
# 44th Annual Genetics Graduate Student Symposium

February 14th, 2020
Stanley G. Stephens Room
3503 Thomas Hall

**The GGSA welcomes you to the**
**44th Annual Genetics Graduate Student Symposium**

**Session I - Moderator: Kara Carlson**

9:00 am Opening Remarks – Jen Baltzegar

9:15 am Aldo Carmona Baez
*Identifying candidate genes for trophic level adaptation in the African cichlid gut*

9:30 am Allison Schloop
*Feedforward and feedback regulation in Drosophila dorsal-ventral patterning*

9:45 am David Bullock
*Exploration of the molecular basis of the fast ethylene response*

10:00 am Aiden Jones
*Identification of long non-coding RNAs involved in HIV-1 infection of human CD4+ T cells using large scale CRISPRi screen*

10:15 am Monica Zeynalzadeh
*Non-coding genetic variation in the hsp90ab1 locus contributes to developmental and environmental exposure susceptibility differences*

10:30 am Coffee Break

**Session II - Moderator: Joseph Tolsma**

11:00 am Morgan Maly
*Genomic approaches to endangered species management: Population analysis and gut microbiome characterization of the ex-situ cheetah (Acinonyx jubatus)*

11:15 am Eddie Lauer
*Genetic analysis via high-density linkage mapping and bulked segregant RNAseq identifies multiple candidate broad-spectrum fusiform rust genes in Pinus taeda L.*

11:30 am Kay Diveley
*Using high-throughput approaches to identify functional SNPs derived from GWAS studies*

11:45 am GGI Update - Dr. Martha Reiskind and Dr. Fred Gould

12 pm Lunch

**Session III - Moderator: Anna Rogers**

1:00 pm Sam McMillan
*Engineering of Escherichia coli MP1 for delivery of bile salt hydrolases for prevention and treatment of Clostridiodes difficile infection*

1:07 pm Uchechukwu Chimeh
*Characterization of geno-by-environment (GxE) effect on disease susceptibility*

1:15 pm Fausto Rodriguez Zapata
*Assessing population genetics evidence for local adaptation to phosphorous availability in maize*

1:22 pm Kristen John
*Characterization of long non-coding RNAs in influenza A virus replication*

1:30 pm Sam Harp
*Using the four core genotypes mouse model to investigate the role of sex chromosome genes in drug addiction*

1:37 pm Sarah Hudadoff
*Sex-specific transcripts of a novel gene in Lucilia cuprina*

1:45 pm Kristen Adcock
*Understanding Temperature Sensitivity in Yeast*

1:52 pm Outreach Update

2:00 pm Jake Deslauriers
*Identifying the role of cyfip2 in regulation of the acoustic startle threshold*

2:15 pm Elle Rooney
*FlySection: A database of gene expression patterns in embryonic Drosophila*

2:30 pm Coffee Break

**Session IV - Moderator: Jake Deslauriers**

3:00 pm Erin Peterson
*A supernumerary chromosome produces a 0W/00 sex determination system in a cichlid fish*

3:15 pm Megan Williamson
*Transgenic male-only system development in the sheep blowfly, Lucilia cuprina*

3:30 pm Joseph Tolsma
*Influence of the circadian clock on the Arabidopsis gravitropic response*

4:00 pm Kara Carlson
*Population structure and evolution of immune genes in two ancient ray-finned fishes*

4:15 pm Grace Parker
*Genomic regulation of limited lifespan and postponed reproductive senescence in Drosophila melanogaster*

4:30 pm Anna Rogers
*From Genomes to Fields: Exploring genotype-by-environment interactions and environment specific prediction in maize hybrids*

4:45 pm Closing Remarks - Dr. Reade Roberts

**Identifying candidate genes for trophic level adaptation in the African cichlid gut**

Aldo Carmona-Baez
Advisor: Reade Roberts

Trophic specialization is key for the phenotypic and species diversity observed across life. Previous studies have explored the contribution of morphological, physiological, and behavioral changes to this phenomenon; nevertheless, the role of the gastrointestinal system in dietary adaptation remains unexplored. In this study, we compared the gene expression profiles of gut tissue from four different Malawi cichlid species and one outgroup in order to look for candidate genes and pathways involved in intestinal dietary adaptation. This analysis showed that the carnivore species, Aulonocara koningsi, has a more distinct gene expression profile compared to the omnivore and herbivore species. Furthermore, the gene ontology enrichment procedure identified DNA-replication, oxidative phosphorylation, and metabolism of xenobiotics as enriched terms in the differentially expressed gene sets. Future functional validation studies of these physiological differences will contribute to our understanding of trophic specialization in this group of species.

**Feedforward and feedback regulation in *Drosophila* dorsal-ventral patterning**

Allison Schloop
Advisor: Greg Reeves

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 (NCSU CBE), 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.

**Exploration of the molecular basis of the fast ethylene response**

David Bullock

Advisors: Alonso JM, and Stepanova AN

Ethylene is a gaseous phytohormone involved in multiple aspects of plant growth, development, senescence, and stress response. Ethylene causes significant changes at the molecular level in gene expression at both transcriptional and post-transcriptional levels. It is known the initial phase of the response to ethylene 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. In parallel, we are using a pharmacological approach to inhibit gene transcription during the ethylene treatment and examining the ethylene response. 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 of this project is investigating the role of ETHYLENE INSENSITIVE2 (EIN2), a key ethylene signaling molecule, in the fast ethylene response. Cumulatively, my project has to expand our knowledge of the ethylene-triggered fast response.

**Identification of long non-coding RNAs involved in HIV-1 infection of human CD4+ T cells using large scale CRISPRi screen**

Aiden Jones
Advisor: Xinxia Peng

Currently, over 16,000 annotated long non-coding RNAs (lncRNAs) and over 35,000 transcripts are uncharacterized. Functional identification and full annotation of these sequences provide a potential source of novel targets. To determine which portion of these lncRNAs are involved in HIV-1 infection, we need a high throughput method to streamline this process. By using CRISPR interference (CRISPRi), we propose a high throughput method to analyze approximately 9,400 genes for association with HIV-1 infection of human CD4+ T cells. From this screen, we expect a subset of lncRNAs whose correlation can be confirmed using qPCR and individual gene knockdown. In further studies, we plan to define transcript structures and functions using 5' RACE, SHAPE-MaP, RNASeq, and various pulldown assays. This experimental pipeline can be used in future studies to find related lncRNAs related to infection with different viruses and in different cell types.

**Non-coding genetic variation in the *hsp90ab1* locus contributes to developmental and environmental exposure susceptibility differences**

Monica Zeynalzadeh
Advisor: Antonio Planchart

Genetic variation contributes substantially to the variation in drug and xenobiotic susceptibility across human populations and impacts human traits and diseases. The chaperone heat-shock protein 90 (Hsp90) interacts directly or indirectly with many client proteins and is known to buffer genetic and environmental variation by stabilizing them, thereby uncoupling phenotype from genotype. Results from our lab indicate that an intronic deletion in the heat shock protein 90 alpha-class B member 1 (*hsp90ab1*), an isoform of *hsp90*, is correlated with different phenotypic outcomes. We tested the ability of zebrafish embryos carrying this mutation to adjust and adapt to environmental stressors such as heat shock, or exposure to the aryl hydrocarbon receptor (AhR) ligand, leflunomide. Exposure to leflunomide or heat shock resulted in different outcomes that depended on the zygosity of the *hsp90ab1* deletion. Analysis of mRNA and protein expression did not show significant differences in the expression levels of *hsp90ab1* in deletion-bearing homozygotes. However, results from comparative global proteomics showed significantly different abundance of several proteins in *hsp90ab1* deletion-bearing homozygotes compared to non-deletion-bearing homozygotes. This suggests that the deleted region in *hsp90ab1* harbors regulatory elements (enhancers and/or repressors) that affect the transcription of neighboring genes. Our data elucidate the effects of a deletion in *hsp90ab1* on developmental processes and provide insights into how variant alleles of *hsp90ab1* may affect variation in population susceptibility to common environmental stressors independent of the actual levels of *hsp90ab1* mRNA or protein levels.

**Genomic approaches to endangered species management: Population analysis and gut microbiome characterization of the *ex-situ* cheetah (*Acinonyx jubatus*)**

Morgan Maly
Advisors: Reade Roberts and Matthew Breen

The cheetah is an excellent ambassador for wildlife conservation and education. Due to poaching, habitat loss, and the illegal wildlife trade cheetah populations are dwindling. Only ~7,100 individuals remain *in-situ*, illuminating the importance of maintaining captive breeding populations as insurance against extinction. A broader understanding of cheetah biology and health is critical for captive populations to become self-sustaining. Our goal is to combine felid genetics and bacterial metagenomics to better understand and improve cheetah health and management. We hypothesize the use of species-specific genetic markers will inform *ex-situ* breeding programs to increase population genetic variability. We developed a panel of 16 tetranucleotide markers developed from the cheetah genome to assess the empirical relatedness of the *ex-situ* North American Species Survival Plan (SSP) cheetah population. Gastrointestinal (GI) diseases affect a large portion of *ex-situ* cheetahs, with few reports in the wild. Due to significant dietary differences between the two populations and the link between diet, microbiome, and disease, we hypothesize that GI diseases in *ex-situ* cheetahs are associated with unique gut microbial profiles. To address this second hypothesis, we will characterize the GI microbiome of cheetahs across varying diet types using 16S rRNA sequencing, and compare results with wild Namibian cheetahs. These data will be utilized to more thoroughly understand dietary factors influencing the gut microbiome and GI disease, providing new strategies to mitigate management and health challenges.

**Genetic analysis via high-density linkage mapping and bulked segregant RNAseq identifies multiple candidate broad-spectrum fusiform rust resistance genes in Pinus taeda L.**

Eddie Lauer
Advisor: Fikret Isik

Fusiform rust, caused by the fungus Cronartium quercuum f. sp. fusiforme, is the most important disease affecting loblolly pine plantations in the United States, resulting in multi-million-dollar annual losses to the forestry and wood products industry. In this report, we detail the first discovery of alleles which confer broad-spectrum fusiform rust resistance. Using an integrated approach combining high-density linkage mapping with bulked-segregant RNAseq, we mapped these alleles with high resolution in the pine genome, identified the sequence of candidate genes, and determined their effects on disease outcome at the population level. Within-family QTL analysis using a controlled inoculation on two full-sib families of 1000 individuals indicated two resistance loci in both families which differed markedly in their genomic localization and resistance profile. In the most resistant family, both alleles reduced the odds of infection by around 20-fold, and the alleles exhibited additive gene action with respect to the odds of infection. RNAseq using bulked samples of resistant and susceptible seedlings revealed nonsynonymous substitutions in two TIR-NBS-LRR genes and one CC-NBS-LRR gene, all of which completely cosegregated with disease status. Fine mapping is currently underway in order to determine allele content and gene order of these loci, as well as to identify markers which can be used in developing the first true breeding disease resistant pine varieties.

**Using high-throughput approaches to identify functional SNPs derived from GWAS studies.**

Kay Dively
Advisor: Xinxia Peng

Genetic research into complex diseases heavily relies on Genome-Wide Association Studies (GWAS) to identify significant regions of variation, such as Single Nucleotide Polymorphisms (SNPs). However, GWAS results provide statistical associations to disease phenotypes without confirming causation or explaining the underlying functionality of each genetic variant.

To fill this gap, researchers traditionally choose to characterize a few selected SNPs and their functional contribution to a disease of interest. While useful, this would be extremely expensive and time-consuming to investigate the large number of SNPs identified from a typical GWAS study. High-throughput approaches have since been established to experimentally screen thousands of candidate SNPs in parallel to determine the causal variants and their functionality. These methods include the use of tandem SNP-sequencing and Flanking Restriction Enhanced Pulldown (FREP).

Currently, I am utilizing SNP-Seq to identify causal genetic variants from a pool of over 4,000 candidate SNPs located in the Fc gamma receptor locus that may affect HIV-1 vaccine efficacy. Results from this research may facilitate the development of efficacious HIV vaccines that account for natural variations in important immune genes.

**Engineering of *Escherichia coli* MP1 for delivery of bile salt hydrolases for prevention and treatment of *Clostridioides difficile* infection**

A.S. McMillan
Advisor: C.M. Theriot

*Clostridioides difficile* is a gastrointestinal pathogen seen most frequently after antibiotic treatment. Antibiotic treatment alters the gut microbiota and the bile acid metabolome decreasing colonization resistance against *C. difficile* in the gut. While some conjugated bile acids act as germinants for *C. difficile* spores (ex. TCA), deconjugated bile acids (ex. CDCA, and DCA) can inhibit *C. difficile* growth and prevent toxin production. The first step in deconjugating bile acids is done by Bile Salt Hydrolases (BSHs). Previous work suggests that re-establishing bacteria encoding BSHs can improve outcomes in patients with recurrent *Clostridioides difficile* infection (CDI). However, delivering such bacteria to the colon is challenging. We sought to engineer *Escherichia coli* MP1 for successful expression of an exogenous BSH. Growth of *E. coli* MP1 in the presence of both the glycine and taurine conjugated (GCA, GCDCA, GDCA, TCA, TCDCA, TDCA) as well as the unconjugated (CA, CDCA, DCA) bile acids was observed even at high physiologic concentrations (20 mM). The resistance to both the substrates and products of BSHs makes *E. coli* MP1 a promising vector for BSH delivery. Future work will include cloning BSHs from both *L. crispatus* (glycine specific) and *L. aviarius* (taurine specific) into *E. coli* MP1 for *in vitro* assays with *C. difficile* to determine how they inhibit *C. difficile* pathogenesis. *E. coli* MP1 strains encoding active BSHs will also be evaluated *in vivo* in a mouse model of CDI. If successful, this engineered bacterial strain encoding novel BSHs could aid in the prevention and treatment of CDI.

**Characterization of Gene-by-Environment Interaction (GxE) Effect on Disease Susceptibility**

Uchechukwu Chimeh
Advisor: David Aylor

Interactions between an individual's genotype and environmental exposures influence susceptibility to complex diseases. Typically, investigations of gene-by-environment interactions (GxE) involve candidate gene studies or agnostic genome-wide association studies (GWAS). Advances in GWAS technology have led to discovery of GxE loci across various diseases, despite challenges including underpowered studies and difficult-to-measure exposures. These interactions, a few of which researchers have validated in replicated functional studies, often involve metabolic genes and lifestyle factors such as diet and smoking. However, many GxE interactions remain unvalidated and there remains a need for discovery of unique interactions. Recent studies have underutilized advanced functional genomics methods including annotated omics data, CRISPR/Cas9 genome editing, and innovative culture and model systems. We propose optimization of cell culture models to conduct reproducible exposure studies and elucidate the mechanisms of GxE interactions. Various candidate loci have been identified in regulatory regions; therefore, interactions should be validated in tissue-specific contexts. Discovery of novel GxE interactions and validation of existing candidates would lead to a better understanding of the mechanisms of complex diseases. Consequently, this would improve customization of disease prevention and treatment for individual susceptibility based on genotype and environmental risk.

**Assessing Population Genetics Evidence for Local Adaptation to Phosphorus Availability in Maize.**

Fausto Villafrade Rodríguez Zapata
Advisor: Ruben Rellán-Alavarez

Plant phosphorus deficiency reduces yield and disables the plant capacity to cope with stress. However maize has been able to adapt to locations such as Mexican highlands where phosphorus deficient volcanic soils and low temperatures combine into an unfavorable production environment. There at high elevations highland maize outyields lowland maize. I propose that divergent selection in loci involved in phosphorus starvation response (PSR) can partially explain this pattern of local adaptation. In the absence of opposing evolutionary forces, the main consequence of such 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 solubility potential to geographic locations. With this solubility potential as phenotype I performed environmental GWAS on 3238 georeferenced landraces of Zea mays from Latin America and the Caribbean. I found a significant signal from Inv4m, a previously reported adaptive retrogression from highland teosinte that includes ZmPho1;2a an inorganic phosphate transporter. In order to disentangle the effects of neutral population structure and environmental correlates of soil phosphorus availability from actual allelic effects I propose an analysis of phosphorus response in biparental populations polymorphic for Inv4m.

**Characterization of long non-coding RNAs in influenza A virus replication**

Kristen John
Advisor: Xinxia Peng

Influenza epidemics cause 3 to 5 million cases of severe illness every year, resulting in 291,000 to 646,000 deaths. While much progress has been made to understand the molecular interactions that regulate influenza replication in the human host, the majority of these studies have focused primarily on protein coding genes. Limited evidence suggests that long noncoding RNAs (lncRNAs) regulate host antiviral responses as well, yet the mechanisms by which they modulate viral response still remain elusive. In order to identify potential target lncRNAs that regulate viral replication, we compiled a large collection of RNAseq analysis of influenza infections across multiple human epithelial cell types and influenza A strains. From this analysis, we identified a subset of lncRNAs that were consistently upregulated during infection. We propose that these lncRNAs regulate influenza replication and host response in human cells. To test this hypothesis, we will first determine how viral replication is altered when the expression of these individual lncRNAs is knocked down or activated in human epithelial cells. Further characterizing the function of these lncRNAs would not only elucidate the role of lncRNAs during infection, but would likely provide new targets for influenza intervention.

**Using the four core genotypes mouse model to investigate the role of sex chromosome genes in drug addiction**

Samuel Harp
Advisor: Emilie Rissman

In the past decade, drug addiction has become an increasingly severe public health problem. Many addiction studies in both humans and mice have found behavioral differences between males and females. On average, adult men are more likely to have a drug abuse problem, while adult women escalate doses faster after beginning use. Complex behaviors like drug abuse are influenced by the various social, cultural, and biological factors. Previous research has considered hormonal differences to be the primary biological variable when looking at differences between males and females, but there has not been a focus on the possibility of differences in sex chromosome gene dosage.

In females, one of the X-chromosomes is inactivated in each cell, which would suggest that males and females have equal X-chromosome gene dosage. However, a small number of genes have been found to reliably escape inactivation, leading to higher rates of transcription in females. Many of these active genes have been identified as chromatin remodelers, which affect transcription throughout the genome.

Investigating the effects of these chromatin remodeling genes is essential to understanding the causes of sex differences in drug addiction. Our lab uses the four-core genotype mouse model to separate the effects of sex hormones and sex chromosome genes on drug-taking behavior. By putting these mice through classic drug addiction behavioral paradigms like self-administration and conditioned place preference, we hope to increase our understanding of one of the major causes of sex differences in drug addiction.

**Sex-Specific transcripts of a novel gene in *Lucilia cuprina***

Sarah Hudadoff
Advisor: Max Scott

In Drosophila melanogaster, sex is determined by female-specific expression of sex-lethal (Sxl), which regulates sex-specific RNA splicing of transformer (tra). Downstream of tra are fruitless (fru) and doublesex (dsx), which control the transcription of genes leading to the differences between males and females. Fru and dsx transcripts are spliced differently in males and females, due to the binding of the TRA/TRA2 protein complex in females only. The TRA/TRA2 protein complex binds to sites that are clustered near alternatively spliced exons.

The tra gene in the sheep blowfly, Lucilia cuprina, appears to serve the same function as in Drosophila. Transcripts from the dsx gene are sex-specifically spliced as in Drosophila and the alternatively spliced female exon contains sites that match the TRA/TRA2 consensus. A fru ortholog has been identified in the genome assembly. I will be testing if fru transcripts are sex-specifically spliced as in Drosophila. However, besides dsx and fru, two novel genes could be targets of tra in L. cuprina. The genes have several predicted TRA/TRA2 binding sites clustered in one intron, suggesting transcripts may be sex-specifically spliced.

I am extracting RNA from male and female blowflies at different stages of development in order to generate cDNA preparations for RT-PCR analysis. The transcripts can then be compared, to determine if sex-dependent RNA splicing is occurring. If the genes do produce sex-specific transcripts, they may play important roles in male or female development and could also be potential targets for sex-ratio manipulation for future population control of the blowfly.

**Understanding Temperature Sensitivity in Yeast**

Kristen Adcock
Advisor: Caiti Smukowski-Heil

Over time, environmental stimuli can facilitate evolution of an organism's responses to environmental factors, one being tolerance to nonideal temperatures. However, some of the means used by organisms to adapt have not been elucidated. Two strains of yeast- Saccharomyces cerevisiae and Saccharomyces uvarum are known for their thermotolerance or cryotolerance, respectively. Both encode a temperature sensitive gene, known as PHO84. PHO84 was discovered through utilization of S. cerevisiae and S. uvarum hybrids that were grown during nutrient-limited evolution experiments. In an event known as loss of heterozygosity (LOH), the S. cerevisiae copy of PHO84 was amplified while the S.uvarum allele was lost. PHO84 has since been shown to contribute to S. cerevisiae's thermotolerance and S. uvarum's cryotolerance. To discover the location of beneficial mutations in the PHO84 gene responsible for conferring differential temperature sensitivity, chimeric promoter-coding sequences were created. These chimeric constructs can be monitored on a PHO84 null background of each species to determine if regulatory mutations confer temperature sensitivity. Elucidated mechanisms of temperature sensitivity could be utilized by industry to cultivate strains of S. cerevisiae at colder temperatures.

**Identifying the role of *cyfip2* in Regulation of the Acoustic Startle Threshold**

Jake Deslauriers
Advisor: Dr. Kurt Marsden

Amidst a perpetual stream of sensory stimuli, animals must be able to distinguish between salient and innocuous stimuli in order to navigate their environment. For instance, animals escape from loud, threatening sounds by setting a startle threshold to trigger a widely conserved startle response. Animals must establish an appropriate level for the startle threshold to ensure inconsequential stimuli are ignored, but threats are detected. Dysregulation of the startle threshold is associated with autism and anxiety-related disorders, yet its genetic regulation is poorly understood. A recent forward genetic screen in zebrafish using an unbiased, high-throughput approach and whole-genome sequencing isolated a causal mutation in the gene encoding the *Cytoplasmic Fragile X Mental Retardation Protein Interacting Protein (FMRP) 2* (*cyfip2*), but the molecular and cellular pathways by which *cyfip2* regulates the startle threshold remain unknown. Cyfip2 binds Rac1 to promote actin polymerization and may regulate local RNA translation through its interaction with FMRP/eIF4E. To determine whether Rac1 or FMRP pathways are engaged by *cyfip2* to regulate the startle threshold I will create stable transgenic lines expressing mutant *cyfip2* in which critical residues for Rac1 or FMRP/eIF4E binding are altered. To identify where Cyfip2 activity is required, previous *in vivo* $Ca^{2+}$ imaging experiments revealed that hindbrain spiral fiber neurons (SFNs) are hyperactive in *cyfip2* mutants. I will transgenically express Cyfip2 in SFNs or their upstream inputs, to determine whether Cyfip2 expression in these cells is sufficient to restore a normal startle threshold. This project will demonstrate how a gene can regulate neural circuitry to shape simple decision-making behavior.

**FlySection: A Database of Gene Expression Patterns in Embryonic *Drosophila***

Elle Rooney
Advisor: Gregory T. Reeves

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 present a database, called “FlySection,” 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 will be searchable and available for download. FlySection consists of a JavaScript webapp where users can search for relevant images using a few categorizing labels or quickly create datasets using criteria for parameters extracted from the images during analysis. The app accesses a Firebase Real-time Database where the results of our image analysis are stored. We expect that this database will assist members of the *Drosophila* research community who are studying gene regulatory networks in development to explore existing hypotheses and uncover new hypotheses for further study by collecting a large volume of image data in one place that is easily accessible and sorted by fly genotype, gene, fixed vs. live imaging, protein vs. mRNA imaging, or specific parameter values from which custom datasets can easily be constructed.

We present some examples, including pooled datasets drawn from FlySection that allow for greater statistical power than images acquired and analyzed by a single researcher, datasets that contain a wider scope of images than might be expected to apply to a given question, and novel questions generated by examining datasets created with FlySection.

**A supernumerary chromosome produces a 0W/00 sex determination system in a cichlid fish**

Erin Peterson
Advisor: Reade Roberts

Genetic sex determination is a relatively conserved process among many vertebrates, but appears to evolve rapidly in some taxa, such as East African cichlid fish, where sex can be determined by a variety of genetic loci. B chromosomes, also known as supernumerary chromosomes, are characterized by highly repetitive DNA and in East African cichlid fish show homology with autosomal sequences. Here, we examine a species of cichlid, *Labidochromis caeruleus*, whose sex determination relies entirely on the presence or absence of a B chromosome, producing a female-determining 0W/00 system. While other species of cichlids show interactions of autosomal sex determiners with feminizing B chromosomes, this is the first instance of a B chromosome as the sole sex determiner. Using whole genome sequencing of a female *L. caeruleus*, we identified B chromosome sequences and SNPs. Using B-specific primers, we genotyped for the presence or absence of the B chromosome in both pure species and in hybrid crosses. In pure *L. caeruleus* families, we observe sex associating perfectly with the presence of a B chromosome (n = 44). In two interspecific hybrid mapping crosses, we find lowered transmission of the B chromosome to the F2 offspring, while maintaining the B chromosome as the primary sex determiner. In an intergeneric hybrid cross we observe only 7% female F2 (n = 474), and in an intrageneric hybrid cross, 34% female F2 (n = 162). Our results provide the strongest evidence of a 0W/00 sex determination to date, and suggest an effect of genetic background leading to differential transmission of the B chromosome.

**Transgenic male-only system development in the sheep blowfly, *Lucilia cuprina***

Megan E. Williamson
Advisor: Max J. Scott

The Australian sheep blowfly, *Lucilia cuprina*, is a major agricultural pest in Australia and New Zealand. Genetic approaches, such as the sterile insect technique (SIT), have been considered for use for control. For over 60 years, SIT has been used successfully to control the New World screwworm, a blowfly that is a close relative of *L. cuprina*. However, more efficient genetic systems would facilitate control of NWS in areas where it remains endemic. In SIT programs, females cost money to rear and can hinder the effectiveness of the program by competing with wildtype females to mate with the sterile males. For this reason, transgenic sexing systems have been developed that produce only males by killing the females early in development when fed a diet lacking tetracycline. Our lab has developed a novel conditional transformation system in *L. cuprina* that utilizes RNA interference and an essential gene in the sex determination pathway to transform XX females into XX males. To date, lines have been made using various promoters acting at different points in development, with current lines range from giving no transformation to full transformation of female progeny. This work shows promise to produce more males from a single female, allowing for more efficient mass rearing and therefore, more efficient control programs. As with any control program, many factors must be considered to minimize risk introduced by these systems.

**Influence of the Circadian Clock on the Arabidopsis Gravitropic Response**

Joseph Tolsma
Advisors: Colleen Doherty and Imara Perera

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. Because the circadian clock plays a role in many plant transcriptional responses, we aimed to characterize its role in the plant gravitropic response. An exploratory evaluation of RNA-Seq data from *Arabidopsis* space flight experiments showed a disruption of clock-related genes in microgravity. To characterize the effect of the circadian clock on the gravitropic response, we performed a root-bending assay over a 24-hour time course. We identified consistent differences in the response angle dependent on the time of day and selected the two time points with the greatest difference in response for further study. We also identified circadian clock mutants that exhibited different gravitropic responses compared to wild type (WT) plants. Using the random positioning machine (RPM at Kennedy Space Center), we compared the response of WT plants and plants with constitutive, high-level expression of CCA1 (a core component of the circadian clock). We grew seedlings under staggered lighting conditions so that when the plants were moved to the RPM, it would be at dawn for one set and 10h after dawn for the other set. The relative light conditions were maintained on the RPM for a 48h run in simulated microgravity. This allowed for a direct comparison of the two photoperiods found to have different response angles in the root bending assay. The root growth in response to simulated microgravity was compared between photoperiod and genotype.

**Population structure and evolution of immune genes in two ancient ray-finned fishes**

Kara Carlson
Advisor: Jeffrey A. Yoder

Immunogenetic diversity between individuals, populations, and species affects disease outcomes and characterizing this diversity can provide vital predictors for managing species facing emerging diseases. Novel immune-type receptors (NITRs) are a muti-gene family of innate immune receptors thought to be functionally orthologous to mammalian Natural Killer Receptors (NKRs), which differentiate self, or healthy cells, from non-self, such as infected cells and pathogens. Until recently, NITRs were thought to exist only in teleost species; a clade of fish which includes more than 30,000 species. We have identified NITRs in two holostean species, longnose gar (Lepisosteus osseus), and bowfin (Amia calva), close ancestors to teleosts. Using transcriptomic data, we identified and compared predicted NITR protein domains across 16 individuals from two river basins in North Carolina and Louisiana. Bowfin NITR genes displayed some degree of inter-individual variation including polymorphisms and gene-content differences, which may be a result of a history of geographically specific pathogen interactions. Future work will utilize a similar analysis of NITRs in longnose gar across similar sampling locations to determine whether NITRs display any species-specific or population specific signatures. Additionally, we will determine whether NITRs show evidence of coevolution with other immune genes, such as those encoding MHC class I molecules. Determining genetic diversity of immune genes and patterns of coevolution across species and geographic boundaries will broaden the understanding of how immunogenetic diversity evolves on both a fine and broad scale.

**Genomic Regulation of Limited Lifespan and Postponed Reproductive Senescence in Drosophila melanogaster**

Grace Parker
Advisor: Trudy Mackay

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. 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. We then identified alternative SNPs between the O and B lines that alter promoter function using an in vitro luciferase reporter assay. Additionally, we assessed phenotypic differences in O and B lines with a small CRISPR/Cas9-mediated deletion containing one of these candidate SNPs. 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.

**From Genomes to Fields: Exploring genotype-by-environment interactions and environment specific prediction in maize hybrids**

Anna R. Rogers
Advisor: James B. Holland

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 environment specific performance of individual genotypes.

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.

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