PROGRAM IN GENETICS

9th ANNUAL FALL RETREAT

Monday, August 20, 2018

The Dorothy and Roy Park Alumni Center

The Program in Genetics welcomes you to the 9th Annual Genetics Fall Retreat!

Say hello to some of our new faces!

2018 - 2019 New Graduate Students for the Program in Genetics

Kara Carlson

Kayleigh Dively

Program in Genetics Fall Retreat Schedule

Registration - Foyer/Great Reception (Room 112)

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

Welcome - Great Reception (Room 112)

9:00 AM	Opening remarks by Dr. Reade Roberts, Director of the Graduate Program in Genetics
9:15 AM	Introduction and discussion of the Genetics and Genomics Initiative by Dr. Fred Gould and Dr. Reade Roberts

Session 1 - Great Reception (Room 112)

9:45 AM	Dr. Ross Sozzani, *Gene regulatory networks controlling root stem cells*

10:00 AM Aldo Carmona-Baez (Roberts Lab), *The genetic basis of gut length divergence across trophic levels in cichlid fishes*

10:15 AM Dr. Greg Reeves, *Quantitative approaches in tissue patterning*

Coffee Break - Foyer

10:30 AM Coffee Break - Refreshments provided by the Park Alumni Center
Fall Retreat Group Photo – Outside, front of Alumni Center

Session 2 - Great Reception (Room 112)

11:00 AM Aiden Jones (Peng Lab), *Functional analysis of long non-coding RNA within the context of viral infections*

11:15 AM Christopher McCallough (Reeves Lab), *Natural variation in Drosophila is key to deciphering the complexity of gene regulatory networks*

11:30 AM Dr. Anna Stepanova, *Tailoring hormone responses in plants via synthetic signal integration devices*

11:45 AM Katie Hudson (Cowley Lab), *Epigenetic responses to in utero cadmium exposure*

Lunch - Great Reception (Room 112)

12:00 PM Lunch provided by the Park Alumni Center

Social Activity - Outside (Rain Location: Foyer)

1:00 PM Social Activity – Egg Hunt
Social Leader: Lossie Rooney

Session 3 - Great Reception (Room 112)

2:00 PM Dr. Betty Gardner, *GN 311: A new adventure in distance education*

2:15 PM Dr. Jeff Yoder, *On the function and evolution of novel immune-type receptors (NITRs) in ray-finned fish*

2:30 PM Cameron Parsons (Kathariou Lab), *Emerging IVb-v1 clone (CC554) of Listeria monocytogenes is highly prevalent among strains from suburban black bears (Ursus americanus)*

New Genetics Graduate Student Orientation - Great Reception (Room 112)

2:45 PM New students meet with Dr. Reade Roberts and Jenni Shaw

Poster Presentations/Coffee Break - Foyer

Enjoy Coffee! – Refreshments provided by the Park Alumni Center

2:45 PM Poster Presentations

Farida Akhtari	*Dose response analysis in cell line models for cancer pharmacogenomics*
Jennifer Baltzegar	*Evolution of insecticide resistance of Aedes aegypti from Iquitos, Peru*
Marine Baptissart	*Zac1 and the imprinted gene network in the programming of non-alcoholic fatty liver disease*
Javier Brumos	*Local production of the plant hormone auxin is sufficient to maintain the stem cell niche*
Q. Brent Chen	*Epigenetic regulation of aging in Drosophila melanogaster*
David Bullock	*Exploration of the molecular basis of the ethylene fast response*
Josefina Patricia Fernandez Moreno	*Single-locus multi-hormone reporters for comprehensive plant phenotyping: a synthetic biology approach*
Melissa Lamm	*Uncovering genetic mechanism controlling sex in an African cichlid with polygenic sex determination*
Erin Peterson	*Evolution of Genetic Sex Determination in a Model for Rapid Speciation*

Mark Simmers	*The role of epigenetic dysregulation of imprinted genes in cadmium-induced fetal growth restriction*

Session 4 - Great Reception (Room 112)

4:00 PM	Grace Parker (Carbone and Mackay Lab), *The genomic regulation of limited lifespan and reproductive senescence*
4:15 PM	Dr. Claire Gordy, *Encouraging creativity in lab courses by rethinking the traditional lab report*
4:30 PM	Sam Widmayer (Aylor Lab), *Genomic signatures of hybrid male sterility in the mouse*
4:45 PM	Dr. Heather Shive, *Identification of microenvironmental contributors to carcinogenesis with a zebrafish model*

Adjourn

5:00 PM	Thank you for joining us! We hope to see you next year!
5:15 PM	Take down posters and clean up

Session 1

Gene regulatory networks controlling root stem cells

Dr. Ross Sozzani

Development in multicellular organisms requires not only the production of specialized cell types but also mechanisms of coordination among them. Stem cells are ultimately the source of all cell types, and the balance between self-renewal and differentiation of their progeny regulates organ growth. Transcription factors and cell-to-cell signaling have a key role in coordinating these processes; however, how these transcriptional networks control multicellular development is not completely understood. Moreover, while a number of transcription factors involved in root stem cell maintenance have been described, a comprehensive view of the transcriptional signature of the stem cells is lacking. Here, we used spatial and temporal transcriptomic data to predict interactions among the genes involved in stem cell regulation. To accomplish this, we transcriptionally profiled several stem cell populations, developed gene regulatory network inference algorithms, and leveraged the topology of our networks to infer potential major regulators. Specifically, through mathematical modeling and experimental validation, we identified PERIANTHIA (PAN) as an important molecular regulator of quiescent center function. Current work focuses on identifying additional players in the QC as well as if SHORTROOT and SCARECROW function to regulate QC maintenance. The results presented in this work show that our combination of molecular biology, computational biology, and mathematical modeling is an efficient approach to identify candidate factors that function in the stem cells.

The genetic basis of gut length divergence across trophic levels in cichlid fishes

Aldo Carmona-Baez (Roberts Lab)

Trophic specialization is key to the phenotypic and species diversity observed across life. Several characteristics of gut morphology and physiology correlate with trophic levels. The most common example of these correlations is found in vertebrates, where organisms with a plant-based diet generally have longer digestive tracts compared to animals at higher trophic levels. Despite its importance, very few studies have explored the genetic basis of diet adaptation. In this study, we used recently diverged Malawi cichlid species as a model to identify candidate genes involved in gut length variation with a forward genetics approach. We performed QTL mapping of gut length on an F2 mapping population from a hybrid cross between carnivorous and omnivorous species of cichlids, and identified QTL contributing to variation in gut length. This analysis represents the first identification of naturally evolved, adaptive genetic variants associated with gut length. We are now integrating these mapping results with comparative genomic and transcriptomic studies to pinpoint the genes and gene networks driving evolution of the gut.

Emerging IVb-v1 clone (CC554) of Listeria monocytogenes is highly prevalent among strains from suburban black bears

Cameron Parsons (Kathariou Lab)

Listeria monocytogenes is a facultative intracellular pathogen responsible for the disease listeriosis, which is most commonly foodborne, contracted through the consumption of contaminated food or water. While cases of listeriosis are uncommon, the severity of symptoms such as septicemia, still-births, meningitis or even death in susceptible individuals make it a major cause for public health concern. One of the reasons that L. monocytogenes proves to be so problematic is its wide distribution in the environment, having been isolated from such diverse sources such as humans, other animals, soil, water, and plant matter.

A recent survey of L. monocytogenes prevalence among black bears (Ursus americanus), yielded 786 isolates spread over three years, and spanning three different states (North Carolina, Virginia and Georgia) of the southeastern United States. Whole genome sequencing was carried out on a representative sample (104) of these isolates. MLST analysis revealed that of the 40 serotype 4b (one of the L. monocytogenes serotypes most commonly associated with human disease) isolates sequenced, 18 proved to be from CC554, making it by far the most predominant CC. This CC belongs to the 4bv-1 sertotype 4b subgroup, and has been associated with at least one outbreak, and also tied to at least four cases of listeriosis in North Carolina during this same time period. This CC was repeatedly isolated through all three years of sampling, and was isolated from samples in North Carolina as well as Virginia suggesting that its prevalence is both stable and geographically diverse. These findings also indicate that black bears may prove to be an important wildlife reservoir for L. monocytogenes, potentially playing a role in the evolution and dissemination of novel variants of this pathogen.

Session 2

Functional analysis of long non-coding RNA within the context of viral infections

Aiden Jones (Peng Lab)

The study of genetics has been mainly focused on the effect of protein coding genes. Studies on viral infections have revealed a need to investigate the functions of long non-coding RNAs (lncRNAs). LncRNAs are defined as non-protein coding RNAs of length greater than 200 nucleotides. The functions of lncRNAs are largely unknown and understudied. The focus of this study is to determine the function of human lncRNAs found to be significant from a comparative RNA-Seq analysis of viral infections. Through the utilization of SHAPE-MaP and RNA-Seq, we will probe the secondary structures of these lncRNAs and the changes in their structures during viral infections, therefore inform their functional impact.

Natural variation in Drosophila is key to deciphering the complexity of gene regulatory networks

Christopher McCallough (Reeves Lab)

Gene regulatory networks (GRN) are central to the development of the Drosophila embryo by insuring proper differentiation, morphogenesis and tissue specification. To better characterize the Anterior/Posterior (A/P) GRN during embryogenesis, this project will implement a novel methodology that makes use of the natural variation within *Drosophila melanogaster*. The three aims of this study are: One, to use natural variation to correlate DNA elements to gene expression patterns; two, use natural variation to correlate A/P genes to transcriptomic regulation; and third, construct a comprehensive model of the A/P patterning network. Preliminary data using 13 of the 205 lines in the Drosophila genetics reference panel (DGRP) suggests genetic polymorphisms could explain differences in the expression patterns of Kruppel (Kr) and Even skipped (Eve) in the 13 lines tested. Association mapping of single nucleotide polymorphism (SNP) reveals multiple SNPs in known and unknown cis-regulatory elements (CRE) of Kr and Eve. Reporter analysis of novel CREs identifies a transcription factor not previously known to regulate the Gap gene network.

Tailoring hormone responses in plants via synthetic signal integration devices

Dr. Anna Stepanova

Phytohormones are key regulators of plant growth and development that control nearly every aspect of plant's life, from embryo development to fruit ripening, from organogenesis to pathogen response. By altering the levels and distribution of hormones, plants can change their growth patterns and adapt to different environments, a phenomenon known as phenotypic plasticity. An overarching goal of my research is to understand how plants employ a limited set of hormones to integrate developmental programs with a wide array of environmental signals and produce adequate responses that enable the plants to survive and reproduce in even hostile conditions. I have been using various molecular, genetic, genomic, biochemical, and cell biology approaches in Arabidopsis and other plant species to explore the role of plant hormones in mediating plant phenotypic plasticity, to decipher the molecular mechanisms of auxin biosynthesis and ethylene signaling, to uncover the interaction nodes between the hormonal pathways, and to determine the contribution of translational regulation in hormone signaling and response. Despite the availability of a wide variety of biotechnological tools to manipulate plant growth, it has been challenging to precisely control when and where hormones are produced in a plant. We are developing a new set of CRISPR-based synthetic genetic devices to target expression of genes of interest to specific cell types. The potential utility of this new approach extends far beyond plants.

Epigenetic responses to in utero cadmium exposure

Katie Hudson (Cowley Lab)

Cadmium (Cd) is a toxic heavy metal found ubiquitously in the environment and is of increasing concern to human health. Developmental exposure to Cd is associated with reduced birth weight, essential trace element deficiencies, fetal malformations, and increased risk of metabolic disease. However, the mechanisms behind these changes are unclear. Despite poor transfer of Cd across the placenta, maternal Cd exposure is associated with altered DNA methylation in the fetus. We have identified over 600 differentially methylated regions in newborn children exposed to higher levels of Cd in utero, with regulatory regions of imprinted genes showing significant enrichment. Epigenetic mechanisms regulating imprinted genes may be more susceptible to environmental exposures during in utero development due to their unique DNA methylation dynamics. To test this, we have established a mouse model of in utero Cd exposure. We used two divergent inbred strains of mice to generate hybrid offspring to facilitate our study of imprinted genes, as we can discriminate the parental alleles based on polymorphisms. We found that in utero Cd exposure significantly reduces birth weight, yet significantly increases the size of the brain. The enlarged brains are likely due to a combination of inhibited apoptosis driven by aberrant Hoxb8 expression and an increase in myelin. At adulthood, in utero exposed mice exhibit hyperactive and uninhibited-like behaviors. We are performing Reduced Representation Bisulfite sequencing (RRBS-seq) on newborn brains to measure allele-specific DNA methylation. We will compare DNA methylation changes in mice to those found in newborn children to validate the use of our model. Results from these studies will give insights into potential mechanisms through which developmental Cd exposure causes adverse health effects in adulthood.

Session 3

GN 311: A new adventure in distance education

Dr. Betty Gardner

The “Principles of Genetics” course (GN 311) is a four credit course that serves as the first undergraduate genetics course for genetics majors. It is also taken by students in most science majors at NC State and is designed to give a general introduction to many areas of genetics. GN 311 serves over 1000 students per year. The course has been taught in a classroom (CL) format for years, but was not taught in a distance education (DE) format until spring semester 2018. Comparisons were made between the semester grade distributions and average test scores for the CL and DE sections of the course. In spring 2018, the semester grade distribution appeared to be less favorable for the distance education section of the course. Adjustments were made for summer 2018 to clarify expectations. Guided notes were provided to help students organize their studying efforts. Collaborate sessions were employed less frequently, but more strategically during the semester resulting in an increase in student participation. The summer section had a grade distribution very similar to that of a previous summer classroom section of the course indicating that student performance in the distance education section can compare favorably to that of students in the traditional classroom.

On the function and evolution of novel immune-type receptors (NITRs) in ray-finned fish

Dr. Jeff Yoder

Novel immune-type receptors (NITRs) have been identified in all teleost (bony fish) species examined and are predicted to function as natural killer (NK) cell receptors (NKRs). In mammals, NKR families (e.g. KIRs and Ly49s) include inhibitory and activating receptors that play important roles in differentiating “self” from “non-self”. Mammalian NK cells utilize NKRs to inspect potential target cells to identify and destroy infected and transformed cells while leaving normal cells unharmed. Although teleost NITRs are not direct genetic orthologs of mammalian NKRs, these gene families share many features: NITR and NKR families are encoded in gene clusters, are expressed in lymphocyte lineages, include activating and inhibitory forms, and are recently and rapidly evolving. Furthermore, when expressed on mammalian NK cells, NITRs can modulate inhibitory and activating pathways influencing NK function. Single-cell transcriptome analyses suggest that certain NITRs may provide useful markers for NK cells in zebrafish. Finally, we have shown that NITRs are encoded in more ancient ray-finned fish (gars and bowfin) indicating that this family of receptors is older than originally estimated and may provide a universal function in all ray-finned fish.

Quantitative approaches in tissue patterning

Dr. Greg Reeves

In a developing animal, tissues are often patterned by long-range signals called morphogens. Since the late 1960s, the so-called “French Flag model” posited that cells bathed in a static morphogen gradient could read their local concentration and differentiate accordingly. However, in recent years, it has been discovered that morphogens are not static at all; the implication is that cells must make sense of the ever-changing morphogen signal in order to make a differentiation decision. To tease apart these decision-making processes, we must make live, quantitative measurements of morphogen signaling and gene expression. In my lab, we use these measurements, together with mathematical modeling, to determine how tissues are patterned.

Poster Presentations

Dose response analysis in cell line models for cancer pharmacogenomics

Farida Akhtari (Motsinger-Reif Lab)

The American Cancer Society estimated over 1.6 million newly diagnosed cases of cancer and over 600,000 deaths due to cancer in 2017, in the United States alone. While therapeutic options for several forms of cancer have been improving, predictive markers for selecting the most efficient anti-cancer drug regimen remain elusive. Understanding the genetic factors responsible for the variability in individual response to a drug is critical for cancer pharmacogenomics, as failed treatments are often fatal.

Towards this goal, we are investigating the genetic factors influencing the variation in drug response to 45 commonly used, FDA-approved anti-cancer drugs, which include 15 tyrosine kinase inhibitors (TKIs) and 2 monoclonal antibodies in cell lines derived from the racially and ethnically diverse 1000 Genomes Project. Results from our multivariate genome-wide association study (GWAS) have identified 40 unique SNPs across 21 unique drugs, associated with drug response. These putative markers are in 15 unique genes, several of which, such as *NFAT5* and *NQ01*, are involved in the cell signaling and cell proliferation pathways, thus making them interesting candidates for follow-up in functional validation studies. We will perform small interfering RNA (siRNA) knockdown experiments and use other suitable *in vitro* methods to validate the significant findings from our GWAS results. We will also analyze RNA-sequencing data to determine if differences in gene expression levels are associated with variation in drug response in individuals.

The completion of this study will elucidate the biological mechanisms of action by which the GWAS-identified SNPs and their associated genes influence anti-cancer drug response. Thus, we will be able to identify novel, causal genetic variants, that may have potential clinical relevance in cancer therapeutics.

Evolution of insecticide resistance of Aedes aegypti from Iquitos, Peru

Jennifer Baltzegar (Gould Lab)

The mosquito, Aedes aegypti, transmits yellow fever, chikungunya, zika, and dengue fever, which affect large numbers of people annually. One of the most prevalent methods to control the spread of arboviral diseases is by using insecticides. Pyrethroids, a common class of insecticides, has been implicated in the development of knockdown resistance (kdr) in multiple arthropod species. With frequent and recurrent applications of pyrethroids to control Ae. aegypti populations, increased levels of kdr are expected to occur. This is a major concern for the continued efficacy of this control method. Many genetic loci associated with kdr resistance have been identified; however, two single nucleotide polymorphisms (SNPs), F1534C and V1016I, located in the voltage-gated sodium channel have been shown to be important in Central and South America. This study explores the evolution of these two SNPs across an 18-year period in Iquitos, Peru, which includes all years of pyrethroid use in the city. The results present an intriguing dynamic between resistant haplotypes that improves understanding of insecticide resistance evolution. Through further analysis, significant heterogeneity in fine-scale patterns of insecticide resistance was found, leading to a better understanding of Ae. aegypti population structure. 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.

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

Marnie Baptissart (Cowley Lab)

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. Moreover, 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 high-fat diet primes the infant for NAFLD in later life. However, the molecular mechanisms mediating disease programming remain unknown.

To address this knowledge gap, we have established a mouse model of maternal high-fat diet exposure. Our study design allows for distinction between prenatal and postnatal exposure, providing the opportunity to discriminate the relative contributions of these developmental windows to NAFLD programming. One of our most striking findings is that postnatal, but not prenatal, exposure is associated with hepatic steatosis and early fibrosis at weaning. 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 never been described. By overexpressing Zac1 in cultured hepatocytes, we show that activation of the IGN promotes fibrotic pathways and down-regulation of epithelial markers, key molecular events in NAFLD pathogenesis.

These data argue for a role for the IGN in NAFLD progression in response to maternal high-fat diet specifically during postnatal development. Our future work will study the underlying epigenetic mechanisms that may regulate modulation of the IGN.

Local production of the plant hormone auxin is sufficient to maintain the stem cell niche

Javier Brumos (Alonso Lab)

The plant hormone auxin is involved in nearly every aspect of a plant's life, from embryo development to organ abscission. Plants use the IPyA pathway to produce most of their auxin, indole-3-acetic acid (IAA). The IPyA route is composed of two simple steps. In the first one, the TAA1/TARs family of aminotransferases converts the amino acid tryptophan into the intermediate IPyA. The second reaction is catalyzed by the family of flavin-containing monooxygenases, YUCs that converts IPyA into IAA. The TAA1/TARs and YUCs gene families have been shown to exhibit very specific and dynamic spatiotemporal expression patterns, contradicting former views supporting the idea that IAA is primarily produced in shoot meristems and is then distributed to the rest of the plant via phloem and polar auxin transport establishing the auxin gradients. In the meristems, auxin gradients regulate cell division, elongation, and differentiation leading to downstream organogenesis that shapes shoot and root architecture.

To define the role of local auxin biosynthesis and its contribution in the regulation of plant growth and development in Arabidopsis, we utilized an array of experimental approaches, including 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 indicate that local auxin biosynthesis and auxin transport act synergistically in the establishment and maintenance of robust morphogenic auxin gradients essential for proper meristem maintenance and activity.

Exploration of the molecular basis of the ethylene fast response

David Bullock (Stepanova Lab)

Ethylene is a gaseous phytohormone involved in multiple aspects of plant growth, development, senescence, and stress response. Seedlings that are germinated in the dark in the presence of ethylene undergo specific phenotypic changes known as the triple response. The three elements of this response are the radial expansion and growth inhibition of hypocotyls and roots and an exaggeration of the apical hook curvature. At the molecular level, the developmental effects of ethylene are accompanied by significant changes in gene expression at both transcriptional and post-transcriptional levels. While transcriptional regulation is well established as a critical process in response to ethylene, little is known about the role of ethylene-triggered gene-specific regulation of translation. Through ribosomal footprinting, our group uncovered a critical molecular mechanism that links ethylene perception to the activation of a novel gene-specific translational control mechanism. Characterization of one of the targets of this translational regulation, EBF2, indicated that the signaling molecule EIN2 and the nonsense-mediated decay proteins UPFs play a central role in this ethylene-induced translational response, setting a new paradigm of gene-specific translational control. We aim to test the role of additional candidate genes whose translational efficiency is affected by ethylene. I am characterizing T-DNA knockouts corresponding to ethylene-responsive translational targets and studying their growth kinetics through a growth response kinetic assay. This test relies on an infrared live imaging system to monitor subtle changes in the rates of elongation in hypocotyls and roots of dark-grown seedlings transiently exposed to the ethylene gas. In parallel, I am also exploring changes in the hypocotyl and root elongation in previously characterized ethylene- and auxin-insensitive mutants. Auxin is another vital plant hormone that controls numerous processed in plant's life cycle, from embryo development to fruit ripening. Remarkably, auxin biosynthesis, transport, and signaling are known to be interconnected with the ethylene biosynthesis and signaling pathway. Thus, mutant plants with defects in auxin also show phenotypic deviations in their response to ethylene. I have tested a set of previously characterized auxin mutants regarding their dynamic responses to ethylene to determine which stages of the ethylene response and recovery are compromised. My data indicate that auxin is required for the early stage of the ethylene response (aka the fast response), that is thought to be independent of transcription. However, transcription independence of the fast ethylene response has not been fully proven, as no loss of function mutants are available for all of the candidate transcriptional master regulators of the ethylene

signaling pathway. ETHYLENE INSENSITIVE3/ETHYLENE INSENSITIVE3-LIKE (EIN3/EIL1) are the only well-characterized transcriptional master regulators of the ethylene response. The functions of their putative orthologs EIL2, EIL3, EIL4, and EIL5 are unknown. We aim to generate CRISPR/Cas9 constructs to knock out each homolog in the ein3-1 eil1-1 mutant background to create higher order mutants to reveal their ethylene-related functions, if any. Thus, my project is expected to expand our limited knowledge of ethylene-triggered translational regulation, further illuminate the role of auxin in response to ethylene, and address the nature of the fast ethylene response by shedding light on the remaining EIN3/EIL family members.

Epigenetic regulation of aging in Drosophila melanogaster

Q. Brent Chen (Mackay Lab)

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 the B lines at approximately 70 days and 35 days, respectively. We find that expression of transposable elements, normally silenced in 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 can be utilized to decipher the genetic basis of global heterochromatin state maintenance and aging. We will 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.

Single-locus multi-hormone reporters for comprehensive plant phenotyping: a synthetic biology approach

Josefina Patricia Fernandez Moreno (Alonso-Stepanova Lab)

Phytohormones are growth regulators that govern plant development, control interactions with the environment, and orchestrate plant adaptation and survival in ever-changing environments. In the past two decades, a handful of plant biosensors have been developed that enable live or ex vivo imaging of hormone levels and distribution. Synthetic transcriptional reporters, such as EBS for ethylene or DR5 for auxin, have been successfully applied to characterize the effects of various genetic or environmental perturbations on the spatiotemporal activity patterns of individual hormones. The utility of these hormone-specific sensors is however limited to detecting one growth regulator at a time. To increase the readout capacity of transcriptional reporters, we are multiplexing several synthetic biosensors in a single construct. Using GoldenBraid molecular cloning technology, we have generated a collection of synthetic hormone-responsive promoters, core promoter elements and terminators, as well as multiple versions of red, green and blue fluorescent proteins and subcellular localization signals. We are in the process of assembling, testing in transient assays and combining various hormone-specific transcriptional units for in planta expression. Our immediate plan is to generate and characterize in Arabidopsis and tomato a single-locus ACE (auxin/ethylene/cytokinin) sensor, with the ultimate goal to multiplex nine transcriptional reporters for nine major growth regulators (ACE plus ABA, gibberellins, brassinosteroids, salicylic acid, jasmonate, and strigolactones), with an individual hormone readout distinguishable by fluorescent protein color and subcellular localization.

Uncovering genetic mechanisms controlling sex in an African cichlid with polygenic sex determination

Melissa Lamm (Godwin Lab)

A diversity of genetic and environmental sex determination systems have been identified in vertebrates, but the underlying molecular mechanisms remain unknown in many lineages, including most teleost fishes. Investigating molecular pathways should provide insight into the evolution of sex determination systems and how novel alleles can invade as sex determiners. Astatotilapia burtoni, an outgroup species for the recently- and rapidly-evolved East African cichlids of Lakes Malawi and Victoria, is an important model for sexual behavior and reproduction, yet little is known about the mechanisms underlying sex. Previous studies revealed polygenic sex determination in this species, with an XY system on linkage group (LG)5-14 (chromosomal fusion) and a ZW system on LG13 in laboratory strains, as well as an XY system on LG18 in a wild population. Preliminary results from PCR-RFLP analysis of several laboratory families suggest the XY system on LG5-14 is epistatically dominant to the ZW system on LG13, though additional crosses will be necessary to confirm. Because no previously described sex-determining genes lie within the sex-associated regions, we are utilizing RNA-sequencing to identify candidate genes and pathways. We are currently sequencing gonadal and brain RNAs from ZW and ZZ (LG13) embryos at 14 days post-fertilization, a time point that likely lies within or near the sex-determining window. In the future we also seek to investigate the Y sex-determining pathway on LG5-14 and the interaction between W and Y pathways in families segregating both alleles. These studies should help uncover genetic mechanisms underlying sex and provide insight into the evolution of diverse sex determination systems.

Evolution of Genetic Sex Determination in a Model for Rapid Speciation

Erin Peterson (Roberts Lab)

Sex determination is a fundamental process in sexually reproducing organisms that can be determined by a number of different factors. There is a variety of genetic systems, with multiple, different genes governing the sex of an individual. The species-rich East African cichlid fishes display extensive variation in multiple, different traits as a result of their explosive adaptive radiation, including vast diversity in their sex determination systems. Using species from this group, we have identified both single gene systems and polygenic systems, spanning multiple distinct chromosomes, providing the opportunity to explore how sex determiners can evolve. Polygenic sex determination (PSD) relies on the interaction of genes, providing the opportunity to study epistatic interactions. It is not well known why these different systems have arisen, with hypotheses about kayrotypic rearrangements and transposable elements. Due to the system of multiple sex determiners present in this population, it presents a unique opportunity to study not how different sex determiners arise but also how sex chromosomes evolve among multiple lineages.

The role of epigenetic dysregulation of imprinted genes in cadmium-induced fetal growth restriction

Mark Simmers (Cowley Lab)

Cadmium (Cd) is a toxic transitional heavy metal found throughout the environment. Research has linked in utero Cd exposure to fetal growth restriction, malformation, and spontaneous abortion. In utero Cd exposure has also been linked with fetal DNA methylation changes across regulatory elements at imprinted genes, which are defined by their expression from a single parental allele. Cdkn1c is an imprinted gene and potent growth regulator, in which subtle increases in placental expression can dramatically reduce developmental size. Significant changes to Cdkn1c expression in the placenta have been shown in response to Cd; however, the exact mechanism is yet to be determined. We hypothesize that methylation changes in the promoter of Cdkn1c and neighboring regulatory elements contribute to the mechanism through which Cd affects Cdkn1c expression. To test this hypothesis, we have established a hybrid mouse model to determine the effects of in utero Cd exposure on allele-specific methylation and expression of imprinted genes. We are currently performing RT-qPCR, pyrosequencing, and next generation bisulfite sequencing on placental and fetal tissues to analyze the expression levels and methylation states of Cdkn1c and its neighboring genes. We aim to determine whether relaxation of imprinting could be driving these changes. Results from these experiments will provide insight into the mechanisms through which Cd negatively impacts fetal development.

Session 4

The genomic regulation of limited lifespan and reproductive senescence

Grace Parker (Carbone and Mackay Labs)

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. Our goal is to identify causal genes associated with lifespan and reproductive senescence in Drosophila melanogaster by functional analyses of genetically divergent genes between five long-lived (O) lines selected for postponed reproductive senescence and five unselected (B) lines. Preliminary data assessing productivity of the reciprocal crosses between the O and B lines suggest that genes influencing reproductive senescence are maternally-controlled. Therefore, all candidate genes tested are expressed in the ovaries of females. To determine which candidate genes exhibit a quantitative change in lifespan or productivity, we assessed lifetime reproduction from the Vienna collection of RNAi lines in which gene expression is knocked down in the ovaries and accessory glands and identified genes that both increase and decrease longevity and productivity. Additionally, we identified alternative SNPs between the O and B lines that alter promoter function using an in vitro luciferase reporter assay. Identifying evolutionarily conserved genes affecting lifespan and 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 in populations with increasing lifespans.

Encouraging creativity in lab courses by rethinking the traditional lab report

Dr. Claire Gordy

Undergraduate biology students take numerous lab courses that require written lab reports. In traditional lab courses, these reports are often formulaic, and there is little room for creativity. We recently implemented a novel yeast genetics course-based undergraduate research experience (CURE) in an introductory genetics lab course enrolling 100-130 students/semester. As part of this course redesign, we have replaced traditional lab reports with Data Summaries that focus on visual representations of data. With this change, the focus has shifted from report structure and grammar to construction of accurate and compelling figures. The students in this course (24% sophomores, 38% juniors, 36% seniors; 92% life science majors) have little previous experience using Excel, performing basic statistical tests, creating graphs, and creating figures. The introduction of Data Summaries has thus required development of brief data analysis workshops along with implementation of peer review, instructor feedback, and revision. This cycle of feedback and revision culminates with the completion of a Final Experimental Portfolio that contains polished and presentable versions of the student's figures along with a Graphical Abstract that visually summarizes the student's key findings in a way that could be understood by someone outside of the class. In this talk, we will present examples of student work that demonstrate the both the progression of students' ability to represent their data visually over the course of the semester and the creative thinking that is unleashed when students are released from the confines of the traditional lab report.

Genomic signatures of hybrid male sterility in the mouse

Sam Widmayer (Aylor Lab)

Hybrid male sterility (HMS) is a reproductive barrier that restricts gene flow between two subspecies of mice, Mus musculus musculus and M. m. domesticus. Two major loci have been previously linked to HMS in laboratory crosses, but we observed wide variation in fertility and reproductive traits among hybrids with identical genotypes at those loci. We characterized reproductive trait variation in a panel of hybrid males bred by crossing musculus-derived PWK/PhJ strain females to males from four inbred mouse strains of primarily domesticus origin. These hybrids displayed three distinct trajectories of fertility: complete sterility, complete fertility, and age-dependent fertility. Males that displayed age-dependent HMS were fertile between 15-35 weeks of age with moderate penetrance. These results point to multiple segregating HMS modifier alleles, some of which have an age-dependent mode of action. Whole-testis gene expression patterns distinguished the three fertility trajectories and implicated key regulatory pathways involved in changes to fertility with age. Characterizing the regulatory signatures of each fertility trajectory could inform implicate new molecular mechanisms of reproductive isolation in mice.

Identification of microenvironmental contributors to carcinogenesis with a zebrafish model

Dr. Heather Shive

Cancers do not exist in isolation, but rather are heavily dependent on complex interactions between cancer cells and the surrounding microenvironment. It is accepted that the tumor microenvironment directly impacts cancer cell survival, growth, invasion, and metastasis. However, little is known about molecular mechanisms that operate in the microenvironment to support cancer initiation. We have developed a zebrafish model that experiences a heritable cancer syndrome characterized by high cancer onset in a limited number of cancer-prone sites. Using a reporter construct, we isolated the cells that constitute the cancer-prone microenvironment from putative precancerous and cancerous cells in these cancer-prone sites. We performed RNA-seq analysis of the cellular component of the wild type (control), precancerous, and cancerous microenvironments, and identified genes with significantly different levels of expression between wild type and precancerous samples (842 genes), wild type and cancerous samples (6,711 genes) and precancerous and cancerous samples (4,837 genes). Pathway analysis is underway to (1) identify key genetic networks that define the cancer-prone microenvironment and (2) direct selection of candidate genes that may function in the microenvironment to promote cancer cell growth. Pairing this comparative transcriptomics approach with direct in vivo testing of candidate genes, as is uniquely possible with our zebrafish model, is expected to efficiently identify candidate microenvironmental gene networks that favor cancer onset.

Have a question concerning the Program in Genetics?

Contact one of us below!

Dr. Reade Roberts
Director of the Graduate Program in Genetics
rbrober2@ncsu.edu

Dr. Betty Gardner
Undergraduate Director of Genetics Program
bgardner@ncsu.edu

Jenni Shaw
Graduate Services Coordinator for Genetics
jnshaw2@ncsu.edu

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

Dr. Henry and Diane Schaffer
Dr. Hamid Ashrafi
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Dr. Betty Gardner
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Dr. Kurt Marsden
Dr. Carolyn Mattingly
Dr. Reade Roberts
Lossie Rooney
Dr. Ross Sozzani
Dr. Anna Stepanova

List of Attendees		
Last Name	**First Name**	**Email**
Akhlaghi	Shiva	sakhlag@ncsu.edu
Akhtari	Farida	farida.akhtari@ncsu.edu
Al Asafen	Hadel	hyalasaf@ncsu.edu
Allard	Nicole	necarns@ncsu.edu
Alonso	Jose M	jmalonso@ncsu.edu
Aylor	David	dlaylor@ncsu.edu
Baltzegar	Andy	dabaltze@ncsu.edu
Baltzegar	Jennifer F	jen_baltzegar@ncsu.edu
Baptissart	Marine	mbaptis@ncsu.edu
Bandodkar	Prasad	pbandod@ncsu.edu
Bird	David	bird@ncsu.edu
Brumos	Javier	jbrumos@ncsu.edu
Bullock	David Allen	dabullo2@ncsu.edu
Carlson	Kara	kbcarlso@ncsu.edu
Carmona	Aldo	acarmon@ncsu.edu
Chen	Qichuan	qchen14@ncsu.edu
Conant	Gavin	Gconant@ncsu.edu
Cowley	Mike	macowley@ncsu.edu
Crisci	Elisa	ecrisci@ncsu.edu
Deslauriers	Jake	jcdeslau@ncsu.edu
Diveley	Kayleigh R	Krdivele@ncsu.edu

Doherty	Colleen	cjdohert@ncsu.edu
Fernandez Moreno	Josefina Patricia	jpfernan@ncsu.edu
Gardner	Betty	bgardner@ncsu.edu
Gordy	Claire	clgordy@ncsu.edu
Gould	Fred	Fred_Gould@ncsu.edu
Goyal Gupta	Etika	egoyalg@ncsu.edu
Halweg	Chris	chris.halweg@ncsu.edu
Holland	Jim	james_holland@ncsu.edu
Hudson	Katie	kmbehren@ncsu.edu
Hulse-Kemp	Amanda	amhulse@ncsu.edu
Isik	Fikret	fisik@ncsu.edu
Johnstun	Joel A	jajohn23@ncsu.edu
Jones	Whitney M	wmjones4@ncsu.edu
Jones	Aiden	amjone16@ncsu.edu
Karre Satyanarayana Guptha	Shailesh	skarres@ncsu.edu
Kathariou	Sophie	skathar@ncsu.edu
Lamm	Melissa	maslane@ncsu.edu
Langerhans	Brian	langerhans@ncsu.edu
Little	Joy	jlsnowde@ncsu.edu
Maly	Morgan	Mamaly@ncsu.edu
Marsden	Kurt	kcmarsde@ncsu.edu
Mattingly	Carolyn	cjmattin@ncsu.edu
McCallough	Christopher	clmccal2@ncsu.edu
Nielsen	Dahlia	dahlia@statgen.ncsu.edu

Parker	Grace	gaparke3@ncsu.edu
Parker	Jackson	jpparke2@ncsu.edu
Parsons	Cameron	ctparson@ncsu.edu
Peterson	Erin	enpeters@ncsu.edu
Reeves	Gregory T	gtreeves@ncsu.edu
Roberts	Reade	rbrober2@ncsu.edu
Rogers	Anna	arroger4@ncsu.edu
Rojas-Pierce	Marcela	mrojasp@ncsu.edu
Rooney	Lossie	llrooney@ncsu.edu
Schaffer	Henry	hes@ncsu.edu
Schloop	Allison	aeschloo@ncsu.edu
Shaw	Jenni	jnshaw2@ncsu.edu
Shive	Heather	hrshive@ncsu.edu
Simmers	Mark	mdsimme2@ncsu.edu
Sozzani	Ross	rsozzan@ncsu.edu
Stepanova	Anna Rogers	atstepan@ncsu.edu
Tao	Jing	jtao2@ncsu.edu
Thorne	Jeff	thorne@statgen.ncsu.edu
Tolsma	Joseph	jsjoseph@ncsu.edu
Unselt	Desiree	dmunselt@ncsu.edu
Venkatakrishnan	Sandhya	svenka16@ncsu.edu
Widmayer	Sam	sjwidmay@ncsu.edu
Williamson	Megan	mewilli9@ncsu.edu
Yoder	Jeff	jeff_yoder@ncsu.edu

Zeng	Zhao-Bang	szeng@ncsu.edu
Zeynalzadeh	Monica	mzeynal@ncsu.edu

Thanks for attending the 9th Annual Program in Genetics Fall Retreat 2018!

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