elucidate the evolutionary history of a major metabolic quantitative trait locus (QTL) that we mapped down to a single gene, phospholipase HPC1. Alterations in highland HPC1 are the result of a teosinte mexicana introgression in maize, leading to high phosphatidylcholine levels and improving fitness by accelerating flowering.

1. Barnes AC, F Rodríguez-Zapata et al. 2022. An adaptive teosinte mexicana introgression modulates phosphatidylcholine levels and is associated with maize flowering time. *Proc Natl Acad Sci USA.* **119**(27):e2100036119

Abstracts
Posters

Posters

Avery Roberts, PhD Student in Rodolphe Barrangou Lab, Functional Genomics Graduate Program, CALS

Functional characterization of diverse type I-F CRISPR-associated transposons

CRISPR-Cas systems generally provide adaptive immunity in prokaryotes through RNA-guided degradation of foreign genetic elements like bacteriophages and plasmids. Recently, however, transposon-encoded and nuclease-deficient CRISPR-Cas systems were characterized and shown to be co-opted by Tn7-like transposons for CRISPR RNA-guided DNA transposition. As a genome engineering tool, these CRISPR-Cas systems and their associated transposon proteins can be deployed for programmable, site-specific integration of sizable cargo DNA, circumventing the need for DNA cleavage and homology-directed repair involving endogenous repair machinery. Here, we selected a diverse set of type I-F CRISPR-associated transposon systems derived from *Gammaproteobacteria*, predicted all components essential for transposition activity, and deployed them for functionality testing within *E. coli*. Our results demonstrate that these systems possess a significant range of integration efficiencies with regards to temperature, transposon size, and flexible PAM requirements. Our findings also support the categorization of these systems into functional compatibility groups for efficient and orthogonal RNA-guided DNA integration. This work expands the CRISPR-based toolbox with new CRISPR RNA-guided DNA integrases that can be applied to complex and extensive genome engineering efforts.

Posters #2

Destiny Tyson, PhD Student in Rubén Rellán-Álvarez/ Jim Holland Labs, Genetics Graduate Program, COS

Genetic characterization of a new teosinte self-incompatibility system

Many plant species have genetically controlled self-incompatibility systems that prevent self-fertilization by distinguishing between self vs. non-self pollen. These systems promote outcrossing and limit inbreeding. Cross-incompatibility systems have been described in maize and may have evolved as selfish genes or to prevent the production of unfit inter-population hybrids between maize and its wild relatives, teosintes. Cross-incompatibility also has practical uses in modern maize by protecting organic and specialty corns like popcorn and some sweet corns from being contaminated by undesirable pollen. To date, three cross-incompatibility genes have been described in detail in maize and teosinte: *teosinte crossing barrier 1* (*tcb1*), *gametophyte factor 1* (*Ga1*), and *gametophyte factor 2* (*Ga2*). However, recently, Dr. Jerry Kermicle identified a teosinte-derived line of maize inbred W22 that set seed poorly following self-pollination and likewise after pollination with most of the other inbreds tested. This phenomenon is referred to provisionally as *teosinte self-incompatibility* (*TSI*)". We hypothesize that separate female and male factors interact to determine pollination success. Previous evidence suggests that the female factor that results in self-incompatibility is a single recessive gene. We are analyzing an F_2 population that segregates for the female factor and a backcross-F_1 population segregating for the male factor in an attempt to map the male and female factors. Are they at the same locus or linked? Is *TSI* a new allele of a previously described cross-incompatibility gene, or is it independent? Can we identify a small number of candidate genes for *TSI* using linkage analysis in these populations?

Asa Budnick, PhD Student in Heike Sederoff Lab, Plant Biology Graduate Program, CALS

Identifying Functional CircRNAs in Lotus AM Symbiosis

Understanding how gene expression is regulated is one core of genetic and genomic research. Various classes of RNA molecules have been shown to play important roles in transcriptional and post-transcriptional regulation of gene expression. Circular RNAs are covalently closed single stranded RNA. This unique structure is important to the function of circular RNAs. Circular RNAs participate in gene regulatory networks through a variety of mechanisms which have not been fully established. Some CircRNAs can bind to miRNAs, direct alternative splicing, or regulate translation through antisense activity [1]. Select circular RNAs can form more stable R-loops with genomic DNA than their linear counterparts [2]. RNA sequencing studies have shown that circRNA expression changes across a broad range of stress and developmental conditions in plants. However, in plant systems there are very few circular RNAs with validated functions and mechanisms. We are identifying functional circular RNAs in Lotus japonicus. Lotus japonicus is a model organism for microbial symbioses. We seek to discover and characterize circular RNAs which are important to bacterial and mycorrhizal symbiosis. My poster focuses on the first phase of the project which is to identify circular RNA candidates through applying nanopore sequencing and bioinformatics. Future work will characterize these circRNAs candidates using molecular biology and genetics.

1. Chu, Q. et al. Characteristics of plant circular RNAs. *Briefings in Bioinformatics* **21**, 135–143 (2020).

2. Conn, V. M. et al. A circRNA from SEPALLATA3 regulates splicing of its cognate mRNA through R-loop formation. *Nature Plants* **3**, 1–5 (2017).

Posters #4

Bethany Mostert, PhD Student in Deyu Xie Lab, Plant Biology Graduate Program, CALS

The role of CYP partner enzyme interactions for artemisinin biosynthesis in Artemisia annua

Artemisia annua is a plant that produces a major antimalarial drug known as artemisinin. Since it is important for malaria treatment, research objectives center on generating *A. annua* plants with high artemisinin yields. For these purposes, key enzymes along artemisinin's biosynthetic pathway have been overexpressed in *A. annua*. One such enzyme is CYP71AV1 (amorpha 4, 11-diene oxidase), a member of the CYP superfamily. Like all CYPs, this enzyme oxygenates its substrates through a sequence of electron transfers between its redox partners, itself, and its substrate. In artemisinin biosynthesis, CYP71AV1 accepts electrons from both CPR1 (cytochrome P450 reductase) and CYB5 (cytochrome b5) to convert amophor-4,11-diene to late artemisinin precursors. The specific nature of CYP71AV1-CPR/CYB5 partnerships is still unknown. Uncovering the details of these interactions is important not only for their influence on substrate turnover, but also for reducing oxidative stress caused by enzyme uncoupling or inefficient binding. Thus, improving the effectiveness of the electron transfer between these enzymes is anticipated to increase artemisinin production and decrease oxidative stress. This project intends to further this objective by defining the kinetics of these enzyme interactions and their binding affinity through various biochemical techniques such as surface plasmon resonance (SPR) and spectroscopy.

Hao Wei Teh, PhD Student in Anna Whitfield Lab, Plant Pathology Graduate Program, CALS

Deciphering the interactions between maize mosaic virus and its insect host, Peregrinus maidis

Rhabdoviruses initiate infections by using membrane-embedded viral glycoproteins to attach to cell surface receptors to enter host cells. The interactome between the glycoprotein (G) of maize mosaic virus (MMV) and its insect vector *Peregrinus maidis* was previously documented using a membrane-bound yeast-2-hybrid (MbY2H) assay. Two of the 125 G-interacting proteins, apolipophorin III and cyclophilin A, were selected for further downstream analyses, including validation of their interactions with MMV-G using co-immunoprecipitation assays. A putative antimicrobial peptide (AMP) was also selected from that MbY2H screen for functional analysis. AMPs are products of host innate immune signaling pathways, and have been shown to possess antiviral and antibacterial activity. Our goal is to characterize the AMP and determine its role in the MMV-*P. maidis* interaction. We have performed localization experiments in Sf9 insect cells and validated the efficacy of custom antibodies raised against the AMP. Future experiments are under way to elucidate the function of the AMP in the MMV- *P. maidis* interaction in relation to virus acquisition and transmission. Discovery and characterization of insect-derived AMPs are active areas of research as AMPs are promising candidates for insect pest control strategies.

Posters #6

Amarish Yadav, Postdoctoral Research Scholar in Max Scott Lab, Department of Entomology and Plant Pathology, CALS.

CRISPR-Cas9 based split homing gene-drive targeting doublesex for population suppression of the global fruit pest Drosophila suzukii.

Genetic-based methods offer environmentally friendly species-specific approaches for control of insect pests. One method, CRISPR homing gene drives that target genes essential for development, could provide very efficient and cost-effective control. While significant progress has been made in developing homing gene drives for mosquito disease vectors, little progress has been made with agricultural insect pests. Here we report the development and evaluation of split homing drives that target the *doublesex* (*dsx*) gene in *Drosophila suzukii*, an invasive pest of soft-skinned fruits. In three of the four strains initially produced, females with one copy of the disrupted *dsx* gene were sterile, did not lay eggs and produced the male *dsx* transcript. Females from the fourth strain were fertile and did not make the male *dsx* transcript. DNA sequencing identified a few nucleotide polymorphisms in the region upstream of the transgene that were unique to the fertile strain. Females from four strains with one copy of a modified drive, which included a splice acceptor site, were fertile and did not make the male *dsx* transcript. Homing efficiency was highest in the strains that were hemizygous female fertile but lower than reported in Anopheles species. Finally, mathematical modeling showed that homing gene drive strains could be used for suppression of *D. suzukii* populations with repeated releases at lower release ratios than used for male-only strains. With further improvement in homing efficiencies, our results indicate that CRISPR homing gene drive strains could be used for effective control of *D. suzukii* populations.

Hannah Pil, Undergraduate Student, Rubén Rellán-Álvarez Lab, Department of Molecular and Structural Biochemistry, CALS

Assessing maize HPC1 via heterologous expression in Arabidopsis thaliana HPC1 mutants

Despite its cultivation for thousands of years and across varying climates, the genetic and molecular basis of the adaptation of maize is far from fully understood. We have previously found *Zea mays* to have observable phospholipid variation across maize accessions adapted to different elevations. This is largely attributed to the gene *High PhosphatidylCholine 1* (*HPC1*), which encodes a phospholipase A1 (PLA1) enzyme. The highland *HPC1* allele entered cultivated maize by introgression from wild highland *teosinte mexicana* (*Zea mays ssp. mexicana*) and induces an earlier flowering time in *Zea mays ssp. mays* which is linked to a higher fitness in a cold and high-latitude environment. To further evaluate the function of *HPC1*, we will use mutants of *Arabidopsis thaliana* HPC1 homologs, including SALK lines created via tDNA insertions. The mutant Arabidopsis plants will be assessed against the wild type Columbia-2 for flowering time, cold tolerance, and effects of low phosphate. Additionally, we plan to use the Arabidopsis mutants to investigate the effect of maize *HPC1* alleles of temperate inbred line B73, highland landrace Palomero Toluqueño, and lowland, tropical line CML322 by transforming them into the mutant Arabidopsis homologs. With these studies, we hope to provide further insight into the role of phospholipid metabolism in determining flowering time under different environmental conditions.

Posters #8

Teresa Tiedge, PhD Student in Kelly Meiklejohn Lab, Comparative Biomedical Sciences Graduate Program, CVM

Utilizing soil and dust eDNA to assist in criminal investigations

Soil and dust are often submitted to crime laboratories as trace evidence and can be used to link an individual to a crime scene or to determine an evidentiary sample's origin. Methodologies that are routinely applied to analyze these geologic materials aim to characterize their physical properties (*e.g.* color and pH) and inorganic components (*e.g.* mineral content). However, sample size is often a limiting factor in these analyses; supplemental methods requiring a small amount of geologic material as input could provide additional evidentiary information. DNA metabarcoding is a commonly used approach to identify the biological taxa that are present in various environmental samples by amplifying and sequencing short, informative regions of the genome and is not restricted by sample amount. The goal of this research was to determine the utility and stability of environmental DNA from four biological taxa associated with soil and dust for sample-to-sample comparisons and sample origin. In this study, five mock geologic evidence items were collected monthly from an agricultural and urban location over a one-year period. DNA metabarcoding was applied to characterize bacteria (*16S*), fungi (*ITS1*), arthropods (*COI*), and plants (*ITS2*, *trnL*) associated with each sample (n, 1026). Libraries were generated using custom indexed primers and subsequently sequenced using the Illumina MiSeq. Raw sequencing reads were processed through a bioinformatic pipeline that removes primer sequences, identifies amplicon sequence variants (ASVs) via DADA2, and searches ASVs against GenBank for taxonomic identification. This presentation will focus on the experimental design and workflow and will include preliminary data.

Jacob Deslauriers, Ph.D. Student in Kurt Marsden Lab, Genetics Graduate Program, COS

Cyfip2 controls the acoustic startle threshold through Rac1 and FMRP

Amidst a perpetual stream of sensory stimuli, animals must distinguish between salient and innocuous stimuli to survive. For instance, sudden loud sounds can trigger a highly conserved startle response that enables animals to evade danger. An appropriate threshold must be established such that only threatening stimuli elicit the response. Dysregulation of the startle threshold can produce hypersensitivity associated with autism and anxiety-related disorders, yet the genetic regulation of this threshold is poorly understood. A forward genetic screen in zebrafish identified a hypersensitive mutant harboring a causal nonsense mutation in the *Cytoplasmic Fragile X Mental Retardation Protein Interacting Protein* (*FMRP*) *2* (*cyfip2*). However, the molecular pathway(s) by which *cyfip2* regulates the startle threshold remains unknown. Cyfip2 binds Rac1 to promote actin polymerization and regulates RNA translation through FMRP. To determine which pathway Cyfip2 uses to regulate the startle threshold, we created heatshock-inducible *cyfip2* rescue constructs with specifically mutated residues that impair interaction with Rac1 or FMRP. While wild-type Cyfip2 restores normal sensitivity in *cyfip2* mutants, mutants expressing altered Cyfip2 remain hypersensitive, indicating that Cyfip2 must interact with both Rac1 and FMRP to establish the startle threshold. To identify proteins downstream of Cyfip2-Rac1/FMRP interactions that may directly affect neuronal circuit function to regulate the startle threshold, we have performed a candidate-based drug screen as well as a discovery proteomics approach to define the molecular pathways impacted by loss of *cyfip2*. Together, my work will determine how *cyfip2* regulates a clinically relevant behavior and identify molecular mechanisms that set the acoustic startle threshold.

Posters #10

Cole Butler, PhD Student in Alun Lloyd Lab, Biomathematics Graduate Program, COS

Rethinking mosquito control: Gene drives and the consequences of over-suppression

Suppression gene drives (SGDs) spread a deleterious genetic cargo through a population by biasing their own inheritance. This technology offers a promising solution to the burden posed by crop pests and vectors of important human diseases. Presently, theoretical and experimental studies favor SGD constructs that rapidly eradicate a population. If drive killing occurs faster than drive spreading, however, the target species can be locally eradicated before it is able to spread the SGD to the rest of the population. This phenomenon, in which drive killing hinders overall gene drive performance, is referred to as over-suppression. The consequences of over-suppression can be dire, especially if the target organism is a vector of human disease, such as mosquitoes. How might we balance SGD lethality with spreading potential? To help answer this question, I developed a spatially explicit patch-based model to simulate the spread of a SGD in a wild-type population with heterogeneous population density. Using a simple gene drive example, numerical experiments are performed to establish under what conditions over-suppression occurs. Thus, we can determine how deleterious a gene drive can be before over-suppression occurs. Future work will extend this analysis to more complicated SGDs to assess their potential in more realistic conditions.

Dillon Lloyd, Phd Student in Alison Motsinger-Reif Lab, Bioinformatics Graduate Program, NIEHS

Questionnaire-based polyexposure assessment outperforms polygenic scores for determination of type 2 diabetes in a multi-ancestry cohort

Objective. Environmental exposures may have greater predictive power for type 2 diabetes than polygenic scores (PGS). Studies examining environmental risk factors, however, have included only individuals with European ancestry, limiting the applicability of results. We conducted an exposome-wide association study (ExWAS) in the multi-ancestry Personalized Environment and Genes Study to assess the effects of environmental factors on type 2 diabetes.

Research Design and Methods. Using logistic regression, we identified associated exposures with type 2 diabetes, adjusting for age, BMI, income, sex and race. To compare cumulative genetic and environmental effects, we computed an overall clinical score (OCS) as a weighted sum of BMI and prediabetes, hypertension, and high cholesterol status and a polyexposure score (PXS) as a weighted sum of 13 environmental variables. Using UK Biobank data, we developed a multi-ancestry PGS and calculated it for participants.

Results. We found 76 significant associations with type 2 diabetes, including novel associations of asbestos and coal dust exposure. OCS, PXS, and PGS were significantly associated with type 2 diabetes. PXS had power to determine associations, with larger effect size and greater power and reclassification improvement compared to PGS. Results for all scores displayed differences for Black and White subgroups.

Conclusions. Our findings in a multi-ancestry cohort elucidate how the odds of type 2 diabetes diagnosis can be attributed to clinical, genetic, and environmental factors and emphasize the need to include exposome data in disease-risk association studies. Race-based differences in predictive scores highlight the need for genetic and exposome-wide studies in diverse populations.

Posters #12

Marlonni Maurastoni, Postdoctoral Scholar in Anna Whitfield Lab, Department of Entomology and Plant Pathology, CALS

Identification of tomato spotted wilt virus glycoprotein G_N domain responsible for binding to thrips guts

Tomato spotted wilt virus (TSWV) acquisition is mediated by the molecular interaction between the virus membrane glycoprotein G_N, which serves as a viral attachment protein, and western flower thrips proteins in the thrips midgut. It has been shown that an excess of G_N ectodomain can block virus acquisition by thrips when fed exogenously to thrips prior to or simultaneously with TSWV. Also, transgenic tomato plants expressing a recombinant form of the ectodomain of TSWV G_N glycoprotein, G_N-S, interfere with TSWV acquisition and transmission by thrips. These results support the idea that G_N is the TSWV structural protein required for virus acquisition. To identify the region of G_N interacting with thrips gut cell receptors, we bio-panned thrips with a phage library displaying overlapping peptides covering the entire TSWV G_N protein. After three rounds of bio-panning, we identified three main regions spanning G_N, the first one covering 55 aa on the N-terminal region; the second covering 40 aa on the C-terminal domain (CTD); the third one covering 35 aa on the first transmembrane domain. Ab initio structural predictions show that all three peptides present a conserved β-hairpin followed by a coil region (loop) and a C-terminal α-helix. Superimposition of the peptide's predicted fold onto the crystal structure of the TSWV G_N ectodomain revealed structural similarity between the CTD peptide and the β-sandwich in the CTD of G_N structure. These results support the CTD domain of G_N as a possible attachment domain for receptors in thrips guts. The next step is to use these peptides to study TSWV binding and entry into thrips and for the development of new strategies for virus and pest control.

Montana Knight, PhD Student in Colleen Doherty/Dahlia Nielsen Labs, Bioinformatics Graduate Program, CALS/COS

Linking essential cis regulatory information to gene expression pattern using machine learning

Cis regulatory elements are key components in regulating gene expression patterns. These elements include k-mer sequence motifs located within nearby regulatory regions that aid in recruiting transcription factors. In plants, these motifs are necessary to activate everything from photosynthetic machinery to stress response pathways. Linking motifs to certain gene responses allows for the creation of synthetic promoters that can fine-tune expression for a gene of interest in a number of conditions. However, identifying critical motifs is not an easy task. Popular motif finding tools may use expectation maximization or greedy algorithms to find motifs in regulatory regions. These tools work well and in relatively low computational time, but lack the ability to find repressor motifs, explore combinatorial effects between motifs, or incorporate other valuable information like location. A machine learning approach to motif finding may be able to address some of these pitfalls. This study developed and employed a machine learning method to identify motifs in a synthetic dataset. General machine learning algorithms were tested for their ability to identify artificially inserted motifs. XGBoost, a gradient boosting algorithm, was able to accurately and quickly identify the motifs in the synthetic sequences. Therefore, XGBoost was the method chosen for further development. The identified motifs were then further examined using the R package xgboostexplainer. The development of the XGBoost model demonstrates a new approach to identify motifs linked to gene expression patterns, which can lead to more responsive synthetic promoters in any desired stress condition.

Posters #14

Isabella Livingston, Ph.D. Student in Matthew Breen Lab, Genetics Graduate Program, CVM

Using genetic and genomic tools in health and conservation assessments of the Galápagos sea lion

Darwin's discovery of the Galapagos archipelago revolutionized science and our understanding of the natural world. Since then, the Galapagos islands have remained one of the most preserved natural habitats in the world. Unfortunately, they face growing threats because of anthropogenic impacts. Invasive species and environmental threats threaten the impressive biodiversity and high levels of endemic species, and innovative measures are needed to mitigate these detrimental impacts. In May of 2022, I, along with members of NCSU's CVM, traveled to the "enchanted islands" to conduct health assessments for a characteristic species within the archipelago, the Galapagos sea lion. We collected whole blood samples, nasal swabs, and hair from 28 different specimens and will be utilizing genetic/genomic methodologies to analyze Galapagos sea lion health comprehensively. With genetic tools, I have already validated the identification of canine heartworm disease for the first time in this species and in a wild pinniped. Additionally, I am creating assays for testing this parasite in sea lion whole blood. We will also be conducting next-generation sequencing for broad pathogen and genetic health analyses, and I am developing a protocol for pathogen detection from marine mammal nasopharyngeal swabs. This study will provide context for conservation of a Galapagos pinniped, and methodologies for future analyses of other Galapagos wildlife.

Taylor Kennedy, PhD Student in Orlando Arguello-Miranda Lab, Microbiology Graduate Program, CALS

Multi-Model approach for segmenting images of mixed cell types and microorganisms

Artificial intelligence (AI)-driven image segmentation is a powerful tool for microscopy analysis in cell biology, biomedicine, and microbial ecology. In microscopy, image segmentation aims to distinguish objects, especially cells, from the image background or other objects. A current challenge for AI-based segmentation is the analysis of complex images composed of diverse cell types or microorganisms. Here, we developed an approach that integrates multiple deep-learning models to produce high accuracy segmentations of complex microscopy images with minimal human input. We acquired extensive data sets composed of time series for life cycle transitions of the yeast *Saccharomyces cerevisiae*. We then used U-NET and Cellpose model architectures to train Convolutional Neural Networks (CNNs) based on human-generated training datasets. The final model combined the results from CNNs tailored to different life cycle stages or cell types of *S. cerevisiae*. This approach enabled the segmentation and tracking of individual cells throughout their entire life cycle despite size and morphological changes. Furthermore, our CNN model could segment images from other organisms such as *Candida glabrata* and *Colletotrichum acutatum* conidia with high accuracy, indicating the applicability of a combined CNN segmentation model to microorganisms with varied morphologies. Our image segmentation approach will expand the capacity to study complex systems, such as microbial communities, host-pathogen interactions, and tissues; which are relevant for cell biology, biomedicine, and microbial ecology.

Posters #16

Ozge S Kuddar, Ph.D. Student in Kelly Meiklejohn/Benjamin Callahan Labs, Bioinformatics Graduate Program, CVM

Curation a plant-focused reference sequence database for ITS2 and trnL regions

With advances in sequencing technologies, DNA metabarcoding offers new perspectives in biodiversity research. The current project looks to compare DNA metabarcoding with traditional morphological approaches for identifying pollen from diverse surface soils. To achieve this, the nuclear *ITS2* and the chloroplast *trnL* (UAA) intron regions were utilized as barcodes. While there are ~94 million available plant sequences on INSDC Genbank, downloading this entire dataset for comparison of unknown sequences was not feasible. The data consists of different molecule types, data classes and sequences from various genetic compartments. Additionally, Genbank sequences differ in terms of sequencing methods, format, and quality. To enable straight-forward and accurate identification of pollen in this study, it was imperative to curate a plant-focused reference *ITS2* and *trnL* sequence database.

OBITools3-ecoPCR (in silico PCR) (https://metabarcoding.org/obitools3) was used to build a reference database. First, the nucleotide sequences (separated by data classes) and the taxonomy file (from NCBI) were downloaded and imported to obitools3's ad hoc data management system. ecoPCR, a script in obitools3 that simulates PCR reactions, was used to identify sequences spanning *ITS2* and *trnL* targeted in this study. Custom Python and bash scripts were used to remove duplicate sequences for a single taxon. The final step was the curation and addition of partial *ITS2* and *trnL* partial sequences, those that lack the primer binding sites, into the database. This poster will provide more details on the steps of this workflow and the resulting number of reference sequences in the curated database.

Sydney Harned, PhD Student in Martha Burford Reiskind Lab, Biology Graduate Program, COS

Let's talk about sex, baby: Using genomic tools to characterize temperature-dependent sex determination in southern flounder

Targeted both in commercial and recreational fisheries, the high demand for flounder harvest has led to overexploitation and severe population decline throughout their range. This continued decline is likely due to a greater number of males compared to females in wild populations. Uneven sex ratios are a result of temperature dependent sex determination (TSD), where genetically female flounder possessing two X chromosomes develop into males when exposed to high temperatures early in development. The proposed study will use genetics to investigate temperature-dependent sex determination in southern flounder. Specifically, two goals will be addressed: 1) investigate population structure throughout the range of southern flounder using high-throughput genomic sequencing, and 2) identify male sex-determining genes. Results from this study will illuminate stock structure and signatures of selection across populations of southern flounder. Additionally, identifying sex-determining genes will allow for selective breeding of females with XX sex-reversed males in aquaculture settings, resulting in female-only broodstock of large, fast-growing fish.

Posters #18

Carley Huffstetler, PhD Student in Nanette Nascone-Yoder Lab, Genetics Graduate Program, CVM

Investigating left-right asymmetry in extracellular matrix glycoprotein distribution during Xenopus stomach curvature

Proper left-right (L-R) asymmetry is crucial for normal organogenesis; thus, understanding the embryonic events that generate L-R asymmetry is vital for elucidating the etiology of laterality-related birth defects. In the digestive tract, morphological asymmetry is first evident as the stomach develops its hallmark leftward curvature. This shape change is believed to be driven by L-R asymmetric endoderm cell shape changes and rearrangement events that preferentially expand the left stomach epithelium more than the right. Because the extracellular matrix (ECM) is known to be intimately involved in cell shape change and rearrangement, I hypothesized that the ECM composition in the developing stomach may also be L-R asymmetric. To test this possibility, immunohistochemical staining patterns of the ECM glycoproteins laminin and fibronectin were analyzed on transverse sections through the *Xenopus* stomach during curvature. While no L-R differences were observed in the number of layers of laminin, the space between the laminin layers was found to be asymmetric, i.e., greater on the right side, corresponding with a previously unrecognized thickening of the right mesoderm. Moreover, at stages prior to curvature, and persisting throughout curvature morphogenesis, fibronectin was more broadly distributed, diffuse, and disorganized in the right mesoderm while, in the left side, fibronectin fibrils were compact and highly organized. These novel L-R differences suggest that the endoderm cell dynamics responsible for inducing stomach curvature may be driven by asymmetric secretion of ECM components in the adjacent mesoderm. Future studies will test the functional roles of ECM glycoproteins in stomach curvature morphogenesis.

Antonio Alonso, High School Student in Albert Keung Lab, Department of Chemical and Biomolecular Engineering, COE

Improving DNA-based information storage systems with solid-phase transfers

Every day, humans produce over 2.5E9 GB of data. Meeting humanity's exponentially growing demand for archiving data requires a breakthrough in the density and longevity of information storage technologies. DNA as a storage medium offers orders of magnitude improvements in data density compared to current electronic storage technologies. This property of DNA, in addition to its longevity, makes it a promising candidate for extreme-scale archival data storage. Still, key limitations have prevented the adoption of DNA as a storage medium. Notably, current DNA synthesis and sequencing methods are bottlenecks that make scaling up DNA's use as a storage medium prohibitively expensive. As opposed to synthesizing DNA one nucleotide at a time, as is currently performed, we aim to assemble DNA from small chunks with the help of laser printer technology. Laser printers print substantially faster than inkjet printers and typically offer better-quality prints, making their underlying technology promising for helping to co-locate small chunks of DNA accurately. Here, we demonstrate electrostatic transferring of DNA in a manner similar to laser printing, and we analyze the impact of toner on enzymatic reactions. Through this work, we seek to accelerate the adoption of DNA as a storage medium by harnessing the useful properties of solid-phase electrostatic printing.

Posters #20

Cesar A. D. Xavier, Postdoctoral Scholar in Anna Whitfield Lab, Department of Entomology and Plant Pathology, CALS

Knockdown of exportin 1 negatively affects reproduction of the corn planthopper, Peregrinus maidis

Exportin 1 is a nuclear receptor mediating the nuclear export of hundreds of proteins and RNA from the nucleus to the cytoplasm. It regulates several processes in the cell, including a general antiviral response and larval progression in *Drosophila melanogaster*. We evaluated the effect of exportin 1 knockdown on physiology of the corn planthopper, *Peregrinus maidis*. Analysis of exportin 1 expression in whole bodies of insects across different life stages, nymphs (N1 to N5) and adults (males and females), revealed no difference in gene expression. However, exportin 1 did exhibit tissue specific expression patterns with higher expression in the ovaries compared to the guts of adult females. Survival rate was significantly lower for exportin 1 knockdown females, compared to controls, but no effect was observed for males. Adult females with exportin 1 knockdown were significantly heavier and had a larger abdomen compared to controls at four, eight and 12 days after dsRNA injection. Consistent with increase in weight, glyceride content was specifically and significantly increased in exportin 1 knockdown females, but not in males. Exportin 1 knockdown negatively affected *P. maidis* reproduction. Ovary development was significantly inhibited, and mature eggs were not observed in adult females with exportin 1 knockdown. Consistent with a major role of exportin 1 in ovary function and egg production, oviposition and egg hatch in plants was dramatically reduced in dsRNA exportin 1 treated insects. Altogether, these results suggest that exportin 1 is a positive regulator of *P. maidis* reproduction.

Anna Yaschenko, PhD Student in Jose Alonso/Anna Stepanova Labs, Plant Biology Graduate Program, CALS

Leveraging synthetic promoters to control gene expression

Understanding gene expression regulation is central to the development of biotechnological solutions for several pressing agricultural problems. Synthetic biology methods can be applied in a variety of agricultural research projects to create a multiplex of customizable promoters that drive the expression of genes of interest. However, the relationship between promoter elements within the promoter of a gene and the level of that gene's expression is not well defined. To begin tackling this question, we are building synthetic promoters harboring up to ten transcription factor binding sites using GoldenBraid technology and cloning these promoters upstream of a reporter gene. This is done by inserting one to ten copies of a protospacer, a 23bp recognition sequence for the dCas9-based synthetic transcription factor, into a neutral promoter sequence that has no known transcription factor binding sites in plants. In this study, we assemble the reporter gene and transiently co-express it along with a dCas9 activation system in Nicotiana benthamiana to test the effects of multiple promoter characteristics on reporter expression. Preliminary results show that constructs with protospacers in either the sense or antisense orientation confer comparable levels of gene expression, an increase in protospacer copy number boosts reporter activity, and that there may be a decrease in expression as the distance between the protospacer and the core promoter increases. This work is expected to shed light on the rules of nature dictating promoter architecture that, in turn, determine gene expression levels, ultimately paving the way to the creation of tunable expression systems.

Posters #22

Clara Tyson, Undergraduate Student in Anna Whitfield Lab, Department of Entomology and Plant Pathology, CALS

RNAi-mediated knockdown of RUVBL1 negatively influences reproduction and supports increased maize mosaic virus accumulation in its insect vector Peregrinus maidis

RUVBL1 (TIP49, Rvb1, Pontin) encodes an ATPase of the AAA+ protein superfamily involved in several cellular functions, including chromatin remodeling, control of transcription, and cellular development (motility, growth, and proliferation), likely in part via target gene repression and potentially via DNA helicase activity. RUVBL1 demonstrated broad antiviral activity against animal arthropod-borne viruses, including positive- and negative-sense RNA viruses, in a RNAi screen in *Drosophila*. Maize mosaic virus (MMV) is an *Alphanucleorhabdovirus* transmitted in a circulative propagative manner by the corn planthopper *Peregrinus maidis* (*P. maidis*). Considering the antiviral role of RUVBL1 and its broad cellular functions, we evaluated the effect of *RUVBL1* knockdown on MMV accumulation and physiology of its insect vector *P. maidis*. *RUVBL1* knockdown was associated with increased MMV accumulation in *P. maidis*, with significantly higher viral titer observed at 12, 15 and 18 days after dsRNA and virion co-injection. *RUVBL1* knockdown was correlated with visible morphology changes in female insects, with significant increases in body mass observed at 12-days post dsRNA injection. *RUVBL1* knockdown significantly affected ovary morphology, with no mature oocytes observed at eight and 12 days after knockdown. Significantly fewer eggs were laid in plants at 12 days post-injection, and dramatic reductions in egg hatching were observed at four, eight and 12 days after *RUVBL1* knockdown, in comparison to controls injected with dsGFP. These results suggest that RUVBL1 has antiviral activity against phytoarboviruses, and indicate an extension of its functions as a positive regulator of *P. maidis* reproduction.

Yu-Ming Lin, PhD Student in Fikret Isik Lab, Forestry and Environmental Resources Graduate Program, CNR

Correspondence between a low-density targeted genotyping-by-sequencing (GBS) and high-density SNP array in loblolly pine

A good genotyping technology can facilitate genomic selection (GS) for quantitative traits, and it should have characteristics of being repeatable, reliable, high-throughput and cost-effective. A medium-density genotyping SNP Axiom array (Pita50K) was released in 2019 and has been utilized in routinely for loblolly pine (*Pinus taeda* L). A small subset of high quality SNP markers from the array were amplified using the AgriSeq™ Genotyping-By-Sequencing (GBS) technology by Thermo Fisher for quality control operations in pine breeding. The utility of the AgriSeq markers (995 SNP) was validated on a 192 pine samples with known pedigree with respect to (1) marker quality measures, (2) realized genomic relationships, and (3) genetic clustering of families in comparison with two marker sets from Pita50K array. Realized genomic relationships constructed from the array, a subset of SNP loci (995) from the array, and AgriSeq995 markers produced similar coefficient of coancestry. The inbreeding coefficients from AgriSeq995 markers were slightly inflated, with a mean of 1.078. Three marker sets also clustered full-sib families similarly based on the principal component analysis. The AgriSeq995 targeted GBS platform is highly cost-efficient, reliable, and quick to genotyped large number of trees for pedigree control. Research is underway to validate the platform for other applications in pine breeding.

Posters #24

Megan N. Dillon, PhD Student in Martha Burford Reiskind and Matthew Breen Labs, Genetics Graduate Program, COS/CVM

Population structure and search for outlier loci in dogs living in Chernobyl

The 1986 disaster at the Chernobyl Nuclear Power Plant (NPP) released ~5,000 petabecquerels of radioisotopes into the surrounding environment. Tens of thousands of residents in nearby towns were evacuated, and an area comprising a roughly 30 km radius surrounding the NPP was established at the Chernobyl Exclusion Zone (CEZ). Early remediation efforts and the abandonment of various military and industrial complexes have further contributed to the contamination of the area, with the release of heavy metals, organics, pesticides, and other environmental toxins. Despite culling attempts towards domestic animals and livestock shortly after the accident, hundreds of dogs, presumably descendants of abandoned household pets, now inhabit the CEZ. For this study, we focused on two populations of these dogs: one living within the NPP area and one living approximately 16.5 km away in Chernobyl City. Through genetic analyses, we aimed to quantify the degree of local adaptation to the prolonged exposure to various hazardous materials. We assessed the genetic structure of these two populations and identified outlier loci which highlight genomic regions under candidate positive selection. Despite some overlap and genetic similarity, we found evidence of significant genetic differentiation. Outlier loci pointed towards putative candidate genes, with 52 genes having potentially relevant Gene Ontology terms towards the exposures, such as response to ionizing radiation. We will investigate these genes to search for variants that could be under selection. This study will add to the understanding of how prolonged exposure to radiation, heavy metals, and other contaminants can impact the genome.

Nirwan Tandukar, PhD Student in Rubén Rellán Álvarez Lab, Functional Genomics Graduate Program, CALS

Improving Candidate Gene Discovery by Combining Multiple Genetic Mapping Datasets

Complex biological processes involve multiple phenotypes. The Genome-Wide Association Studies (GWAS) are useful for detecting associations between SNPs and the phenotype. Comprehensive research on the matter is laborious, costly, and labor extensive due to the overwhelming number of genes and their regulatory networks. Hence, the need for developing a robust statistical framework that can combine individual p-values to aggregate multiple small effects and redefine the order of emphasis of genes. We will use the Cauchy distribution to define a test statistic as a weighted sum of Cauchy transformation of individual p-values. This will be used to combine p-values across different datasets. We are currently working towards creating such a framework through R packages which will be publicly available. The role of lipid variation in adaptation to low phosphorus and cold environments in Sorghum is not well known. We have previously identified lipid variation in maize associated with adaptation to Mexican highlands. We intend to test whether lipids in Sorghum play a similar adaptive role in Africa, and whether there is convergence in molecular mechanisms between the two crops. We will use three distinct high-dimensional genetic datasets to perform environmental GWAS for availability of soil Phosphorus (soilP, R package), metabolomics GWAS using various lipid concentrations (LC/MS), and Fst measurement between accessions adapted to high and low Phosphorus. Finally, a selection experiment field trial under low and high phosphorus, will tell us if the best and the worst performing plants have difference in allelic frequencies in association hits related to adaptation.

Posters #26

Khushi Patel, Undergraduate Student in Kelly Meiklejohn Lab, Department of Population Health and Pathobiology, CVM

Interlaboratory study to assess practical utility of OSAC proposed standard 2021-S-0006 (Standard for the Use of GenBank for Taxonomic Assignment of Wildlife)

Wildlife forensic laboratories are frequently asked by law enforcement to confirm the taxonomic identity of material submitted as evidence. When taxonomic assignment based on morphology is not possible, DNA-based approaches are typically employed. Practitioners often rely on comparing their unknown evidence sequence to public sequence databases such as GenBank, to permit taxonomic assignment. To address the use of GenBank in forensic casework, the Organization of Scientific Area Committees for Forensic Science (OSAC) Wildlife Forensic Biology subcommittee developed *2021-S-0006 Standard for the Use of GenBank for Taxonomic Assignment of Wildlife* (currently an OSAC Proposed Standard). To address the practical utility of *2021-S-0006*, an interlaboratory study was conducted. To execute this, Sanger sequence data for informative mitochondrial loci were generated from forty diverse wildlife species encountered in forensic casework. Eleven wildlife forensic laboratories from across the globe were recruited to participate, and were sent ten unknown sequences to analyze using *2021-S-0006*. Taxonomic assignments generated by each laboratory were compared a) to the true identity to determine correctness, and b) across laboratories to assess congruence. Results were also independently evaluated to determine whether laboratories were interpreting the criteria of the standard correctly and reporting to the appropriate taxonomic level (*i.e.*, species level or higher). When following *2021-S-0006*, laboratories that reported to the species level were correct in 98.4% of cases and high congruence was observed between laboratories. In this presentation, details on the outcomes of the interlaboratory study will be presented along with lessons learned for conducting interlaboratory studies.

Jessica Martinez-Baird, PhD Student in Caroline Laplante Lab, Genetics Graduate Program, CVM

Robust mechanism of tension production compensates for increasing the dosage of myosin Myp2 in the cytokinetic contractile ring

Cytokinesis partitions a dividing cell via the constriction of a contractile ring of actin filaments and myosin motor proteins. Yet, how myosins drive the production of tension in the contractile ring remains unknown. In fission yeast, the type II myosin Myp2 contributes to ~30-40% of the total tension of the constricting contractile ring. Based on this role, we hypothesized that the amount of tension generated by the contractile ring scales up when *myp2* is doubled (*2xmyp2*). Fluorescence intensity comparisons showed that integrating a second copy of *mEGFP-myp2* into a cell expressing endogenous mEGFP-myp2 increased the amount of fluorescence signal by ~1.27X. This incremental increase in Myp2p had no measurable impact on the constriction rate of rings in *2xmyp2* cells, which suggests the total amount of tension generated by *2xmyp2* rings is comparable to wild-types. However, increasing Myp2p caused its distribution in the ring to shift from an uneven crescent shape in wild-types to a relatively uniform circle. This re-arrangement suggests an underlying change in the mechanical properties of contractile rings in *2xmyp2* cells, which we measured using laser ablation. Our data showed *2xmyp2* rings had an increase of ~90% in viscous drag, consistent with Myp2 crosslinking actin filaments. These results highlight both the strong relationship between Myp2 and viscous drag, such as a ~27% increase in Myp2p resulted in a dramatic increase in viscous drag, and the robust nature of the compensatory mechanisms in the contractile ring that can overcome significant increases in viscous drag to conserve constriction rate.

Posters #28

Nathan Harry, PhD Student in the Zakas Lab, Functional Genomics Program

The making of a developmental dimorphism

The mechanisms underlying the divergence and evolution of developmental programs are poorly understood. Finding a suitable model to study these mechanisms alone is a challenging barrier to progress in this area. Species too extensively diverged make poor models due to the noise introduced by genetic and regulatory differentiation unrelated to developmental programs. The marine annelid *Streblospio benedicti* provides a glimmer of hope because it is poecilogonous: there are two developmental pathways exhibited by members of this single species. Using bulk RNAseq, differential expression analysis, and expression profile clustering, we explore how the two developmental morphs found in *S. benedicti* differ over the course of 6 key time points throughout their development. We find that the differences in early development are characterized by many small expression changes which likely indicate small shifts in the timing of developmental events. These small differences even out over the course of development and resolve into far fewer, but much larger expression differences in later stages the functions of which match expectations based on morphological differences at those stages. This study provides evidence that early developmental divergence happens by way of small shifts in the expression of regulatory genes that have large effects on a small number of pre-existing structural genes that mediate morphological changes which are subsequently selected upon. It does not provide evidence to support the hypothesis that developmental divergence involves extensive gene co-option or by the emergence of novel genes.

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Have a question? Contact one of the Executive Committee members!

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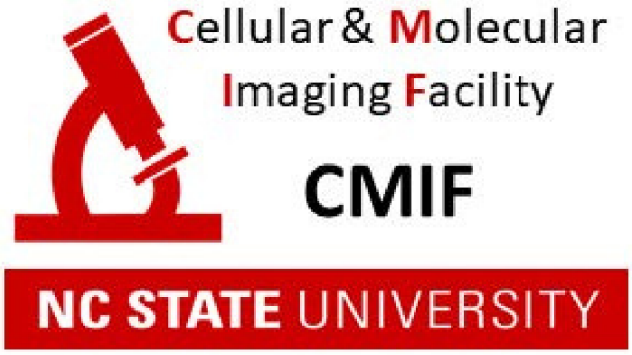

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