Genetics and Genomics Initiative

2nd ANNUAL RETREAT

Monday, August 19, 2019

The Dorothy and Roy Park Alumni Center

Sponsors:

The Genetics and Genomics Initiative (GGI) welcomes you to the 2nd Annual Retreat!

Schedule of Events

Registration - Foyer/Great Reception (Room 112)

8:30 AM	Registration check-in Poster Presentation set-up in Foyer and Great Reception Breakfast buffet provided by the Park Alumni Center

Welcome - Great Reception (Room 112)

9:00 AM	Opening remarks by Dr. Fred Gould Welcome from Dean Linton, Dean McGahan, and Associate Dean Meurs

The Future of GGI - Great Reception (Room 112)

9:20 AM	Updates from GGI Committees and Activities - Seminar Series - Research Interest Groups (RIG) - Graduate Program and Student Recruitment - Future Leadership

Coffee Break - Foyer

10:30 AM	Enjoy Coffee! – Refreshments provided by the Park Alumni Center

Keynote Speaker - Great Reception (Room 112)

11:00 AM	Dr. Francis Ligler, Lampe Distinguished Professor of Biomedical Engineering, NCSU *On being a Gemini: Combining science and engineering to help people*

Lunch and Poster sessions - Great Reception (Room 112)

12:00 PM — Lunch provided by the Park Alumni Center
Poster presenter numbers and names listed on pg. 5-7

Science Talks: Session 1 - Great Reception (Room 112)

1:30 PM — Dr. Catherine Hoyo, Professor and Director of Epidemiology and Environmental Epigenomics Laboratory, Co-Director of Integrative Health Science Facility Core in the Center for Human Health and Environment
Dept. of Biological Sciences, COS
Gestational Cadmium Exposure, Epigenetic Response and Metabolic Dysfunction in Black, Hispanic and White offspring

1:45 PM — Dr. Jason Delborne, Associate Professor of Science, Policy, and Society, Dept. of Forestry and Environmental Resources, CNR
Public engagement when biotechnology goes wild

2:00 PM — Dr. Javier Brumos (Alonso-Stepanova Lab), Postdoctoral Scholar, Dept. of Plant and Microbial Biology, CALS
Local auxin biosynthesis is a key regulator of plant development

2:15 PM — Dr. Martha Buford Reiskind, Research Assistant Professor / Coordinator for the GGI Graduate Program, Dept. of Biological Sciences, COS
Evolution in Changing Environments

2:30 PM — Dr. Nanette Nascone-Yoder, Associate Professor, Dept. of Molecular Biomedical Sciences, CVM
Functional Analyses of Candidate Human Birth Defect Genes

2:45 PM — Dr. Sumit Dhole (Gould Lab), Postdoctoral Scholar, Dept. of Entomology and Plant Pathology, CALS
Tethered homing gene drives for localized population suppression

Poster Presentations/Coffee Break - Foyer

Enjoy Coffee! – Refreshments provided by the Park Alumni Center

3:00 PM Poster Presentations
Poster presenter numbers and names listed on pg. 5-7

Science Talks - Session 2 - Great Reception (Room 112)

3:30 PM Dr. Jack P. Wang, Assistant Professor, Director of Forest Biotechnology Group, Dept. of Forestry and Environmental Resources, CNR
Strategic Engineering of Populus For Bioenergy and Bioproducts Through Multi-Omics Integration In Lignin Biosynthesis

3:45 PM Dr. Caiti Smukowski Heil, Assistant Professor, Dept. of Biological Sciences, COS
The influence of genomic architecture and the environment on hybrid genome evolution

4:00 PM Dr. Nick Buchler, Associate Professor, Dept. of Molecular Biomedical Sciences, CVM
It's an animal! It's a fungus! It's a Chytrid!

4:15 PM Jennifer Baltzegar (Gould Lab), PhD Graduate Student in Genetics, Dept. of Entomology and Plant Pathology, CALS
Spatial and temporal dynamics of Aedes aegypti pyrethroid resistance in Iquitos, Peru

4:30 PM Dr. David Rasmussen, Assistant Professor, Dept. of Entomology and Plant Pathology, CALS
Estimating mutational fitness effects across environments and scales from population genetic data

4:45 PM Dr. Albert Keung, Assistant Professor, Dept. of Chemical and Biomolecular Engineering, COE
Epigenetics & Bioengineering

Adjourn

5:00 PM Thank you for joining us! We hope to see you next year!

List of Poster Presenters

1. **Kimberly Bellingham-Johnstun**, Research Associate, Dept. of Molecular Biomedical Sciences, CVM
   *The Anillin Homolog Mid1p Is Dispensable For Cytokinetic Node Assembly In Fission Yeas*t
   Lab: Caroline Laplante
2. **Dr. Javier Brumos**, Postdoctoral Scholar, Dept. of Plant and Microbial Biology, CALS
   Local auxin biosynthesis is a key regulator of plant development
   Lab: Dr. Jose Alonso and Dr. Anna Stepanova
3. **David Bullock**, PhD Graduate Student in Genetics, Dept. of Plant and Microbial Biology, CALS
   Exploration of the molecular basis of the ethylene fast response
   Lab: Dr. Jose Alonso and Dr. Anna Stepanova
4. **Q. Brent Chen**, PhD Graduate Student in Genetics, Dept. of Biological Sciences, COS
   Epigenetic Regulation of Aging and Transposable Element Expression in Drosophila melanogaster
   Lab: Dr. Trudy Mackay
5. **Dr. Michael Cowley**, Assistant Professor, Dept. of Biological Sciences, COS
   Zac1 and the imprinted gene network in the programming of non-alcoholic fatty liver disease
6. **Dr. Pathy Fernandez-Moreno**, Postdoctoral Researcher, Plant and Microbial Biology, CALS
   THE HORMOMETER: A synthetic biology toolbox to study hormone interactions in plants
   Lab: Dr. Jose Alonso and Dr. Anna Stepanova
7. **Khushi Goda**, PhD Graduate Student in Genetics, Tree Improvement Program, CNR
   OPTIMAL MATING IN PINUS TAEDA
   Lab: Dr. Fikret Isik
8. **Sydney Graham**, MS Graduate Student in Crop Science, Dept. of Crop and Soil Sciences, CALS
   Identification of St. Augustinegrass Quantitative Trait Loci Associated with Freeze Tolerance
   Lab: Dr. Susana Milla-Lewis
9. **Yue Hao**, PhD Graduate Student in Bioinformatics, Dept. of Biological Sciences, COS
   Baby genomics: tracing the evolutionary changes that gave rise to placentation
   Lab: Dr. Gavin Conant
10. **Mohamed Moshtohry**, PhD Graduate Student in Physics, Dept. of Physics, COS
    Laser ablation uncovers the mechanical properties of the constricting contractile ring in fission yeast
    Lab: Dr. Caroline Laplante and Dr. Mary Etling

11. **Grace Parker**, PhD Graduate Student in Genetics, Dept. of Biological Sciences, COS
    Identification of St. Augustinegrass Quantitative Trait Loci Associated with Freeze Tolerance
    Lab: Dr. Trudy Mackay
12. **Jackson Parker**, PhD Graduate Student in Functional Genomics, Dept. of Biological Sciences, COS
    Early-life TCDD Exposure Shapes Gene Expression Across the Life Course of Mice
    Lab: Dr. David Aylor
13. **Dr. Luis Rivera-Burgos**, Postdoctoral Research Scholar, Dept. of Crop and Soil Sciences, CALS
    "Fine mapping of powdery mildew resistance gene Pm54 in soft red winter wheat (Triticum aestivum L.)
    Lab: Dr. Gina Brown-Guedira
14. **Fausto Rodriguez Zapata**, PhD Graduate Student in Genetics, Dept. of Molecular and Structural Biochemistry, CALS
    Genome-environment associations suggest highland introgression as a source of local adaptation to soils with low phosphorus availability in maize.
    Lab: Dr. Rubén Rellán Álvarez
15. **Anna Rogers**, PhD Graduate Student in Genetics, Dept. of Crop and Soil Sciences, CALS
    From Genomes to Fields: Understanding Genotype-By-Environment Interactions in Maize Hybrids
    Lab: Dr. James Holland
16. **Lossie (Elle) Rooney**, PhD Graduate Student in Genetics, Dept. of Chemical and Biomolecular Engineering, COE
    Prasad Bandodkar, PhD Graduate Student in Chemical Engineering, Dept. of Chemical and Biomolecular Engineering, COE
    FlySection: A database of gene expression patterns in embryonic Drosophila
    Lab: Dr. Gregory T. Reeves
17. **Allison Schloop**, PhD Graduate Student in Genetics, Dept. of Chemical and Biomolecular Engineering, COE
    Feedforward and feedback regulation in Drosophila dorsal-ventral patterning
    Lab: Dr. Gregory T. Reeves
18. **Ryan Spurney**, Research Assistant, Dept. of Electrical and Computer Engineering, COE
    Lisa Van den Broeck, Postdoctoral Researcher, Dept. of Plant and Microbial Biology, COE
    Identification of St. Augustinegrass Quantitative Trait Loci Associated with Freeze Tolerance
    Lab: Dr. Rosangela Sozzani

19. **Joseph Tolsma**, PhD Graduate Student in Genetics, Dept. of Biochemistry, CALS
    Influence of the Circadian Clock on the Arabidopsis Gravitropic Response
    Lab: Colleen Doherty
20. **Yukun Jennifer Zhang**, Research Associate, Dept. of Molecular Biomedical Sciences, CVM
    SPIZELLOMYCES PUNCTATUS: A NEW FUNGAL MODEL ORGANISM FOR STUDYING CELL CYCLE EVOLUTION

Abstracts

Science Talks: Session 1

Dr. Catherine Hoyo, Professor and Director of the Epidemiology and Environmental Epigenomics Lab, co-Director, Integrative Health Science Facility Core in the Center for Human Health and Environment

Gestational Cadmium Exposure, Epigenetic Response and Metabolic Dysfunction in Black, Hispanic and White offspring

Cadmium (Cd) is a ubiquitous environmental pollutant associated with a wide range of health outcomes including cancer. However, obscure exposure sources often hinder prevention efforts. Further, although epigenetic mechanisms are suspected to link these associations, gene sequence regions targeted by Cd are unclear. Aberrant methylation of a differentially methylated region (DMR) on the MEG3 gene that regulates the expression of a cluster of genes including MEG3, DLK1, MEG8, MEG9 and DIO3 has been associated with multiple cancers. In 287 infant–mother pairs, we used a combination of linear regression and the Getis-Ord Gi* statistic to determine if maternal blood Cd concentrations were associated with offspring CpG methylation of the sequence region regulating a cluster of imprinted genes including MEG3. Correlations were used to examine potential sources and routes. We noted a significant geographic co-clustering of elevated prenatal Cd levels and MEG3 DMR hypermethylation in mixed leukocytes in cord blood (p=0.01), and these findings were substantiated in our statistical models (p=0.03). These associations were strongest in those born to African American women (p=0.01) compared with those born to white women (p=0.56) or Hispanic women (p=0.34). Consistent with Cd bioaccumulation during the life course, blood Cd levels increased with age (b¼0.015 mg/dl/year (p=0.003), and Cd concentrations were significantly correlated between blood and urine (p<0.01), but not hand wipe, soil or house dust concentrations (P>0.05). Together, these data support that prenatal Cd exposure is associated with aberrant methylation of the imprint regulatory element for the MEG3 gene cluster at birth. However, neither house-dust nor water are likely exposure sources, and ingestion via contaminated hands is also unlikely to be a significant exposure route in this population. Larger studies are required to identify routes and sources of exposure.

Dr. Jason Delborne, Associate Professor of Science, Policy, and Society, Dept. of Forestry and Environmental Resources, CNR

Public engagement when biotechnology goes wild

Developments in genetic engineering and synthetic biology have enabled new visions of using biotechnology in new contexts, outside of agriculture, medicine, and industrial production. Specifically, biotechnologies released into unmanaged, wild environments may offer new tools for conservation or biodiversity restoration. This talk discusses research on the importance of public engagement in these realms, how it has been conducted, and how it contributes to governance processes for emerging biotechnologies.

Dr. Javier Brumos (Alonso-Stepanova Lab), Postdoctoral Scholar, Dept. of Plant and Microbial Biology, CALS

Local auxin biosynthesis is a key regulator of plant development

Brumos J., Alonso J., and Stepanova A.

Auxin is a major plant hormone that controls nearly every aspect of plant development and coordinates plant responses to the environment. Plants produce auxin, indole-3-acetic acid (IAA), from the aromatic amino acid tryptophan via the indole-3-pyruvic acid (IPyA) pathway, a simple two-step route catalyzed by tryptophan aminotransferases TAA1/TARs and flavin-containing monooxygenases YUCs. Influenced by the animal field, classical views supported the idea that most of the IAA is produced in shoot meristems and is then transported to the rest of the plant establishing morphogenic auxin gradients which govern cell fate decisions and underlie plant phenotypic plasticity. However, the TAA1/TAR and YUC gene families have been shown to exhibit exquisite spatiotemporal expression patterns suggesting that local sources of auxin may contribute to the formation of the auxin gradients.

To define the role of local auxin biosynthesis and its contribution to the regulation of plant development, we performed an array of complementary experiments that employed pharmacological treatments with chemical inhibitors of auxin biosynthesis and transport, a set of auxin transport and production mutants, ectopic expression of auxin biosynthetic genes under the control of tissue-specific promoters, inducible Cre-Lox systems, recombineering-based whole-gene fusions with protein reporters, and grafting. Our results revealed that local auxin biosynthesis and transport act synergistically and are individually dispensable for root meristem maintenance. In contrast, root responses to the stress hormone ethylene and flower fertility require local auxin production that cannot be fully compensated for by transport in the generation of auxin maxima.

Dr. Martha Buford Reiskind, Research Assistant Professor / Coordinator for the GGI Graduate Program, Dept. of Biological Sciences, COS

Evolution in Changing Environments

Research in our lab focuses on understanding the interaction of genes and environment in shaping the evolutionary trajectory of species. We address relevant questions on how species adapt to rapid changes in the abiotic or biotic environments. We focus on small population dynamics, landscape genomics, and evolution of native and invasive species with a wide variety of taxonomic groups. Today, I will talk about some of our recent work on adaptation and movement of native and introduced species to better understand the process of how species respond to new regions or new biotic interactions.

Dr. Nanette Nascone-Yoder, Associate Professor, Dept. of Molecular Biomedical Sciences, CVM

Functional Analyses of Candidate Human Birth Defect Genes

Heterotaxy (HTX) is a complex birth defect in which the left-right (LR) asymmetrical arrangement of internal organs is highly abnormal. HTX patients exhibit unusually symmetric or mirror-image anatomical features in multiple organ systems, often accompanied by complex heart defects, and substantial neonatal morbidity and mortality. To identify candidate HTX genes, we have leveraged exome sequence data from a well-defined cohort of HTX patients from the National Birth Defects Prevention Study. We are now utilizing a powerful vertebrate animal model (Xenopus) to efficiently query the in vivo function of high priority candidate HTX genes in the development of normal and abnormal left-right asymmetry. Our goal is to reveal new genes that may confer risk for some of the most severe and common birth defects, and to generate novel hypotheses about their potential pathogenetic mechanisms.

Dr. Sumit Dhole (Gould Lab), Postdoctoral Scholar, Dept. of Entomology and Plant Pathology, CALS

Tethered homing gene drives for localized population suppression

Optimism regarding potential epidemiological and conservation applications of modern gene drives is tempered by concern about the possibility of unintended spread of engineered organisms beyond the target population. In response, several novel gene drive approaches have been proposed that can, under certain conditions, locally alter characteristics of a population. One challenge for these gene drives is the difficulty of achieving high levels of localized population suppression without very large releases in the face of gene flow. We present a new gene drive system, tethered homing (TH), with improved capacity for both localization and population suppression. The TH drive is based on driving a payload gene using a homing construct that is anchored to a spatially restricted gene drive. We use a proof-of-concept mathematical model to show the dynamics of a TH drive that uses engineered underdominance as an anchor. This system is composed of a split homing drive and a two-locus engineered underdominance drive linked to one part of the split drive (the Cas endonuclease). We use simple population genetic simulations to show that the tethered homing technique can offer improved localized spread of costly transgenic payload genes. Additionally, the TH system offers the ability to gradually adjust the genetic load in a population after the initial alteration, with minimal additional release effort. We discuss potential solutions for improving localization and the feasibility of creating TH drive systems. Further research with models that include additional biological details will be needed to better understand how TH drives would behave in natural populations, but the preliminary results shown here suggest that tethered homing drives can be a useful addition to the repertoire of localized gene drives.

Science Talks: Session 2

Dr. Jack P. Wang, Assistant Professor, Director of Forest Biotechnology Group, Dept. of Forestry and Environmental Resources, CNR

Strategic Engineering of Populus For Bioenergy and Bioproducts Through Multi-Omics Integration In Lignin Biosynthesis

A multi-omics quantitative integrative analysis of lignin biosynthesis can advance the strategic engineering of wood for bioenergy and bioproducts. Lignin is polymerized from three monomers (monolignols) produced by a grid-like pathway. The pathway in wood formation of Populus trichocarpa has at least 21 genes, encoding enzymes that mediate 37 reactions on 24 metabolites, leading to lignin and affecting wood properties. We perturb these 21 pathway genes and integrate transcriptomic, proteomic, fluxomic and phenomic data from 221 lines selected from ~2,000 transgenics (6-month-old). Stem-differentiating xylem of the transgenics and wildtype were analyzed by 239 full transcriptomes and 239 proteomes to regress the abundances of transcripts and proteins. Using recombinant proteins from the 21 monolignol pathway genes, we determined 207 reaction and inhibition enzyme kinetic parameters to predict the effects of protein abundances on pathway metabolic-fluxes and metabolite concentrations. To determine the effects of metabolic-fluxes and metabolite concentrations on lignin and wood properties, we quantified the chemical composition of 220 wood samples, and 76 lignin samples using 2D HSQC NMR for lignin composition and structures. We measured the growth of 221 lines, the modulus of elasticity of 416 wood samples, the density of 213 wood samples, and tested 236 wood samples for saccharification efficiency. All these data were then systematically integrated to describe the transduction of biological information from the 21 monolignol genes through transcripts, proteins, metabolic-fluxes, and metabolite concentrations, leading to 25 wood traits, including lignin, tree-growth, density, strength, and saccharification. The analysis then predicts improvements in any of these 25 traits individually or in combinations, through engineering expression of specific monolignol genes. The analysis may lead to greater understanding of other pathways for improved growth and adaptation.

Dr. Caiti Smukowski Heil, Assistant Professor, Dept. of Biological Sciences, COS

The influence of genomic architecture and the environment on hybrid genome evolution

Genome sequencing has revealed introgression to be present in fungal, animal, and plant genomes, such that hybridization is now recognized as a common phenomenon across the tree of life. We are interested in understanding the forces that govern hybrid genome evolution and adaptation, specifically, how genetic variation provided by hybridization can help a population adapt to new environments, and how genomic architecture and the environment influence the distribution and persistence of introgression in a hybrid genome over time. We study these questions utilizing genomics and experimental evolution in the budding yeast Saccharomyces cerevisiae and its relatives. We are currently working to characterize recombination rates in domesticated and natural populations of Saccharomyces uvarum to understand how and why recombination rate differs between populations and species. Future work will use experimental evolution of hybrids under divergent environmental regimes to test if linked selection eliminates introgression in regions of low recombination.

Dr. Nick Buchler, Associate Professor, Dept. of Molecular Biomedical Sciences, CVM

It's an animal! It's a fungus! It's a Chytrid!

Chytrids are early-diverging Fungi with chitinous cell walls and hyphal-like structures, which have also retained features of the animal-fungal ancestor such as flagellated zoospores and amoeboid movement. Chytrids play diverse ecological roles as algal predators in aquatic food chains, as cellulose-degrading gut symbionts in ruminants, and as pathogens of amphibians. Here, we developed a protocol for the Agrobacterium-mediated genetic transformation of a non-pathogenic soil Chytrid, Spizellomyces punctatus. We fused histone H2B and LifeAct with a fluorescence reporter to measure live cell nuclear and actin dynamics over a full life cycle. I will show that nuclear cycles occur after germination and are synchronous during sporangiogenesis, and that actin is localized to the leading edge of amoeboid fronts in crawling zoospores. This work is a first step towards establishing Chytrids as tractable organisms for addressing important questions in fungal cell biology, evolution, and pathogenesis.

Jennifer Baltzegar (Gould Lab), PhD Graduate Student in Genetics, Dept. of Entomology and Plant Pathology, CALS

Spatial and temporal dynamics of Aedes aegypti pyrethroid resistance in Iquitos, Peru

Aedes aegypti, the Yellow Fever mosquito, is responsible for transmitting several arboviruses that infect and sicken large numbers of people annually. Pyrethroids target the voltage-gated sodium channel in arthropods and have been an effective method of controlling mosquito populations for several decades. However, they have been implicated in the development of knockdown resistance (kdr) in multiple arthropod species. Many genetic loci associated with kdr resistance have been identified; however, two single nucleotide polymorphisms (SNPs), F1534C and V1016I, have been shown to be important in Central and South America. This study explores the spatial and temporal dynamics of these two SNPs across an 18-year period in Iquitos, Peru. The results present an intriguing dynamic between resistant haplotypes that improves understanding of insecticide resistance evolution. Further, this study provides evidence for significant heterogeneity in fine-scale population structure of *Aedes aegypti*. Together these data provide crucial information to develop mosquito control programs for delaying widespread insecticide resistance and for improving the empirical evidence used to model emerging mosquito control techniques.

Dr. David Rasmussen, Assistant Professor, Dept. of Entomology and Plant Pathology, CALS

Estimating mutational fitness effects across environments and scales from population genetic data

The fitness effects of new mutations strongly shape the long-term evolutionary potential of a population, including the ability to adapt to new selective pressures. But mutational fitness effects are notoriously difficult to quantify and are typically both environment and scale-dependent. We have therefore developed new phylogenetic methods based on multi-type birth-death models that can be used to estimate the joint distribution of fitness effects across different contexts. We show how these methods can be applied to RNA viruses in order to estimate the fitness of mutations in different host environments and fitness at both the within and between-host scales. Our preliminary analysis of mutational effects in Ebola and human influenza virus suggest that while fitness effects are often highly positively correlated, the magnitude of these fitness effects can greatly vary across scales.

Dr. Albert Keung, Assistant Professor, Dept. of Chemical and Biomolecular Engineering, COE

Epigenetics & Bioengineering

Engineering approaches in biology, including from the fields of Synthetic Biology and Bioengineering, have harnessed the information stored in DNA sequences to solve biomedical, societal, and environmental challenges. This is driven by the fact that the genomic sequence is indeed a fundamental layer of information and that recombinant DNA engineering tools facilitated its manipulation. Yet, perhaps just as important as the DNA sequence itself is when, where, and how the genomic information is expressed. And engineering methods to control these layers of regulation will be crucial for a range of fundamental and applied applications. As an example from natural eukaryotic systems, the epigenome is the collection of RNAs, proteins, and their chemical modifications bound to genomic DNA. It serves as a layer of regulation literally on top of genomic DNA, controlling when and where genes are expressed. Our research group is interested in engineering methods to control and track the epigenome as well as other 'layers' of information. I will highlight this concept through synthetic molecular memory circuits involving DNA methylation, identifying important neurodevelopmental time periods of epigenetic imprinting in human cerebral organoids, engineering tools to bind and induce chemical modifications on histone proteins and DNA, and developing a completely abiotic information storage system or hard-drive using DNA.

Poster Presentations

1. Kimberly Bellingham-Johnstun, Research Associate, Dept. of Molecular Biomedical Sciences, CVM

Lab: Caroline Laplante

*The Anillin Homolog Mid1p Is Dispensable For Cytokinetic Node Assembly In Fission Yeas*t

Kimberly Bellingham-Johnstun, E. Casey Anders, Christina Bruinsma, John Ravi, and Caroline Laplante

The contractile ring is a machine built of actin, myosin, and other highly conserved proteins that constricts during cytokinesis. In fission yeast, nodes, protein complexes containing the Anillin homolog Mid1p and the Myosin-II molecule Myo2, assemble in a broad band at the site of cell division and coalesce into a contractile ring. Mid1p is thought to be essential for node assembly because broad bands of nodes are not detected in Δmid1 cells. Rather, strands made of the same cytokinetic proteins as nodes loop to form the contractile ring in these cells. Mid1p leaves the contractile ring prior to the onset of constriction giving rise to the assumption that the node structure is not maintained during constriction. How proteins organize within the constricting contractile ring remains unclear mainly because of the limited tools to probe molecular organization in live cells. We used quantitative high-speed Fluorescence PhotoActivation Localization Microscopy (hsFPALM) in live cells to probe protein organization at the nanometer scale, a ~tenfold improvement in resolution over confocal microscopy. We hypothesized that contractile rings and strands contain nodes that are packed too densely to be resolved by confocal microscopy. Using hsFPALM, we found that both the strands and contractile rings of Δmid1 cells contain nodes. We found that rings assemble from two different types of strands in Δmid1 cells: pre-existing and de novo strands. Pre-existing strands are present in cells that have previously failed at cytokinesis, can form contractile rings independently of the cell cycle stage, and require actin polymerization by the formin For3p. De novo strands assemble as expected during anaphase and are independent of For3p. The molecular organization of nodes in the contractile rings of Δmid1 and wild-type cells and in the broad bands of wild-type cells is comparable. However, the distribution of Myo2 heads is more compact in the nodes of preexisting strands in Δmid1 cells. This difference in the spreading of the Myo2p heads is actindependent. In conclusion, our work 1) shows that Mid1p is dispensable for node formation and maintenance, 2) indicates that nodes are components of the contractile ring, and 3) suggests that node protein organization is governed by the molecular composition and organization of the underlying actin network.

2. Dr. Javier Brumos, Postdoctoral Scholar, Dept. of Plant and Microbial Biology, CALS

Lab: Dr. Jose Alonso and Dr. Anna Stepanova

Local auxin biosynthesis is a key regulator of plant development

Brumos J., Alonso J., and Stepanova A.

Auxin is a major plant hormone that controls nearly every aspect of plant development and coordinates plant responses to the environment. Plants produce auxin, indole-3-acetic acid (IAA), from the aromatic amino acid tryptophan via the indole-3-pyruvic acid (IPyA) pathway, a simple two-step route catalyzed by tryptophan aminotransferases TAA1/TARs and flavin-containing monooxygenases YUCs. Influenced by the animal field, classical views supported the idea that most of the IAA is produced in shoot meristems and is then transported to the rest of the plant establishing morphogenic auxin gradients which govern cell fate decisions and underlie plant phenotypic plasticity. However, the TAA1/TAR and YUC gene families have been shown to exhibit exquisite spatiotemporal expression patterns suggesting that local sources of auxin may contribute to the formation of the auxin gradients.

To define the role of local auxin biosynthesis and its contribution to the regulation of plant development, we performed an array of complementary experiments that employed pharmacological treatments with chemical inhibitors of auxin biosynthesis and transport, a set of auxin transport and production mutants, ectopic expression of auxin biosynthetic genes under the control of tissue-specific promoters, inducible Cre-Lox systems, recombineering-based whole-gene fusions with protein reporters, and grafting. Our results revealed that local auxin biosynthesis and transport act synergistically and are individually dispensable for root meristem maintenance. In contrast, root responses to the stress hormone ethylene and flower fertility require local auxin production that cannot be fully compensated for by transport in the generation of auxin maxima.

3. David Bullock, PhD Graduate Student in Genetics, Dept. of Plant and Microbial Biology, CALS

Lab: Dr. Jose Alonso and Dr. Anna Stepanova

Exploration of the molecular basis of the ethylene fast response

Bullock DA, Rogers A, Alonso JM, and Stepanova AN

Ethylene is a gaseous phytohormone involved in multiple aspects of plant growth, development, senescence, and stress response. At the molecular level, the developmental effects of ethylene are accompanied by significant changes in gene expression at both transcriptional and post-transcriptional levels. The initial phase of the response to ethylene is thought to be independent of transcription, but its molecular nature is currently unknown. This project aims to shed light on the molecular basis of the fast ethylene-mediated response. Towards this goal, we are working on completing the following five objectives. First, we implemented our high-throughput live infrared imaging pipeline to monitor seedling growth dynamics with the help of a custom-made MatLab software developed in our lab. Auxin is another phytohormone that is involved in nearly all aspects of plant growth and developmental processes. There is a connection between ethylene signaling and preception and auxin signaling, biosynthesis, transport, signaling, and preception. Second, using a semi-automated pipeline, we have assessed the ethylene phenotypes of a set of previously characterized auxin mutants and wild-type plants treated with an auxin inhibitor kynurenine and discovered that auxin is required for the fast response to ethylene. Third, we are working on confirming that the initial ethylene response is independent of gene transcription and to achieve this aim our goal is to generate CRISPR/Cas9 knockouts of the remaining family members in the *ein3-1 eil1-1* mutant background and to examine ethylene response in these higher-order mutants. The fourth objective is to address the possibility that the fast response to ethylene is regulated at the level of gene translation. We have characterized T-DNA knockouts corresponding to ethylene-responsive translational targets, and we have determined that these candidate mutants do not play a role in the initial response to ethylene. Our fifth and last objective is to address the role of ETHYLENE INSENSITIVE2 (EIN2), a key ethylene signaling molecule, in the fast ethylene response. Our next objective is to use our live imaging pipeline to explore other treatment specific growth dynamics.

4. Q. Brent Chen, PhD Graduate Student in Genetics, Dept. of Biological Sciences, COS

Lab: Dr. Trudy Mackay

Epigenetic Regulation of Aging and Transposable Element Expression in Drosophila melanogaster

As the average lifespan of the world population continues to increase, deciphering the biological underpinnings of natural variation in aging and lifespan is critical to managing aging-related diseases. Recent studies have strengthened the heterochromatin loss model of aging: organisms exhibit a global loss of heterochromatin over time that results in the aberrant expression of silenced genes and the inability to maintain homeostasis. Here, we use five Drosophila melanogaster lines selected for postponed reproductive senescence for over 170 generations (O lines) and five lines from the same base population maintained without selection (B lines) to assess differential chromatin states between long lived and normal lifespan flies. The O lines have twice the lifespan compared to the B lines at approximately 70 days and 35 days, respectively. We find that expression of transposable elements, normally silenced within heterochromatic domains, are higher in the B lines than the O lines at both young and old age. Additionally, expression of transposable elements in old O line flies are comparable to young B line flies. These findings suggest that the O and B lines maybe be utilized to decipher the genetic basis of global heterochromatin state maintenance and aging. We will also use ATAC-seq to determine changes in open chromatin from a variety of tissues in both sexes of the O and B lines at one and five weeks and ChIP-seq to target histone modifications. In conjunction with previous genomic, transcriptomic, metabolomic, and phenotypic data, we will derive putative causal relationships between epigenetic modifications and natural variation in lifespan.

5. Dr. Michael Cowley, Assistant Professor, Dept. of Biological Sciences, COS

Zac1 and the imprinted gene network in the programming of non-alcoholic fatty liver disease

Marine Baptissart, Evan Walsh, Brie Jones, Sierra Moorefield, Dereje Jima, and Michael Cowley

Non-alcoholic fatty liver disease (NAFLD) ranges from excess lipid accumulation in hepatocytes (steatosis) to advanced fibrosis. With a prevalence reaching 30% worldwide, NAFLD is rapidly becoming a public health concern and understand the etiology of the disease is now a priority. The diagnosis of NAFLD is occurring at increasingly younger ages, suggesting an early-life origin influenced by environmental factors. A wealth of epidemiological and model system data has shown that maternal metabolic syndrome (MetS) primes the infant for NAFLD in later life. However, the molecular mechanisms mediating NAFLD susceptibility remain unknown.

To address this knowledge gap, we established a unique mouse model of maternal MetS. Importantly, our study design allows for a clean distinction between exposure during prenatal and postnatal development, providing the opportunity to test the relative contribution of these developmental windows in the programming of NAFLD.

With this model, one of our most striking findings is that postnatal, but not prenatal, exposure to maternal MetS is associated with histological evidence of hepatic steatosis and molecular signature of liver fibrosis. Transcriptomic analysis identified the Imprinted Gene Network (IGN), including its master transcription factor Zac1, as being up-regulated in the liver of these same mice. The function of the IGN in liver has not been described previously. By overexpressing Zac1 in cultured hepatocytes, we show that activation of the IGN promotes fibrotic pathways and extracellular matrix deposition, key events driving NAFLD progression.

Epigenetic dysregulations at imprinted genes have been proposed to contain the memory of developmental exposures influencing lifelong metabolic health. Our results suggest that the IGN may be a novel transcriptional network linking maternal MetS to NAFLD specifically during postnatal development. Our future work will study the underlying epigenetic mechanisms responsible for IGN modulation.

6. Dr. Pathy Fernandez-Moreno, Postdoctoral Researcher, Plant and Microbial Biology, CALS

Lab: Dr. Jose Alonso and Dr. Anna Stepanova

THE HORMOMETER: A synthetic biology toolbox to study hormone interactions in plants

The interaction between endogenous plant growth regulators is a key process in the integration of environmental and developmental signals. How different plant hormonal pathways talk with one another is, however, poorly understood, and new phenotyping tools that enable simultaneous detection of the activity of multiple pathways are urgently needed. Taking advantage of the GoldenBraid gene multiassembly technology, we are building the hormometer, a multi-hormone sensor that permits detection of the transcriptional output of several growth regulators at once. An ideal hormometer should consist of a single construct comprised of multiple hormone-specific transcriptional reporters for all nine major non-peptide plant hormones arranged in tandem. Each individual reporter would contain five DNA elements (phytobricks): a hormone-specific distal promoter, a synthetic core promoter (+5'UTR), a subcellular localization tag, a fluorescent protein coding sequence, and a synthetic terminator (+3'UTR). A combination of three fluorophores and three subcellular localization tags provides enough multiplexing power to monitor the nine growth regulators simultaneously. Towards this objective, a collection of nearly 120 phytobricks has been generated in our lab, a majority of these parts have been assembled in tester transcriptional units, and to date about a third have been functionally validated in transient assays in tobacco. Using some of the parts from our collection, we have also built two different versions of the ACE hormometer that harbor fluorophore- and localization-tag-compatible reporters for auxin, ethylene and cytokinin, along with a selectable marker, in a single binary vector construct. The upcoming characterization of ACE activity in the resulting Arabidopsis and tomato stable transgenic lines will provide the first proof of concept for our multiplexing approach and offer a new streamlined tool for monitoring the three hormones in parallel.

7. Khushi Goda, PhD Graduate Student in Genetics, Tree Improvement Program, CNR

Lab: Dr. Fikret Isik

OPTIMAL MATING IN PINUS TAEDA

Khushi Goda, Fikret Isik

Loblolly pine (Pinus taeda) is the most important tree crop in the US, planted over 25 million acres in the south. It is responsible for 58% of domestic wood supply and 16% of the global wood supply. The Tree Improvement Program at North Carolina State University manages the genetic improvement of Loblolly pine. Loblolly pine has a high genetic load and suffers greatly from inbreeding depression. It is a challenge to balance two important but contrasting goals of capturing as much genetic gain as possible while managing short- and long- term inbreeding. While methods and algorithms for animal breeding are well-established, an efficient algorithm suited to this species remains elusive. Developing an algorithm to design mating that optimizes genetic gain whilst putting constraints on relatedness is imperative for loblolly pine breeding. Towards this goal, we have adopted evolutionary genetic algorithms for optimized mating design. The optimization algorithm developed can utilize pedigree-based relationships to create optimal mating list for future breeding. Modified differential evolution (DE) algorithm has been applied to create mating lists that can be realized to give maximum return of genetic gain in future progeny while minimizing the increase in average co-ancestry in the population. Using the algorithm and optimizing the mating list from 964 monoecious loblolly pine tree candidates, resulted in 69.8% increase in genetic gain and no inbred progeny. The completion of this study will see the development of a suite of software that is able to not only utilize genetic relationships from pedigree but also utilize genomic relationships derived from Single Nucleotide Polymorphisms (SNPs). The framework and methods adapted for loblolly pine breeding have relevance to breeding of other monoecies species as well.

8. Sydney Graham, MS Graduate Student in Crop Scienc), Dept. of Crop and Soil Sciences, CALS

Lab: Dr. Susana Milla-Lewis

Identification of St. Augustinegrass Quantitative Trait Loci Associated with Freeze Tolerance

S.E. Graham, J.A. Kimball, X. Yu, T.D. Tuong, Y. Zheng, D.P. Livingston, S.R. Milla-Lewis

St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntz) is a warm-season turfgrass commonly used in home lawns in the southern United States. A lack of freeze tolerance has limited the spread of St. Augustinegrass further north into the transition zone. 'Raleigh', released in the early 1980s, is still the industry's standard for cold tolerance. Despite the identification of freeze tolerant germplasm, limited progress has been made in increasing the available pool of freeze tolerant cultivars for the species.

In order to elucidate the genetic control of freeze tolerance, previous research developed a SSR-based linkage map for a 'Raleigh' by 'Seville' (RxS) mapping population. The map was used in conjunction with field and lab-based freezing data and identified multiple QTL on linkage groups 1, 2, 3 and 4. A linkage map of 2871 SNPs has since become available for this previously-studied population. The objectives of the present study were to 1) improve the resolution of the previously identified freeze tolerance QTL and 2) validate these QTL in a separate population.

A 126 SSRs linkage map of a F1 population of 'Raleigh' (RxR) was developed and freeze tests yielded data on surviving green tissue and regrowth. Composite interval mapping of least-squares means was used for the analysis of both populations and the significance thresholds were determined through permutation. The RxS population yielded 42 QTL with significant QTL identified in each linkage group. QTL for spring green-up and winterkill were co-localized on linkage groups 8 and 9. In the RxR population, four significant QTL were identified on linkage groups 1, 3, 5 and 9, which align with the previously identified QTL. Several of these QTL could be used as potential targets for marker assisted selection, reducing the need for multi-year field evaluations.

9. Yue Hao, PhD Graduate Student in Bioinformatics, Dept. of Biological Sciences, COS

Lab: Dr. Gavin Conant

Baby genomics: tracing the evolutionary changes that gave rise to placentation

Yue Hao, Hyuk Jin Lee, Michael Baraboo, Katherine Burch, Taylor Maurer, Jason A Somarelli and Gavin C Conant

It has long been a challenge to determine the molecular mechanisms behind morphological innovations and striking evolutionary transitions such as the origin of the mammalian pregnancy. Using an orthology assignment pipeline that employs both robust, distance-based measures of gene relationships and synteny, we inferred orthology relations between human genes and genes from each of 43 other vertebrate genomes, resulting in ~18,000 orthologous pairs for each genome comparison. By identifying genes that are associated with the origin of placental mammals, we sought a subset of the genome that was enriched for genes that play a role in placental evolution in therian mammals. We thus reconstructed the ancestral states of these orthologs and pinpointed orthologs that appeared before and after the divergence of eutherian mammals from marsupials. Reinforcing previous suggestions, we found that much of the genetic toolkit of mammalian pregnancy evolved through the repurposing of genes with pre-existing roles. These genes then acquired regulatory controls for novel roles from a group of regulatory genes. Importantly, many of these regulatory genes did in fact originate as eutherian-specific duplicates: orthologs shared by the eutherian ancestor but not by earlier ancestors are enriched in functions such as transcription regulation by Krüppel-associated box ZNFs, innate immune responses and the melanoma-associated antigen protein class. Since the cellular mechanisms of invasive placentae are similar to that of the metastatic cancer cells, we also explored the overlap between genes identified as functioning in placenta invasion in our evolutionary analysis and those known to be involved in cancer metastasis.

10. Mohamed Moshtohry, PhD Graduate Student in Physics, Dept. of Physics, COS

Lab: Dr. Caroline Laplante and Dr. Mary Etling

Laser ablation uncovers the mechanical properties of the constricting contractile ring in fission yeast

Cytokinesis in animals, fungi and amoebas is a robust process that requires the constriction of a contractile ring of actin, myosin, and other conserved proteins. Recent studies have begun to unravel the molecular architecture of the contractile ring. However, we still do not understand how this organization, and its dynamics, support force generation due to the experimental challenges of probing force in live cells. We overcome this challenge by using laser ablation to cut the contractile ring and measuring the kinetics of the resulting severed ends. After cutting the contractile ring, the free ends recoil away from each other, briefly stall, and move back toward each other to heal the severed ring. The profile of recoil follows an exponential relaxation with interruptions when the recoiling ends stall for short periods of ~10 s. These data support that severing the contractile ring releases internal tension and that the profile of recoil of the severed ends is influenced by drag forces caused by anchoring of the contractile ring to the plasma membrane/cell wall. We hypothesized that defects in tension production and ring anchoring would alter the kinetics of the severed ends of the contractile ring in response to laser ablation. We cut contractile rings in control, tension-defective and anchoring-defective cells and find distinct responses in each background. In control cells, the cut free ends of the contractile ring recoils by ~700 nm over the first 50 s after ablation, after which the recoiling stops and the cut ring heals over time. In tension defective cells (Δmyp2 myo2-E1), no recoil was observed after laser ablation and the cut ring healed rapidly. In anchoring defective cells (Cdc15p depleted cells), the cut ring continuously recoiled over the entire imaging period. We tracked these severed rings for ~500 s, until the signal was complete photobleached or the severed ends of the contractile ring left the imaging plane. By the end of our imaging, the severed ends had recoiled ~ 950 nm and healing of the ring was never observed. This powerful tool uncovers the mechanical properties of the contractile ring and ascribes mechanical functions to cytokinetic proteins.

11. Grace Parker, PhD Graduate Student in Genetics, Dept. of Biological Sciences, COS

Lab: Dr. Trudy Mackay

Identification of St. Augustinegrass Quantitative Trait Loci Associated with Freeze Tolerance

Limited lifespan and senescence are near-universal phenomena. These quantitative traits exhibit variation in natural populations due to the segregation of many interacting loci and from environmental effects. Due to the complexity of the genetic control of lifespan and senescence, our understanding of the genetic basis of variation in these traits is incomplete. Here, we analyzed the pattern of genetic divergence between long-lived (O) Drosophila melanogaster lines selected for postponed reproductive senescence and unselected control (B) lines. We quantified the productivity of the O and B lines and found that reproductive senescence is maternally-controlled. We therefore chose 57 candidate genes that are expressed in ovaries, 49 of which have human orthologs, and assessed the effects of RNAi knockdown in ovaries and accessary glands on lifespan and lifetime reproduction. All but two candidate genes affected at least one life history trait in one sex or productivity week, 41 affected lifespan and 41 affected productivity. Interestingly, ten genes had sex-specific effects on increased lifespan and five had sex-antagonistic effects on lifespan. In addition, 14 genes had antagonistic pleiotropic effects on lifespan and productivity. Identifying evolutionarily conserved genes affecting increased lifespan and delayed reproductive senescence is the first step towards understanding the evolutionary forces that maintain segregating variation at these loci in nature and may provide potential targets for therapeutic intervention to delay senescence while increasing lifespan.

12. Jackson Parker, PhD Graduate Student in Functional Genomics, Dept. of Biological Sciences, COS

Lab: Dr. David Aylor

Early-life TCDD Exposure Shapes Gene Expression Across the Life Course of Mice

Jackson P. Parker, Thomas I. Konneker, Jacob D. Fredenburg, Nicole E. Allard, Brian Horman, Alexias Safi, Heather B. Patisaul, Gregory E. Crawford, and David L. Aylor

2,3,7,8-Tetracholorodibenzodioxin (TCDD) is a potent environmental toxin that is generated as a byproduct of industrial operations involving high temperature processing of organic material. It enters into environmental systems as a constituent of solid waste and flue gas. In vertebrate systems, TCDD activates the AhR-mediated xenobiotic response which modulates transcription of numerous genes responsible for metabolizing toxic compounds. The World Health Organization recognizes links between early-life exposure to TCDD and late-onset pathologies including neurological disability, reproductive impairment, and increased cancer risk.

Our goal is to understand the consequences of early-life TCDD exposure on the molecular state of multiple tissues. Mice were exposed to TCDD from preconception through gestation and lactation. Tissue samples were taken three weeks, five weeks, twenty weeks, and forty weeks after birth. From our measurements of transcriptional profiles, we show that gestational TCDD exposure shapes gene expression both in the short-term and in the long-term. Furthermore, we performed analyses of open chromatin using ATAC-seq to contrast chromatin accessibility with associated gene expression.

Early-life exposure to TCDD resulted in substantial changes in gene expression after accounting for tissue, age, and sex. A total of 1497 genes were differentially expressed in liver combined over both sexes and ages. Blood showed few differentially expressed genes at three weeks but substantially more in adult mice. The effects of TCDD differed dramatically between males and females. Furthermore, there is no overlap between the response liver and blood. Though there were clear gene expression signatures of TCDD exposure at both ages, the changes observed at three weeks did not persist into adulthood. We conclude that a complex cascade of gene regulatory events are set in motion by early-life TCDD exposure that result in long-term gene expression differences in adult mice.

13. Dr. Luis Rivera-Burgos, Postdoctoral Research Scholar, Dept. of Crop and Soil Sciences, CALS

Lab: Dr. Gina Brown-Guedira

Fine mapping of powdery mildew resistance gene Pm54 in soft red winter wheat (Triticum aestivum L.)

Powdery mildew, caused by the obligate biotroph Blumeria graminis (DC) Speer f. spp. tritici emend. E. J. Marchal (Bgt), is an important disease-causing increasing damage in USA wheat (Triticum aestivum L.) production regions. A major QTL on chromosome 6BL linked to powdery mildew resistance was reported by Hao et al. (2015). They identified the cultivar AGS2000 as a source of a new resistant powdery mildew gene, designated as Pm54. A recent genome-wide association study in southern soft red winter wheat conducted by Sarinelli (2017), discovered and validated several powdery mildew resistance genes. In the study, the 6BL QTL was associated with a marker (SNP) at position 695,007,016 bp. Thus, the interval containing the 6BL QTL linked to the powdery mildew gene Pm54 is being targeted for fine mapping in a recombinant inbred line population derived from the cross between the susceptible and the resistant cultivars LA95135 and AGS2000, respectively. First, we screened 256 recombinant inbred lines (LA population) under greenhouse and growth chamber conditions. We used the isolate "NCF-D-1-1" of Blumeria graminis f. sp. tritici to induce uniform and strong epidemics. Disease severity was scored on a scale of 0 – 4 (0=resistant and 4= susceptible). LA95135 showed moderate to susceptible reaction while the AGS2000 moderate to resistant reaction. The progeny segregated in a ratio of 3:1 (resistant:susceptible) indicating the presence of 2 genes in the LA population. Second, we exploited exome capture data to discover new polymorphisms for marker saturation in a 10 Mbp region flanking the Pm54 locus. We were able to identify 135 annotated genes and 396 new polymorphisms (SNPs) between LA95135 and AGS2000. We selected two SNPs every 1 Mbp and a total of 20 KASP assays were developed to genotype the LA population. Based on the genotypic and phenotypic results, we identified three genes annotated as disease resistance proteins (RPM1) located nearby to the reported Pm54 gene as the possible cause of the resistance displayed in AGS2000. Further work will be done to identify and valid the polymorphism underlying the Pm54 resistance allele.

14. Fausto Rodriguez Zapata, PhD Graduate Student in Genetics, Dept. of Molecular and Structural Biochemistry, CALS

Lab: Dr. Rubén Rellán Álvarez

Genome-environment associations suggest highland introgression as a source of local adaptation to soils with low phosphorus availability in maize.

Plant phosphorus starving response (PSR) is a coordinated set of biochemical, physiological and developmental reactions to low phosphorus supply. I expect divergent selection in loci involved in PSR if it is the result of local adaptation. Given the absence of opposing evolutionary forces, the main consequence of this selection is an increased frequency of adaptive alleles in populations exposed to low phosphorus availability. Here I use a reverse ecology approach to identify loci that might be involved in PSR and be subject to divergent selection. First I built soilP, an R package for assigning soil phosphorus retention potential to geographic locations. With this retention potential as phenotype I performed environmental GWAS on 3238 georeferenced landraces of Zea mays from Latin America and the Caribbean. This resulted in the detection of significant signal from the 13 Mb span of Inv4m, a previously reported adaptive retrogression from highland teosinte that includes ZmPho1;2a an inorganic phosphate transporter. Finally, I propose analysis of phosphorus response in biparental populations polymorphic for Inv4m in order to disentangle population structure from local adaptation or other environmental covariates.

15. Anna Rogers, PhD Graduate Student in Genetics, Dept. of Crop and Soil Sciences, CALS

Lab: Dr. James Holland

From Genomes to Fields: Understanding Genotype-By-Environment Interactions in Maize Hybrids

Plant breeding programs are often faced with challenges in making initial selections among breeding materials based on evaluation in a single environment, with the ultimate goal of creating new varieties that will later be planted across multiple, more diverse conditions. In some cases, genotypes that initially seemed very promising are observed to vary dramatically for important agronomic traits across diverse environments. Genotype-by-Environment interactions (GxE) underlie relative differences in performance across environments but are difficult to predict without understanding how genotypes respond to specific environmental covariates. Recent advances in genomics and prediction modeling have accelerated the ability to perform selections using genomic data, but little has been done to incorporate environmental data into such modeling. Including environmental variables in G×E analysis often results in issues with multicollinearity, caused by presence of large numbers of predictors that are often highly correlated, each of which only explains a small amount of variance. Development of methods to incorporate both genomic and environmental data into genomic prediction models should provide ability to predict genotypic performance in specific new environments.

Using publicly available data for 1,919 maize hybrids spread across multiple locations over three years in North America, we explore GxE modeling using a mixed models approach incorporating high density DNA marker data and weather covariates. Using these data, we gain a clearer insight of what GxE means in context of plant development and response to fluctuating environmental conditions, and explore the possibility of predicting hybrid phenotypes in previously untested environments.

16. Lossie (Elle) Rooney, PhD Graduate Student in Genetics, Dept. of Chemical and Biomolecular Engineering, COE
Prasad Bandodkar, PhD Graduate Student in Chemical Engineering, Dept. of Chemical and Biomolecular Engineering, COE

Lab: Dr. Gregory T. Reeves

FlySection: A database of gene expression patterns in embryonic Drosophila

Fluorescence microscopy images are frequently used for quantitative genetics and modeling of gene regulatory networks (GRNs) in the Drosophila blastoderm; however, few consolidated sources of these data exist that allow easy curation of datasets. We propose to create a database that will be publicly available for access through the Reeves' lab website to contain quantitative data on gene expression patterns extracted from images generated by our lab. These data and the original images will be searchable and available for download. We expect that this database will assist the Drosophila research community in exploring existing hypotheses and uncovering new hypotheses for further study, and we present some examples.

17. Allison Schloop, PhD Graduate Student in Genetics, Dept. of Chemical and Biomolecular Engineering, COE

Lab: Dr. Gregory T. Reeves

Feedforward and feedback regulation in Drosophila dorsal-ventral patterning

Development of an organism is dependent upon proper regulation of gene expression. Initiation of gene expression often relies on long-range signals referred to as morphogens; these morphogens form concentration gradients that aid in specific activation of genes responsible for proper body patterning. In Drosophila, one such morphogen is Dorsal (Dl), a transcription factor that helps with patterning of the dorsal-ventral (DV) axis in the early embryo. The impact of Dl is further refined by gene regulatory loops that help to control the dynamics of the Dl gradient. Two regulatory loops of interest are the negative feedback loop with Cactus (Cact) and the feed forward loop with Twist (Twi). Cact is initially bound to Dl, sequestering it to the cytoplasm, but Toll signaling on the ventral side of the cell degrades Cact and allows Dl to enter the nucleus. There, Dl can activate target genes, one of which is Cact, suggesting that Dl may regulate its own inhibition. In addition, Dl activates Twi, which is a transcription factor that co-regulates, with Dl, expression of genes on the ventral side of the developing embryo.

Our work currently focuses on establishing a system through which Cact and Twi can be examined in live embryos. Protein expression and use during development is very rapid; the turnover of Cact and the late zygotic expression of Twi both happen too quickly for standard live imaging techniques, like fluorescent protein fusions. Fluorescent proteins like GFP do not have enough time to mature and fluoresce before the associated protein is degraded. With the help of the Rao Lab, we plan to use a FRET system to detect Cact and Twi in live embryos and examine their effects on the dynamics of the Dl gradient. We also plan to use a new system referred to as LlamaTags as another way to detect these two proteins.

18. Ryan Spurney, Research Assistant, Dept. of Electrical and Computer Engineering, COE
Lisa Van den Broeck, Postdoctoral Researcher, Dept. of Plant and Microbial Biology, COE

Lab: Dr. Rosangela Sozzani

Identification of St. Augustinegrass Quantitative Trait Loci Associated with Freeze Tolerance

Despite the increased use of inference methods, existing computational approaches for predicting gene regulatory networks (GRNs) often do not integrate RNA-sequencing data analysis, are not automated, and/or are restricted to users with bioinformatics and programming backgrounds. To address these limitations, we have developed TuxNet, an integrated user-friendly platform which allows the user to process raw RNA-sequencing data from any organism with an existing reference genome using a modified Tuxedo pipeline and infer GRNs from these processed data. We performed two case studies featuring the versatility of TuxNet when using different types of gene expression data to infer networks as well as its accessibility as a pipeline for non-bioinformaticians to analyze and handle transcriptome data, predict causal regulations, assess network topology, and identify important regulators.

19. Joseph Tolsma, PhD Graduate Student in Genetics, Dept. of Biochemistry, CALS

Lab: Colleen Doherty

Influence of the Circadian Clock on the Arabidopsis Gravitropic Response

Joseph Tolsma, Imara Perera, Colleen Doherty

Circadian rhythms are regular oscillations of an organism's physiology with a period of approximately 24 hours. In Arabidopsis, circadian rhythms regulate a suite of physiological processes including transcription, photosynthesis, growth, and flowering. An exploratory evaluation of RNA-seq data from Arabidopsis space flight experiments showed an enrichment of clock-related genes involved in the response to microgravity conditions. Further evaluation using a root-bending assay provided further evidence that the circadian clock is involved in the response to gravity. The time of day when the root bending assay was performed affected the response angle in plant roots. Circadian clock mutants also exhibited different gravitropic responses compared to WT plants. Finally, WT plants and a circadian clock mutant, CCA1 OE, were grown on a random positioning machine at the Kennedy Space Center. During a single RPM run, WT and CCA1 OE seedlings were grown under two different lighting schedules. This allowed for direct comparison of two photoperiods found to have differential gravitropic responses in the root bending assay. These plants were exposed to simulated microgravity for 48 hours. Root phenotypes of the WT and CCA1 OE mutants were compared as well as differences between the two photoperiods.

20. Yukun Jennifer Zhang, Research Associate, Dept. of Molecular Biomedical Sciences, CVM

SPIZELLOMYCES PUNCTATUS: A NEW FUNGAL MODEL ORGANISM FOR STUDYING CELL CYCLE EVOLUTION

Living cells precisely control the reproduction of their genetic material through a fine-tuned regulatory network known as the cell cycle. The regulatory mechanism of the cell cycle is highly conserved across all Eukaryotes. Whereas animals and plants have E2F-Rb as the major regulators of G1/S gene expression, fungi utilize SBF-Whi5 (unrelated to E2F-Rb in origin) to perform an equivalent role. Our previous work suggested that fungi acquired SBF-Whi5 and later lost the ancestral E2F-Rb regulatory system. Spizellomyces punctatus is an early-diverging fungus (known as a chytrid) that has both E2F-Rb and SBF-Whi5 and should provide insights on how a fungal ancestor used both systems and why SBF-Whi5 replaced E2F-Rb during fungal evolution. To this end, we engineered a transgenic line with nuclear-localized fluorescent protein mCitrine (NLS-mCitrine) via Agrobacterium-mediated gene transformation. Time-lapse microscopy with the NLS-mCitrine transgenic Spizellomyces showed that the nucleus divides every 2.5 h and takes less than 24 h to complete a full life cycle. We also created a transgenic line expressing membrane-localized fluorescent protein TdTomato-CAAX to measure membrane dynamics during Spizellomyces development. We are synchronizing the Spizellomyces cell cycle and monitoring the synchrony by DNA staining and flow cytometer. Our goal is to use RNA-seq to measure cell cycle gene expression and to make Spizellomyces a model organism for understanding the evolution of the cell cycle and the cell biology of other chytrids, such as the pathogenic chytrids that infect amphibians or plant-degrading symbionts in the rumen. Our research is of great interest to veterinary medicine.

Thank you for coming to the Genetics and Genomics Initiative 2nd Annual Retreat!

Have a question concerning the Genetics and Genomics Initiative?

Contact one of the Executive Committee members!

Dr. Fred Gould
Professor
fgould@ncsu.edu

Dr. Reade Roberts
Associate Professor
rbrober2@ncsu.edu

Dr. Anna Stepanova
Associate Professor
atstepan@ncsu.edu

Dr. Jeff Thorne
Professor
thorne@ncsu.edu

Dr. Jeff Yoder
Professor
jayoder@ncsu.edu

Visit our website!

ggi.ncsu.edu

A Special Thank You to This Year's Retreat Sponsors!