

13th Annual

# Ecological Genomics

# Symposium

## Poster Abstracts

**November 6-8, 2015 · Hilton Garden Inn · Manhattan, Kansas**

### Posters 1-16 are from undergraduate students

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#### **Histone Reduction During the Evolution of Multicellularity**

Jaden Anderson\*, Erik Hanschen and Bradley JSC Olson  
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Multicellular organisms have evolved multiple times throughout the history of life on Earth. The most recent transition to multicellularity occurred about 200 MYA within the volvocine algae, with phenotypes ranging from unicellular, undifferentiated multicellular and differentiated multicellular all with sequenced genomes. Interestingly, while the genomes of these organisms are very similar showing only a small increase in the number of genes as morphological complexity increases, the number of histone genes is reduced as morphological complexity increases. Compared to *Chlamydomonas reinhardtii*, unicellular, and *Gonium pectorale*, colonial multicellular, which have 125 and 133 histones respectively, *Volvox carteri*, differentiated multicellular, only has 54 histone genes. This reduction in histones is unexpected since increasing in complexity and increasing cellular differentiation should require more histones to regulate the differential expression between tissues. One possible explanation is that this results from a complexity drain where functional demands to a higher levels of biological organization such as the transition to multicellularity, may reduce or render many parts of the lower level of less use and thus loss of those functions is favored by selection (McShea 2002). To investigate the evolutionary history of these genes, phylogenetic, synteny and expression analyses were conducted to dissect the evolutionary history of histones in the volvocales. We found saturating synonymous mutations for H2A, H2B, and H4 histones which strongly suggests that they are undergoing a birth/death process.

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#### **Investigating the Evolution of Germ and Somatic cells in *Pleodorina starrii* using Comparative Genomics**

Julie Cooper\*, Yi Li and Bradley JSC Olson

Kansas State University (JC), Kansas State University (YL), Kansas State University (BO)

The evolution of multicellular organisms is a major evolutionary transition. To understand the molecular mechanisms responsible for this transition, particularly the evolution of the division of labor between germ and somatic cells, we are using the volvocine algae as a model system which include species of a stepwise progression of phenotypes: unicellular, undifferentiated multicellular, and differentiated multicellular. The latter species have independently evolved this germ-soma division of labor such as those in the genera *Pleodorina* and *Volvox*. To understand how this germ-soma differentiation evolved we are sequencing and then comparing the genomes and cell cycle gene expression in these organisms. We hypothesize that the genes promoting multicellularity and tissue differentiation in *Pleodorina* have been co-opted in *Volvox* to promote full germ-soma differentiation. Because little is known about the life cycle of *Pleodorina*, we are first

characterizing its life in detail to understand when developmentally important stages occur, specifically when germ lines are established. Once we characterize these stages, we will synchronize the life cycles of these organisms and perform quantitative RNA sequencing on developmentally important time points.

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### **A forward genetic screen for multicellular genes in *Gonium pectorale***

Katherine Johnson\*, Tina Ding, Dr. Bradley Olson

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The evolution of multicellular organisms on Earth is a major evolutionary state transition. Despite being fundamental for much of the life on this planet, very little is known about what genes are required for multicellular organisms to evolve. The Volvocine algae are an excellent model system for understanding the evolution of multicellularity because the member species represent a recent example of a group of organisms evolving multicellularity. More importantly, the genomes of three species within the group have been sequenced, and each of these three species represents a key transition towards multicellularity: unicellular *Chlamydomonas reinhardtii*, colonial multicellular *Gonium pectorale* and differentiated multicellular *Volvox carteri*. The genomes of all three organisms are highly similar suggesting that the genetic basis for evolving multicellularity requires one or few genes. The focus of this research is to find genes in *Gonium pectorale* that are important for the initial transition from unicellularity to colonial multicellularity by performing a forward genetic screen for unicellular *Gonium* mutants. Three unicellular *Gonium* mutants have been isolated, back crossed to the parental strain, and two of the mutant's genomes have been re-sequenced. We hypothesize that the genes identified by re-sequencing the unicellular *Gonium* mutants will be important for cell cycle regulation or cell cycle regulated cell-cell adhesion.

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### **Reproductive trait variation in big bluestem (*Andropogon gerardii*) ecotypes across the Great Plains: a multi-year reciprocal garden study**

Olivia Parrish\*, Alexandria McChesney\*, Matthew Galliard, Sara Baer, Nora Bello, Keri Caudle, Brian Maricle, Evan McCrea, Sofia Sabates, David Gibson, Laurel Wilson, Loretta Johnson

Kansas State University (OP), Kansas State University (AM), Kansas State University (MG), Southern Illinois University (SB), Kansas State University (NB), Fort Hays State University (KC), Fort Hays State University (BM), Southern Illinois University (EM), Kansas State University (SS), Southern Illinois University (DG), Southern Illinois University (LW), Kansas State University (LJ)

Midwest grasslands are dominated by the C<sub>4</sub> grass big bluestem, *Andropogon gerardii*, which persists across a strong precipitation gradient (500 to 1200 mm/yr rainfall, western KS to IL). Knowledge of grasslands' responses to drought is critical for conservation and rangeland productivity. We used a reciprocal garden to investigate ecotype-specific seed production and phenology across a precipitation gradient. Three ecotypes (central KS, eastern KS, southern IL) were reciprocally planted in Colby, Hays, and Manhattan, KS, and Carbondale, IL. From 2012-2014, we recorded time to flowering and collected seed. We aim to determine annual variation in seed production and time to flowering in response to ecotype and precipitation. We predict ecotypes will have greatest seed production in their home environment. Presumably, drought-adapted CKS ecotype would show advanced flowering as an adaptation to drought. In 2012, the CKS ecotype flowered on average 20 days earlier than EKS and SIL ecotypes, independent of planting site. Moreover, when planted in Colby, the CKS ecotype was the only flowering ecotype. A less pronounced pattern of early flowering in CKS ecotype was evident in Kansas sites in 2013 and 2014 with no apparent difference in flowering time in Illinois site. Early flowering of the CKS ecotype in dry sites and years may be an adaptation to drought. CKS and SIL ecotypes both showed a home site advantage, having greater seed production in Hays and Carbondale. Results provide insight into adaptability of *A. gerardii* fitness in drier climates and will also help to identify best-suited ecotypes for restoration.

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### **Use of propidium iodide dye and GFP-labeled bacteria to assay diffuse accumulation of *S. maltophilia* JCMS in *C. elegans***

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Like all animals, the bacterivorous nematode *Caenorhabditis elegans* evolved in the presence of microbes, some beneficial and some harmful. Natural populations of *C. elegans* are found in rotting vegetation amongst a variety of microbes and interestingly occupy multiple trophic levels in this community of decomposers. On the one hand, they prey upon the microbes around them, yet at the same time, they can also carry a risk of infection and thus be consumed by their food sources. JCMS is a strain of the bacteria *Stenotrophomonas maltophilia* that was isolated locally by members of the Herman lab. We have found that JCMS is pathogenic to *C. elegans*. *S. maltophilia* JCMS is more pathogenic than other *S. maltophilia* isolates and the typical laboratory food *E. coli* OP50. In addition, we have found that *S. maltophilia* JCMS is able to evade a major *C. elegans* defense pathway.

We introduced a plasmid that expresses the Green Fluorescent Protein (GFP) into bacteria to allow us to track the accumulation of bacteria in the *C. elegans* intestine. When *C. elegans* is fed GFP versions of *S. maltophilia* JCMS and *E. coli* OP50, noticeable differences are observed in the resulting intestinal accumulation patterns: *E. coli* accumulates in a punctate pattern, while *S. maltophilia* accumulates in large masses of diffuse GFP accumulation. It is not clear whether these large masses are made up of living or dead bacterial cells. In order to better understand the nature of the diffuse GFP accumulation, we are using propidium iodide dye to differentially stain dead bacterial cells. The end goal of this research is to better characterize this nematode-bacterial interaction and explain how *S. maltophilia* is able to avoid the innate immune defenses of *C. elegans*.

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### **Investigation of Virulence Functions of Type III Effectors in the Cassava Pathogen *Xanthomonas axonopodis* pv. *manihotis* (Bondar) Using Bioluminescence Imaging**

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Cassava, *Manihot esculenta* Crantz, is a dietary staple in many tropical countries, accounting for a third of calories consumed in the world. Cassava is grown agriculturally in large populations making it susceptible to spread of several diseases. The primary bacterial pathogen is, *Xanthomonas axonopodis* pv. *manihotis* Bondar (*Xam*) that causes the disease Cassava Bacteria Blight (CBB). *Xam*, like many other bacteria, interacts with the host cell using the Type 3 Secretion System. This system has a needle-like protein complex structure that is inserted and secretes effector proteins in the host cell. Preliminary bacterial growth assays in the host were done with knockout mutant of these different effector proteins and showed subtle changes in phenotypes. The result of these assays showed slight differences between wild type and mutant strains. To explore the virulence through other phenotypes we utilized imaging techniques that allow us to observe spatial and temporal dimensions of the infection. To look at the *Xam* motility within the vascular system, bioluminescence imaging was used. There is a notable difference in how fast the disease spreads and even which strain spreads first. Future studies will consider (1) Proximity to major vein when being inoculated. There might be a smaller distance the strain has to travel before it integrates itself into the host. (2) The developmental stage of the inoculated leaf. Some leaves may be actively taking up more nutrients and have a strong pull in the vascular system. (3) Lastly, the environmental factors, such as temperature, and humidity.

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### **An Approach to Analyzing Changes in Gene Expression of Non-model Plant Species Grown Under Elevated CO<sub>2</sub> and Soil N Levels**

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The use of genetic techniques is a contemporary approach used to answer ecological questions. A long-term, large-scale field study in Minnesota, USA, called BioCON, manipulates CO<sub>2</sub> and soil nitrogen availability. The physiological responses of several non-model plant species grown in BioCON have been previously observed; however, the gene expression responses of these species have not been studied and may reveal mechanisms that drive these physiological responses. Leaves were sampled from three native species grown in factorial combinations of atmospheric CO<sub>2</sub> (ambient, ambient

+200ppm) and soil nitrogen (ambient (low), ambient +4gm<sup>-2</sup>y<sup>-1</sup>). RNA from these leaves is currently being extracted and analyzed and will ultimately be used to assess gene expression patterns. We are specifically interested in the interactive effects of elevated CO<sub>2</sub> and elevated nitrogen levels on the expression of genes involved in nitrogen metabolism and photorespiration, as photorespiration is directly affected by CO<sub>2</sub> and is linked with nitrogen assimilation and metabolism. These efforts will provide a better understanding of the natural vegetation responses to changing resource availability associated with future global change.

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### **Yellow dwarf virus vectors and their role in the movement of virus in Northeastern Kansas**

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Yellow dwarf virus (YDV) is a major plant disease in North America caused by viruses in the family *Luteoviridae*. The virus is named for the symptoms displayed by infected plants: yellowing (chlorosis), reddening, and stunting. The goals of this project are to determine the incidence of YDV and its aphid vectors in agricultural and native grass systems in Kansas, as well as YDV transmission occurring between the two systems. Aphid populations were monitored throughout the year using yellow sticky cards. Many of the major aphid vector species have been found in Kansas during this project: *Schizaphis graminum* (greenbug), *Sitobion avenae* (English grain aphid), *Rhopalosiphum padi* (bird cherry oat aphid) and *Rhopalosiphum rufiabdominalis* (rice root aphid). The most common vector in wheat was found to be *R. padi*, with *R. rufiabdominalis* and *S. avenae* as the second and third most common aphids, respectively. The aphid species that were found to overlap between wheat and big bluestem were *R. padi* and *S. avenae*, with these being the first and second most common aphids in big bluestem, respectively. Results from summer collection on wheat and sorghum showed *R. padi* to have the largest overlap of the vector species and introduced a new vector that had not been previously significant on wheat or big bluestem, *Rhopalosiphum maidis*, the corn leaf aphid. The highest aphid populations, and therefore possibly the most important fall, spring, and summer hosts for the vectors, were wheat and sorghum. Wheat, native grass, and aphid samples were collected from fields across Northeastern Kansas and tested via a multiplex reverse transcriptase PCR (RT-PCR) for the presence of YDV species. Plant and aphid samples tested positive for many of the YDV viruses, with the virus species occurring in plants and aphids individually or as co-infections of the same insect vector or plant host. Samples from sorghum, corn, and big bluestem fields will be tested similarly in order to identify overlap between virus species, especially between the agricultural and native grass plots. The next steps of the project will repeat the experiments of the last year as we continue to monitor aphid patterns and virus infection throughout the wheat cycle, from planting to harvest.

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### **Soil Fungal Communities Respond Rapidly to Low and High Intensity Fire**

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Rapid climate change has created potential for high-severity forest fires that exceed their historical distribution in the western United States. For instance, forests in California that are currently experiencing widespread intense fires. These events have dramatic effects on the soil microbial communities and their composition. In this study, we describe the rapid responses of soil fungal communities to experimental high and low intensity fires. We utilized research infrastructure in Pringle Falls Experimental Forest in Oregon, where ten pairs of equidistant sites were assigned to either high burn (HB) or low burn (LB) intensity treatments. In these treatments, plots were broadcast burned (LB) or had stacked log complete combustion (HB), generating soil temperatures of approximately 100°C (LB) or up to 700°C (HB) at the soil surface. We sampled soils (0-10cm) at these sites before and three weeks after the burn treatments and Illumina MiSeq-analyzed fungal Internal Transcribed Spacer 1 (ITS1) PCR-amplicons to deeply dissect the fungal communities. Our data indicate dramatic and rapid responses in community diversity and evenness in the HB treatment; and similar diversity responses, but to a lesser degree, in the LB treatment. Nonmetric Multidimensional Scaling (NMS) ordinations and analyses of taxon frequencies reveal a substantial community turnover and corresponding near complete replacement of the dominant basidiomycetes by ascomycetes in the HB treatment. Similar trends were visible, but much weaker in the LB treatments. These coarse-level taxonomic responses were primarily attributable to few fire-responsive (phoenicoid) fungi, particularly *Pyronema* sp. and *Morchella* sp., whose frequencies increased more than 100-fold following the fire in the HB treatment. Our study highlights the strong and rapid fungal community responses to fires, differences among fires of different

intensities, and leads to questions on the function of post-fire communities and the ecosystem services that they may provide.

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### **Genetic diversity of the rare plant species *Phlox pungens* (Polemoniaceae).**

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The genus *Phlox* is an ecologically diverse group of herbs and subshrubs with a center of diversity in western North America. A current focal study investigates population genetics of *P. pungens* Dorn, a rare species occurring in cold deserts of the Wyoming Basins Ecoregion in west central Wyoming. Samples of *P. pungens* were collected across the entire species range (including parts of the Green River and Wind River Basins), with care taken to include diversity relative to morphology (which exhibits geographic patterns). Microsatellite data have been generated for these populations (10 populations total), as well as for populations of additional *Phlox* species from the region (6 additional species, 7 populations total). We present these data and discuss their implications to our understanding of 1) genetic variation within *P. pungens*, including among the eastern and western sets of populations, and 2) genetics of *P. pungens* within the broader context of genetic diversity of *Phlox*. Results of this research may hold implications for the long-term conservation of the rare *P. pungens*, and they also contribute to broader work investigating taxonomic diversity of the genus *Phlox*.

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### **Population differences in seasonality via diapause regulation among the apple maggot fly *Rhagoletis pomonella***

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Physiological mechanisms controlling diapause have been well studied, but little is currently known about how variation in these mechanisms give rise to diapause adaptation. Diapause adaptation is important as insects expand into new geographic regions or experience changes in seasonality, the latter of which has been occurring as global climates change at an increased rate. *R. pomonella* has rapidly evolved into two genetically distinct populations that infest host plants with different fruiting times. Differences in the timing of diapause allow each population to synchronize with their respective host plant. Previous studies suggest that regulatory mechanisms underlying this shift in seasonality may act during the winter independent of any overt cues for diapause termination, such as the typical change from colder to warmer temperatures during the winter-spring transition. To test for potential physiological differences, we sampled diapausing pupae in a narrow time window encompassing three time points: 1) directly out of an overwintering cold treatment, 2) 24 and 3) 48 hours after transfer into a warm treatment. The results from performing RNAseq on the samples show that, although temperature has strong effects on gene expression (consistent with the idea that temperature is a diapause termination cue), there were also pronounced differences in expression between the two populations that persisted across all time points, including prior to warm temperature exposure. It can be concluded from our results that a timing mechanism independent of temperature cue likely causes population differences in seasonality via diapause timing. Further, we discuss candidate genes and pathways underlying population differences in diapause regulation.

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### **Search for Local Relatives of Genetic Model Organism *Caenorhabditis elegans***

Jasmine Sharp\* and Michael Herman  
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The free-living bacterivorous nematode, *Caenorhabditis elegans* is a major genetic model organism used to study all aspects of biology, in particular questions relating to developmental biology, gene regulation and behavior that are of medical relevance. While these studies have provided many biological insights, there is still limited knowledge about *C. elegans* ecology and natural history. For example, it was long thought the *C. elegans* was a soil nematode. However, it was recently discovered that *C. elegans* inhabits rotting vegetation substrates such as fruits, stems, and flowers where it feeds off of the microflora found in decomposing material. Furthermore, we have only begun to learn about their geographic distribution. To date, sampling efforts have been limited, with *C. elegans* being collected from primarily orchards in France and rotting vegetation in the tropics. With this in mind, we set out to identify *C. elegans* relatives from

local samples in order to further our knowledge of the biogeography and diversity of the genus as well as to establish local populations to facilitate research of their environmental interactions, specifically with bacteria.

In a preliminary sampling effort we focused on Konza Prairie Biological station, but include other sites in the Manhattan, Kansas area such as Cico Park, the Kansas State University campus as well as some other areas around the city. Collectively we obtained 111 isolates from 40 substrates. Nine isolates came from the KSU campus, four came from areas around Manhattan and the remainder were collected from Konza Prairie. All the isolates were selfing species, which facilitated the establishment of 40 unique lines from 26 substrates that were suitable for morphological and molecular identification. We used morphological criteria including body shape, size, mouth morphology, pharynx morphology, tail shape and location of the vulva to begin to distinguish the various isolates. Most of the isolates had characteristics common to the family *Rhabditidae* and eight appeared to have a pharyngeal morphology similar to other members of the *Caenorhabditis* genus. We are using molecular identification by amplification of the 18s ribosomal RNA (rRNA) gene to obtain more precise classifications. Thus far, products have been amplified from 26 isolates, three full-length and three partial DNA sequences have been analyzed and determined to be most similar to *C. briggsae*. Molecular identification and evolutionary relationships of our other isolates have yet to be determined. Our work will contribute towards understanding the biogeography of *C. elegans* and in due course, can aid in interpreting what drives *C. elegans* distribution and diversity within and between species.

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### **Evolution of multicellularity by co-opting cell-cell adhesion genes**

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Multicellular evolution is a major transition that has occurred for more than twenty-five times in many eukaryotic taxa. However, the genetic basis of the transition to multicellularity is unknown in any of these taxa due to high divergence between unicellular and multicellular relatives. The Volvocine algae, however, are a group that have recently transitioned to multicellularity about 200 million years ago that have species ranging from unicellular (e.g. *Chlamydomonas reinhardtii*), to colonial groups of like cells (e.g. *Gonium pectorale*), to those with differentiated tissues (e.g. *Volvox carteri*). Since these organisms recently evolved in multicellularity and have not diverged significantly, their genomes are surprisingly similar. In an effort to identify genes that may be important for multicellularity, we hypothesize that cell wall genes in unicellular *Chlamydomonas* were co-opted to promote cell-cell adhesion in colonial organisms such as *Gonium*. To identify these genes, we created comprehensive phylogenies of cell wall genes in *Chlamydomonas*, *Gonium*, and *Volvox* to identify genes whose evolutionary signature suggested co-option. From this analysis, I identified five candidate genes that may be important for cell-cell adhesion. After functionally testing two candidate *Gonium* genes and transforming them in *Chlamydomonas*, the phenotypic ratio expressed almost one hundred percent in unicellularity. Our results mean that these two candidate genes are probably not important for multicellularity. I am currently functionally testing other candidate genes for importance in cell-cell adhesion using transformation.

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### **Unlocking the Secrets of Plant Evolution: A Role for Homeodomain Transcription Factors**

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Over 450 million years ago land plants evolved from algae. This transition from aquatic to terrestrial environments was aided by two groups of transcription factors, namely, class III and class IV homeodomain leucine zipper (HD-Zip) transcription factors that are highly conserved in all land plants. HD transcription factors are master regulators of cell-type differentiation in both plants and animals. HD-Zip transcription factors from plants additionally regulate the biosynthesis of secondary metabolites including plant-specific phenolic compounds such as flavonoids. Class III HD-Zip transcription factors are associated with formation of the shoot apical meristem, initiation of lateral budding, and evolution of leaves and other lateral organs. Class IV HD-Zip transcription factors are essential for epidermal development and confer the properties of homoiohydry and poikilohydry to plants, which pertains to the ability to regulate internal cell water content, and to tolerate dehydration to low cell or tissue water content, respectively. Taken together, these HD-Zip transcription factors are thought to be highly critical for the evolution and survival of land plants. The primary objective of this project is to study the occurrence of these HD-Zip transcription factors and flavonoid biosynthesis enzymes in the genomes of five major Charophycean green algae (CGA) taxa, which comprise the closest ancestral lineages to the land plants. From the bioinformatic analysis of the transcriptome data sets, we identified five new class III and seven new class IV HD-Zip genes in the representative species of the CGA, namely, *Coleochaetales orbicularis*, *Nitella mirabilis*, *Penium margaritaceum*, *Spirogyra pratensis*, *Klebsormidium flaccidum*, and *Mesostigma viride*. We found evidence for one class III and one class

IV HD-Zip gene in each of the algal species, except for *Mesostigma viride*, which appears to contain two class IV HD-Zip-related genes but no recognizable class III HD-Zip gene. This finding is consistent with the postulate that class IV genes predated class III genes. Our current work is focused on exploring the roles of these transcription factors in the CGA. By cloning and expressing cDNA sequences in *Arabidopsis*, we are probing the activity and subcellular localization of these transcription factors. We will also be using RNAi knockouts in *Penium margaritaceum* to investigate their functions. By investigating the role of these genes along with conservation of flavonoids and their biosynthetic pathways in algae, we will be able to gain valuable insight into the genetic mechanisms that led to the evolution of land plants, which thereby paved the way for human existence.

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### **Genome Size Variation in the Wild Sunflower Genus *Helianthus***

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Genome size varies greatly across flowering plants (angiosperms), ranging approximately 2,400-fold. Considerable variation in genome size also can be found among species that are evolutionarily closely related. Elucidating the mechanisms that have given rise to genome size variation is important for understanding the organization and evolution of plant genomes. The objective of the current work is to generate a database of genome size estimates for all species of the wild sunflower genus *Helianthus*. This genus consists of 49 species, native to North America, that differ in ploidy level, geographic range, and life history. Wild sunflowers have become a model system for ecological and evolutionary genomics. The current work will contribute to available resources and provide a framework for future studies investigating mechanisms of genome size evolution in this group of plants.

Estimates of genome size are being determined by flow cytometry. This method requires isolation of plant nuclei via chemical and manual manipulation followed by sample analysis in a flow cytometer. This method determines nuclear genome size estimates based upon the comparison of a known internal standard that is co-processed with the sample. Three biological replicates of three different accessions from sunflower species will be evaluated.

To date, genome size estimates have been determined for 37 species: 29 diploids, 3 tetraploids, and 5 hexaploids. Genome size among diploid species range from  $2C=6.3-23.7\text{pg}$ , variation among tetraploid species range from  $2C=21.8-25.9\text{pg}$ , and hexaploid species range from  $2C=20.8-25.9\text{pg}$ . Genome size estimates among individuals of the same accession vary by less than 10%. Considerable variation in genome size exists among species of the same ploidy level. For example among diploids, genome size ranges 3.5 fold. Ongoing research is examining the mechanisms responsible for the evolution of genome size variation in sunflowers.

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### **Identification and Cloning of Genes Involved in the Regulation of Energy Metabolism of Burying Beetles**

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Reproduction is always associated with energetic costs because of the energy necessary to produce and care for offspring. Animals bear these costs by eating more, increasing energy efficiency, and/or building up energy reserves. As most insects simply lay eggs and leave their eggs, research on molecular and physiological mechanisms underlying energy metabolism during reproduction have focused on insects such as fruit flies and mosquitoes that are lacking parental care. Although rare, parental care occurs in most insect taxa. Burying beetles (*Nicrophorus spp.*), for example, provide extensive parental care to their offspring. Burying beetle reproduction begins with an adult beetle using chemosensors to locate small vertebrate carrion, which will serve as the primary food resource for their larvae. After locating the carrion, a physiological response is seen in the female beetle within a few hours, as the size of its ovaries increases two to three times their original size. To prepare the carrion, either the female, or the male if present, will bury the carrion and remove its exterior coat. After burying the carrion, the female will then lay her eggs in the soil next to it. Both, male and female parent will guard the brood from predators and feed the larvae by regurgitating carrion into their mouths. After three days, the larvae no longer need to be fed and the male leaves before larvae development completes, the female, however, remains with the brood until larval development is completed.

For burying beetles, parental care has large fitness costs. Beetles that provide parental care have substantially shortened life spans compared to beetles that only mate or produce. To understand how parental care causes these fitness costs requires knowledge of the molecular and physiological mechanisms underlying parental care. Energy metabolism is a strong candidate as a mechanism underlying the generation of fitness costs of parental care. This is suggested by the 10% increase in mass of burying beetles during carrion preparation and egg laying. When the larvae hatch, the parent

beetle's mass returns to normal levels. Further, the resting metabolic rate is about two times higher in beetles providing parental care than in beetles that do not.

The goals of this project are 1) to identify and clone genes that are involved in regulation of energy metabolism in reproducing burying beetles using a candidate gene approach and 2) to verify whether the cloned genes are expressed in tissues relevant for reproduction and energy metabolism (ovaries, fat body, and brain) in burying beetles.

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### **Phenotypic distribution models incorporate ecotypic variation of the dominant prairie grass *Andropogon gerardii* to predict response to climate change in Midwest grasslands**

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*Andropogon gerardii* is an ecologically dominant grass in the Midwest. With wide distribution across a precipitation gradient (40 -119cm/yr), we expect ecotypic variation in drought tolerance and local adaptation. Understanding ecotypic variation will help predict how a dominant prairie grass may respond to current and predicted future climate change and may inform sourcing of plant materials for restoration. Current practice uses species distribution modeling to predict an organism's response to climate change but fails to incorporate ecotypic variation within a species. Based on results from reciprocal gardens, we have shown evidence of local adaptation in big bluestem ecotypes as well as genetically-based adaptive divergence. Our study characterizes phenotypes of 37 geographically distributed populations across the Midwest to incorporate intraspecific variation into maximum entropy modeling software, (MAXENT) of current and predicted distribution under climate change. For each of the 37 geographically distributed populations, we grew plants from seed under greenhouse conditions and measured phenotypes; blade width, height, biomass, and chlorophyll absorbance. PCA analyses shows a phenotypic cline across populations that can be partially explained by longitude, mean annual precipitation or vegetation type at the source collection site. We fed PCA scores into a phenotypic distribution model to predict current and future phenotypes across the Midwest. Using climate projected for 2070, phenotypes from dry areas (short stature, low biomass, narrow leaves) were predicted to expand through the Midwest, eclipsing phenotypes from wet areas (robust, wide leaves), provided adequate migration. This novel phenotypic distribution model greatly refines current species distribution models that assume no ecotypic variation and may more accurately predict species' response to climate change.

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### **A genome wide scan for incompatibilities in hybrid fish populations**

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A fundamental question in Evolutionary Biology is the question of how species form and remain distinct. According to the Biological Species Concept, species are groups of interbreeding natural populations that are reproductively isolated from other such groups and interbreeding between species is prevented by a variety of isolating mechanisms. Defining speciation in terms of the evolution of reproductive isolation allowed Dobzhansky and Muller (and before them Bateman) to propose the so called "BDM incompatibility" model to explain what has been called "Darwin's Dilemma" -- that is, how reproductive isolation could evolve without traversing a fitness valley. The answer according to this model is epistasis -- notably, derived substitutions can accumulate along two lineages, by whatever mechanism, and it is only when hybrids are formed that derived (or ancestral) substitutions will be exposed to each other for the first time, potentially causing a reduction in the fitness of hybrids. Negative epistatic interactions between genes from different parental genomes decrease the fitness of hybrids and can limit gene flow between species. However, little is known about the number and genome-wide distribution of genetic incompatibilities separating closely-related species. To detect interacting genes, we perform a high-resolution genome scan for linkage disequilibrium between unlinked genomic regions in naturally occurring hybrid populations of swordtail fish. We estimate that hundreds of pairs of genomic regions contribute to reproductive isolation between these species, despite them being recently diverged. Many of these incompatibilities are likely the result of natural or sexual selection on hybrids, since intrinsic isolation is known to be weak. Moreover, patterns of genomic

divergence at these regions imply that genetic incompatibilities play a significant role in limiting gene flow even in young species.

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### **Footprints of natural selection on proteins of the respiration pathway vary among organisms with differences in aerobic performance.**

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Oxidative phosphorylation (oxphos) is the primary source of ATP in eukaryotes and serves as a mechanistic link between variation in genotypes and energetic phenotypes. Increased aerobic capacity may be facilitated by numerous factors, including increased expression of oxphos genes, increase in number and morphology of mitochondria, enhanced O<sub>2</sub> transport within the body, and structural variation in oxphos proteins. Directional (positive) natural selection on particular amino acid sites may reveal important functional variation in oxphos proteins. Although evidence of natural selection on mitochondrial oxphos genes has been documented in several animal groups, the relationship between selection and oxphos function has not been well characterized. Because organismal performance should be a function of oxphos functional efficiency, we investigated this relationship by examining patterns of natural selection on oxphos genes in several groups of mammals and fishes with extreme differences in aerobic performance.

We acquired oxphos and Krebs cycle gene sequences from de novo transcriptome assemblies or from databases. Taxa represented a range of energetic habits from largely sedentary to moderately active to high-speed, extended-duration locomotion. Included were 74 oxphos genes (13 mitochondrial and 61 nuclear), and 11 Krebs cycle genes. Estimates of synonymous (dS) to non-synonymous (dN) substitution rate ratios under both random-sites and branch-site models were used to assess evidence of natural selection. Specific codon sites under positive selection were identified using the Bayes empirical Bayes procedure.

We found that patterns of natural selection on respiratory pathway genes are complex among fishes and mammals with different locomotive performance or other energetic demands. Positive selection was not consistently associated with high performance taxa and in several cases selection was strong on low-performance taxa. Among pelagic marine fishes the strength of selection was inversely proportional to swimming performance. Within some high-performance lineages, negative selection due to functional constraint appears to predominate. These results provide empirical evidence for fluctuating selection on aerobic respiration pathways associated with divergence in aerobic performance. The broader pattern indicates a complex interplay between organismal adaptations, ATP demand and respiratory function.

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### **Distinct pathways to adaptation to extremely sulfidic environments in closely related populations of fish**

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Similar environmental conditions often lead to molecular convergence in different populations, but the factors that affect the likelihood of molecular convergence are understudied. In theory, short divergence times and strong selective pressures should each increase the chances of parallel adaptation. It follows that recently diverged populations facing the same strong selective pressure would be likely to follow the same evolutionary path to adaptation. Populations of freshwater fish (*Poecilia*) have independently colonized multiple hydrogen sulfide-rich streams. Hydrogen sulfide (H<sub>2</sub>S) is extremely toxic to most organisms; H<sub>2</sub>S inhibits cellular respiration by interfering with the respiratory complex cytochrome c oxidase (COX). We hypothesized that H<sub>2</sub>S-adapted populations of *Poecilia* adapted via a similar pathway in the face of this extreme selective pressure. We performed RNA-sequencing on individuals from three population pairs (adjacent freshwater and sulfidic populations, N=6 for each population) to test whether the same genes were under selection across three independent transitions into H<sub>2</sub>S-rich springs. We identified fixed differences between adjacent freshwater and sulfidic populations and compared those fixed differences across transitions to identify evidence of molecular convergence. There are no shared fixed differences among all three transitions or between the two most recent transitions, however there are shared fixed differences between each of the two recent transitions and the first transition. One of the recent transitions shares fixed differences in genes involved in cellular respiration and COX activity with the first transition, whereas the other recent transition shares fixed differences in genes involved in sulfide detoxification with the first transition. A scan for loci under selection also provided little evidence for convergence between the two recent transitions. These results suggest that independent sulfidic populations have taken two different pathways to adaptation: sulfide resistance and sulfide detoxification. The fish that have lived in sulfidic water the longest show evidence for both

improved detoxification and resistance. Overall, the results of this study imply that adaptation to extreme environmental conditions is unpredictable, and that parallel adaptation is unlikely even in closely related populations.

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### **Large-scale major histocompatibility complex (MHC) organization in Palaeognaths suggests retention of an ancestral avian MHC**

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Class I and class II genes of the major histocompatibility complex (MHC) are widely employed as markers of adaptive genetic variation for studies in ecoimmunology, conservation biology, and evolutionary ecology in birds. However, little is known about large-scale avian MHC genomic organization outside of Galliformes (gamebirds), a situation that impedes our understanding of the evolutionary history of this dynamic genomic region and how sequence context might influence multigene family evolution. High quality *de novo* whole-genome assemblies for seven ratite and three tinamou species were used to reconstruct the core MHC region in Palaeognathae, representing one of the basal lineages of birds and sister to all remaining birds (Neognathae: Galloanseriformes + Neoaves). We found numerous differences between palaeognath MHC structure and the 'minimal essential MHC' paradigm of galliforms, including: presence of MHC class II $\alpha$  loci within the core MHC and their arrangement in  $\alpha/\beta$  gene pairs; existence of an extended MHC class II region (xMHCII) adjacent to the core MHC; and the location of the tapasin gene within this xMHCII region. Similarities between MHC structure in palaeognaths and other amniotes suggest the retention of ancestral avian MHC gene organization in palaeognaths. Retention of this inferred ancestral MHC structure in neoavian species further suggests that the highly rearranged MHC of galliforms likely reflects a lineage-specific event that is not representative of other avian taxa.

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### **Evolution of the thermal niche: functional physiology and genetics across a genus of livebearing fishes (Poeciliidae: Xiphophorus)**

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Temperature is an omnipresent environmental variable influencing survival and reproduction of all living organisms. Understanding how and when species adapt to different thermal regimes can provide insight into the evolution and subsequent maintenance of reproductive isolation via natural selection. We investigated patterns of thermal niche evolution across a genus of livebearing fishes using a multifaceted approach. First, phylogenetically-controlled niche modeling was used to reconstruct the evolution of the thermal niche across the genus using occurrence data. These niche models were used to identify species comparisons for which there was evidence of divergence in thermal biology. Specifically, we focused on replicated species pairs that have diverged at opposite ends of elevational gradients. Using a common-garden approach we tested upper and lower thermal limits in three species pairs, finding evidence of heritable differences in thermal tolerance. Patterns of thermal tolerance were further examined by quantifying gene expression of candidate genes to investigate whether these functionally-important genes also show adaptive, heritable changes corresponding to their respective thermal environments. By integrating correlative analyses of niche modeling with functional assays on lab-reared individuals, we were able to gain insight into adaptive shifts in functional physiology and gene expression that have enabled colonization of novel environments (e.g., high, cool elevations) and that likely have contributed to the subsequent maintenance of reproductive isolation within these species pairs.

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### **Differential gene expression of carbonic anhydrases and local adaptation to pH in *Daphnia pulicaria***

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Understanding the mechanisms that drive acid-base regulation in organisms is important, especially for organisms in aquatic habitats that experience rapidly fluctuating pH conditions. Previous studies have shown that carbonic anhydrases (CAs), a family of zinc metallo-enzymes, are responsible for acid-base regulation in many organisms by catalyzing the

reversible reaction:  $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$ . Here, we characterize three CA isoforms in the keystone aquatic herbivore, *Daphnia pulicaria*. In addition we investigate under common garden conditions the differential expression of those CAs from *D. pulicaria* clones isolated from three North American lakes across a pH gradient (i.e., Frenchman – pH 6.5 Hill – pH 7.9; Madison – pH 8.6). The three *D. pulicaria* CAs were determined to have seven exons, six introns, and highly conserved motifs common to all Animalia CAs. Two of the three *D. pulicaria* CAs (CA1 and CA2) are cytosolic-type CAs, while the third (CA5) is a GPI-anchored CA. CA1 and CA2 have a key substitution at one of three active sites. In addition, we found that CA1 is differentially-expressed across a pH gradient and among lake isolates, showing a signal of local adaptation (i.e., impacting survivorship) in the expression of CA1 in Frenchman and Madison Lake. Further, a significant up-regulation of CA5 in the Frenchman Lake isolates, relative to Hill and Madison Lake isolates across all pH treatments, was observed. Further characterization of potential fitness effects of differential gene expression in this system will be forthcoming.

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### **Reproductive isolation and environmental adaptation shape the phylogeography of Mountain Pine Beetle (*Dendroctonus ponderosae*)**

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The Mountain Pine Beetle (MPB) is an eruptive pest of various pine species native to western North America, reaching from northern Mexico to Canada along the Rocky Mountain and Sierra Nevada ranges. Current MPB ranges are expanding as a result of climate change and infestations cause extensive damage to susceptible forests. Applying genome-wide RAD sequencing to over 700 individuals from 37 populations, we are examining phylogeographic structure, gene flow, and adaptation across the range. Strong population structuring occurs between geographic regions with extensive admixing occurring in the sky island populations residing in the Great Basin. However results also suggest that there are distinct genetic boundaries between some geographically proximate regions. Experimental crossing experiments have previously found postzygotic isolation between geographically distant populations. Moreover crosses between the geographically close populations of Oregon and Idaho also showed a reduction in hybrid male fitness despite their proximity. Population structuring of the autosomes and sex chromosomes show that these populations are permeable to gene flow at autosomal sites but Y chromosome sites show clear disjunction between regions. Population structure suggests that separate genetic lineages occur across similar latitudes in the Rockies and Sierra Nevada. Leveraging a complementary QTL study of development time, a crucial trait in seasonal synchronization, we also discuss tests for parallel adaptation across lineages.

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### **Age-related change in cold stress tolerance in *Drosophila melanogaster***

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Organisms occur in environments that vary spatially and temporally throughout their lifespans, and the genetic architecture of ecologically important traits plays a critical role in the change in trait expression through the aging process. Resistance to cold stress is one important fitness trait that is expected to decline through ontogeny; however this general expectation is based on the response of a small number of *Drosophila* isogenic lines. To further characterize this change in cold stress resistance, we performed rapid acclimation experiments (i.e., rapid-cold hardening or RCH) on 100 isogenic lines of the *Drosophila melanogaster* in the *Drosophila* Genetic Reference Panel (DGRP) at early (one-week-old) and late (four-weeks-old) age. Consistent with previous investigations of RCH and the DGRP lines, we observed significant variation among lines at both early and late age points. In addition, cold stress resistance differs significantly ( $p \ll 0.05$ ) between early and late aged flies. However, the direction of the change in expression of cold stress resistance varies among lines, with several lines increasing in cold stress resistance as they age. The genetic architecture underlying the response to cold stress also changes with age, with several unique SNPs at each age. Analyses of genetic variance indicate that aging in these lines is most likely caused by mutation accumulation (the accumulation of deleterious alleles in

a population that are expressed late in life), and association mapping further supports this hypothesis. Further investigation of fitness consequences associated with cold tolerance suggests that the RCH response is positively correlated with courtship efficiency ( $p < 0.05$ ), while basal levels of cold tolerance are negatively correlated with courtship efficiency ( $p < 0.1$ ).

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### **Selection scans identify genomic regions associated with adaptation to environment in *Populus balsamifera* (L.)**

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*Populus balsamifera* has a large geographic range and local populations occupy distinct locations along strong environmental gradients of climate and photoperiod. Local adaptation to environment is widespread in forest trees, and here, we seek to understand the genomic basis of adaptation to environment across the range of this boreal forest tree. We analyzed 437 individuals collected from 63 populations, and obtained genome-wide SNP data from >150K loci using genotype-by-sequencing at 48-plex. Population structure was estimated using maximum likelihood clustering (ADMIXTURE) and discriminant analysis of principal components (DAPC). Tests for local adaptation manifest as  $F_{ST}$  outliers and SNP-environmental associations were estimated with Bayescan and LFMM. We identified population divergence between eastern North American, central, and western North American populations. In order to minimize the effect of neutral allelic variation on selection scans, we subset our data and focus on core populations in the central range, and search for parallel adaptation in two longitudinal samples. We find genomic regions suggesting novel, locally adapted loci to longer growing seasons and warm, dry environments which may be useful for integrating into poplar breeding programs under future climates.

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### **Evolution of Complex Life Cycles: Is Performance Constrained across Metamorphosis?**

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Organisms are not static over their lifetimes. Characteristic change as the genome unfolds across ontogeny. This change can be extreme, especially over the course of complex life cycles that include abrupt changes in physiology, behavior, and morphology. Larvae and adults of insect species that undergo complete metamorphosis are often subject to distinct environmental conditions, presumably applying selection pressures for highly variable environmental responses, or environmental sensitivity, across development. This begs the question: Is performance under a given set of environmental conditions constrained across metamorphosis or is there the potential for adaptive decoupling in distinct life stages? To answer this question, genetic correlations in lower thermal limits across metamorphosis was estimated for approximately 200 isogenic *Drosophila melanogaster* DGRP lines. Survival curves spanning LD50 and LD90 were estimated for a subset of lines, while survival at -5C for one hour was measured on all lines, proving a reliable proxy for various summaries of survival curves. Next, genetic correlations were determined in larvae vs. adults from each line. Preliminary results show low genetic correlations, suggesting distinct genetic architecture for thermal stress across development. We also discuss the results of genome-wide association analysis (GWAS) to begin to distinguish mechanisms by which thermal tolerance may be ontogenetically decoupled.

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### **Adaptive Ecotypic Variation and Genetic Divergence of a Widespread Grass *Andropogon gerardii* across a Great Plains' Climate Gradient**

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Local adaptation is a fundamental phenomenon in evolutionary, conservation, and global climate change biology, with applications for restoration and climate adaptation. Local adaptation is defined as ecotypes from local populations outperforming non-local transplants. Our research deals with adaptive variation in drought tolerance and local adaptation of an ecologically dominant grass big bluestem *Andropogon gerardii* across the Great Plains' grasslands. This grass

represents as much as 70% of prairie biomass and is widely used in conservation plantings over ~ 5 million acres across the Great Plains. Because of the wide geographic distribution across a precipitation gradient (500-1200 mm/yr from western KS to IL, we expect intraspecific variation in *A. gerardii* in response to precipitation. Objectives are to 1) use reciprocal gardens to investigate ecotype differences in vegetative and reproductive traits, 2) characterize genetic divergence among ecotypes, and 3) identify outlier loci and potential candidate genes responsible for divergence. Three ecotypes (central KS, eastern KS, and Illinois) were reciprocally planted in Colby, Hays, and Manhattan, KS, and Carbondale, IL. We evaluated ecotypic differences in vegetative and reproductive features across sites, predicting ecotypes would perform best at “home”. Canopy area and height increased from west to east, with no evidence for ecotype differences in Colby and Hays. In Carbondale, the IL ecotype showed an increase indicating local adaptation. In Carbondale and Manhattan, the CKS ecotype flowered 20 days earlier than other ecotypes with greater probability of seed in western sites relative to other ecotypes. IL ecotype produced greatest seed in its home site. CKS ecotype shows traits consistent with drought tolerance—reduced canopy, short stature, and early flowering. PCA shows trait variation sorting along a longitudinal precipitation gradient. Morphology was correlated with seasonal mean precipitation and temperature, elevation, and size of precipitation events. To characterize genetic divergence, we used Genotyping-by-Sequencing to identify 4,641 SNPs. STRUCTURE showed evidence for K=3. The IL ecotype existed as a distinct genetic group while the Kansas ecotypes showed admixture. Outlier analysis in Bayescan identified 373 outlier SNPs showing a signature of divergent selection. Of the top 1% of outliers identified in a Bayenv-climate association analysis, SNPs were primarily associated with seasonal diurnal temperature variation and seasonal precipitation. We mapped our GBS tags to the genome of *Sorghum bicolor*, the closest sequenced relative of bluestem. Using TASSEL, we identified SNPs associated with adaptive traits: flowering (1), blade width (1), canopy area (10), leaf nitrogen content (1) and height (22). Key candidate genes of interest that were both outliers and identified in our genotype-phenotype association include: nitrogen content-glutamate synthase (nitrogen assimilation), height-GA1 (internode length), and emergence-WUSCHEL transcription factor (development). Further characterization of these candidate genes may corroborate observed functional differences among ecotypes in nitrogen economy, height and biomass, and development. These results combining phenotypic reciprocal gardens and genetic studies provide insight into plasticity of adaptive traits and genetic divergence and may help inform sourcing for grassland restoration in future drier climates.

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### **Infuence of Heavy Metals in Plant infection and Protection**

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In the last three decades, the industrial and human activities in Crop Plants area of Alexandria have been increased dramatically and resulted in continuous invasion with different types of pollutants. Heavy metals are the most important pollutants in some Agricultural areas in Alexandria governorate, Egypt. The aim of the present study is to determinate the concentrations levels of eight heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in white, corn and rice of certain agricultural sites in Alexandria Sites which collected from Abu-Qir agricultural market, El-agamy agricultural market, the Eastern farms and Western farms

The obtained results clarified that the highest average was for Zn in sites Eastern farms, El-agamy and western farms, respectively. While, highest average of Pb was recorded in Western farms and El-agamy agricultural market sites, respectively. Moreover, Cd showed the highest values in the sites Abu –Qir Agricultural market and El-agamy agricultural market consecutively.

On the other hand, the average values of trace metals ( Cu , Fe, Mn and Ni ) around the agricultural markets and farms areas Alexandria were in levels below the hazard concentrations. Also, the concentrations of the studied heavy metals in all sites were found to be within the safe limits. The target hazard quotients (THQs) values of most investigated metals in crop plants and final products were much smaller than one, which may indicate that there is no health risk.

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### **The effect of abiotic stress on plant chemical defense response to herbivory; variation among high and low elevation populations.**

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Understanding range limit dynamics is a fundamental concern in ecology and evolution, especially for predicting the consequences of climate change. Low elevation “trailing edge” range margin populations typically face increases in both abiotic and biotic stressors that may contribute to range limit development. Here we tested predictions of a hypothesis

that attempts to explain how the stressors may prevent adaptation to lower elevations or to climate change. Within ranges, plants such as *Arabidopsis* respond to temporary abiotic and biotic challenges using different signal transduction pathways, which can be antagonistic to one another. We assume that natural selection may act on signaling pathways at range boundaries for more stable expression of traits. The hypothesis states that antagonistic plastic response pathways may inhibit their simultaneous co-option for more stable expression that is needed for range expansion. To test this hypothesis, we compared high and low elevation populations of *Boechea stricta*, a close relative of *Arabidopsis*, that have diverged for constitutive levels of glucosinolate defenses and root:shoot ratios; neither population has high levels of both traits. If antagonistic signaling underlies this divergence, one would predict that high constitutive levels of traits would coincide with lower plasticity. To test this prediction, we compared the genetically diverged populations in a double challenge drought-herbivory growth chamber experiment. Although a glucosinolate defense response to the generalist insect herbivore *Spodoptera exigua* was attenuated under drought conditions, the plastic defense response did not differ significantly between populations. However, RNAseq analysis did reveal the expected differences between populations. Similarly, although several potential drought tolerance traits were measured, only stomata aperture behavior, as measured by carbon isotope ratio, was less plastic as predicted in the high elevation population that was diverged for high constitutive root:shoot ratios. However, again, RNAseq results supported the hypothesis – gene expression associated with drought tolerance was attenuated in the high elevation population. A negative correlation between carbon isotope ratio and glucosinolate level was also more inducible in the low elevation population. Thus we detected limited phenotypic evidence in support of a negative genetic correlation between constitutive and plastic trait levels, but stronger evidence was found at the gene expression level, suggesting that range limit development may involve the co-option of antagonistic signaling.

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### **Expression and evolution of third chromosome desaturases and elongases in the *Drosophila melanogaster* subgroup**

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Desaturases and elongases have the potential to contribute to speciation through their effects on long-chain fatty acid precursors to pheromones. We identified three desaturases and 11 elongases on the third chromosome as potentially candidate genes for pheromonal cuticular hydrocarbon (CHC) production affecting sexual isolation between *Drosophila simulans* and *D. sechellia*. Focusing on these two species, as well as their sibling species *D. mauritiana* and *D. melanogaster* with *D. erecta* as an outgroup, we measured gene expression for all of the 14 loci in the abdominal cuticle, the site of CHC production. Using qRT-PCR, we found that several of the genes are not expressed in this location indicating that they are not used in CHC production. Two of the genes, a desaturase and an elongase known to be involved in sex-specific differentiation in CHCs in *D. melanogaster*, remain strong candidate genes for species-specific CHC production. Gene tree relationships were determined using the genome sequences for all of the loci. The resulting relationships based only on coding sequences do not reflect the patterns of gene expression, implying that regulatory sequences are more important for species-specific differences in CHC production. This is further supported by allele-specific expression of the two major candidate genes in hybrids of *D. simulans* and *D. sechellia*.

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### **Testing for convergent evolution across disparate fish lineages adapting to toxic hydrogen sulfide**

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Convergent evolution is a common theme in evolutionary diversification, yet the factors promoting or hindering convergent evolutionary outcomes when independent lineages are exposed to similar sources of selection remain largely unstudied. Physicochemical stressors exert strong and consistent selection and have clear consequences at multiple levels of biological organization, facilitating comparative analyses across broad taxonomic and geographic scales. Using eleven lineages of poeciliid fishes that have independently colonized toxic springs rich in hydrogen sulfide (H<sub>2</sub>S), we are studying the mechanisms responsible for convergent evolution across biological levels of organization. Targets of H<sub>2</sub>S are well characterized, providing a clear framework for testing the effects of natural selection from the molecular level to physiological and morphological responses in each lineage of sulfide spring fish. Comparing each lineage's adaptations to sulfidic conditions allows us to determine how phylogenetic relationships and natural selection interact to produce evolutionary convergence at different levels of biological organization. Employing phenotypic analyses and RNA-sequencing, we are addressing three major questions: (1) How do phylogenetic relationships influence the degree of

convergent phenotypic evolution observed across these eleven lineages? (2) Is there convergence in gene expression patterns among lineages exposed to the same environmental conditions? (3) How does adaptation to the same environmental conditions drive patterns of molecular evolution at the level of the nucleotide, amino acid, and gene? Overall, species with close phylogenetic relationships should exhibit high degrees of convergent evolution across all levels analyzed. However, due to the extreme nature of H<sub>2</sub>S and its specific molecular targets, high degrees of convergence might also be observed between more distantly related lineages. Studying both phenotypic and molecular evolution across broad phylogenetic gradients and hierarchical levels of biological organization will help to disentangle the roles of adaptation and evolutionary history in generating convergence. Furthermore, this work offers the opportunity to gain a better understanding of the degree to which evolution is actually predictable.

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### **Using ecological niche modeling to determine landscape barriers of the ornate box turtle on the pine ridge reservation**

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Due to a lack of research, the ornate box turtle (*Terrapene ornata*) is considered a species of greatest conservation need in South Dakota. They are also a sacred species to the people of the Sioux Nation. In response, an ecological study on the Pine Ridge Indian reservation was conducted in order to develop a reliable conservation plan. This is the first genetic research study conducted from within the Pine Ridge Indian reservation. An important component of the conservation plan is an evaluation of known ornate box turtle habitat, identification of additional suitable habitat on the reservation, and identifying any landscape barriers. Based on literature and field observations land cover, sediment type, and elevation were hypothesized as prediction variables. These criteria were measured in ArcGIS 9.3 and analyzed using Maximum Entropy (MaxEnt) and the Boolean Overlay methods. The soils layer and the elevation layer had high box turtle habitat prediction potential. The land use layer was not a good predictor for the model and so it was removed from the analysis. A ranking system was developed and land on the reservation were ranked based on habitat suitability. The resulting map was checked using dogs trained to locate box turtles. In areas that had low potential of finding turtles, the dogs did not locate any turtles. In areas that had high potential of locating turtles, the dogs were able to locate turtles. In the model, soil type had the highest prediction potential with silty sand being the most preferred. These sandy silt areas were often defined by roads and rivers. A genetic landscape analysis was done to determine if they are a significant genetic barrier for the ornate box turtle. Eleven novel microsatellites were used in determinacy of barriers. Although there did not appear to be a difference of population structure (analyzed in STRUCTURE), two of the eleven microsatellites did have all of the all heterozygosity and one of the eleven microsatellites had all homozygosity within the predicted suitable habitat. All of the given information and past conducted research gives reason that the ornate box turtle could be selecting for a silty sand type of sediment. Due to the conservation status and it being a sacred species, it is an optimal species for a genomic study. Future work includes sequencing the genome using radseq to determine if they are selecting for a sandy silt type of sediment.

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### **Evolutionary origins of the nervous system**

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Cnidarians have the simplest of the nervous systems, called the nerve net. To investigate the origin of the nerve net, it is hypothesized that the proteins involved in the nervous system function such as ion channels and neurotransmitters, co-evolved with the muscle proteins and the neurotoxin proteins. To test this hypothesis, the transcriptomes of 28 cnidarian species were computationally tested for co-evolution using phylogenetic trees and statistical correlation between them. It was found that *Heteractis crispa* contained the ancestral protein involved in neurotoxins. It was also found that there was higher correlation between the neurotoxin proteins and the nervous system proteins implying a co-evolutionary arms race scenario between them. The muscle proteins such as actin and myosin showed much less correlation with the proteins involved in the nervous system. However, it needs to be pointed out that correlation, which was what was tested in my study, does not necessarily imply co-evolution.

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### **Ecological Genomics URM – fostering effective learning through hands-on experiences and frequent evaluation**

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The NSF-funded, interdisciplinary Undergraduate Research Mentoring (URM) program in Ecological Genomics at Kansas State University (KSU) is in its fifth and final year. During this time, we have distributed nearly half a million dollars directly to undergraduates in stipends to support their research under supervision of Ecological Genomics Institute (EGI) faculty. These URM funds have been directed to increase the number and diversity of individuals pursuing graduate studies in all areas of biology. The URM program has partnered with the KSU Developing Scholars Program (DSP) to recruit a diverse population of students for a year-long mentoring with EGI faculty in biology, entomology, and plant pathology. URM students may apply for second year of support to continue their research and to serve as peer mentors for beginning scholars. The URM program integrates students tightly into the mentors' laboratories. The mentors' responsibilities include guiding the scholars in professional conduct, ethics, general principles of science as well as introducing them to the research culture in their discipline. A key goal of the URM program is to encourage students to communicate their research. Towards that end, the URM students present their research in national and international meetings with partial, need-based support from the URM program. The URM program also has supported a limited number of non-URM KSU undergraduate students to attend the annual Ecological Genomics Symposium in 2014 and 2015, often as their first exposure to a scientific meeting. The URM program also emphasizes the importance of sharing research findings and the program encourages students to reporting or co-author their research in peer-reviewed journal articles. Frequent evaluation and feedback are central components of the URM program. We utilize informal questionnaires in application materials to identify the most successful recruitment strategies (peer-to-peer contacts, classroom presentations, mentor contacts) and more formally query the URM mentors and mentees through rubrics designed in collaboration with KSU Office of Educational Innovation and Evaluation. These entry and exit surveys have increased our minority recruitment and lead to the development of a program to distribute travel awards to promote science communication in national and international platforms. In addition to these opportunities to share research and research findings, the URM mentees share their experiences through KSU DSP, Summer Undergraduate Research Opportunities program and NSF-REU programs.

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### **QS systems are maintained by interspecies competition**

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Many bacteria use a cell-density dependent communication system called quorum sensing (QS) to coordinate expression of genes in a population-wide manner. QS systems often regulate the production of secreted products such as proteases and antibiotics. These products are public goods that are shared amongst the entire population and vulnerable to cheating by non-producing members of the population (e.g. QS-defective individuals). Additionally, a few of the QS-controlled gene products may be private goods, such as cellular factors, that only benefit the producing individual. QS control of private goods may protect the population from the emergence of cheaters by conferring a disadvantage to cheaters under certain circumstances. Because QS often controls factors thought to be important for competition, such as antibiotics, we are interested in understanding the connection between QS and interspecies competition. We developed a laboratory dual species model with two soil saprophytes, *Burkholderia* and *Chromobacterium*. Previously, we showed that a *Burkholderia* QS-controlled antibiotic (bactobolin) inhibits the growth of *Chromobacterium*, and in *Chromobacterium* QS controls production of several antimicrobials that inhibit the growth of *Burkholderia*. QS also promotes *Chromobacterium* survival during co-culture competition with *Burkholderia*. Here we demonstrate that *Chromobacterium* uses QS to control production of antibiotic-resistance determinants that promote survival in the presence of *Burkholderia*-produced bactobolin and several other antibiotics. To test whether the QS-controlled antibiotic resistance factor confers a public or private benefit, we competed a mixture of QS-intact and QS-defective *Chromobacterium* with *Burkholderia* and determined the frequency of each after co-culture growth. We found that the QS-intact *Chromobacterium* increased in frequency in the presence of *Burkholderia* producing bactobolin, suggesting that bactobolin produced during co-culture selects for QS-intact *Chromobacterium* and that the QS-controlled antibiotic resistance determinant confers a private benefit. Because QS also controls production of antimicrobials, this results in a net increase in the competitiveness of the *Chromobacterium*

population during co-culture growth. These results highlight another important role of QS in interspecies competition. Our results also demonstrate a mechanism where interspecies competition serves to stabilize and protect QS systems, which in turn promotes the competitive ability of the population. This has important implications for how QS systems evolve and are maintained during growth in mixed microbial communities.

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### **Divergent epicuticular wax and transcriptomes of edaphically differentiated *Andropogon* grasses in US Midwest prairies**

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Characterizing adaptive genetic diversity across spatially varying environments provides insight into the role of environment in adaptive differentiation, local adaptation and, ultimately, formation of species. In particular, study of edaphically differentiated populations gives fundamental knowledge about the strength of natural selection in evolution. We focus on two grass varieties adapted to contrasting soils and their divergent morphologies, physiologies, and gene expression. *Andropogon gerardii* (big bluestem) is a widely distributed dominant grass on loamy soils of US Midwest prairies while *Andropogon gerardii* var. *hallii* (sand bluestem) is edaphically restricted to sand dunes. We probed the nature of edaphic adaptation, especially the role of epicuticular waxes (ECW) leading to differences in physiology, morphology, and gene expression between these subspecies. We found that sand bluestem: 1) contains more ECW, comprised of ~20%  $\beta$ -diketones in the form of microtubules (all absent in big bluestem), 2) had decreased light absorbance, and 3) lower minimal stomatal conductance relative to big bluestem, all suggesting greater drought adaptation. Transcriptomes were assembled using miraEST, Velvet and Oases with multiple values of  $k$  (21-61), and OasesMerge and the final merged assembly was used to compare gene expression. Paired end Illumina reads (2x100bp) from four individuals of each subspecies allowed comparison of gene expression differences. Using DeSeq2, 2546 transcripts were differentially expressed between the two subspecies, suggesting fundamental genetic differences in part resulting in divergent epicuticular form and function. Taken together, data indicates adaptation of sand bluestem to sandy soils involves differences in transcriptional expression, leading to divergent cuticle chemistry, morphology, and function.

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### **Understanding nature versus nurture: Using avian song as a model for ecological epigenetics**

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Birdsong has received attention from ecologists and neuroscientists alike because of the discrete nuclei in the brain controlling this complex yet measurable, biologically relevant behavior. This behavior is a secondarily sexually selected trait aiding in territory defense as well as attracting mates. The telencephalic song nuclei responsible for learning and producing those songs are particularly vulnerable to fluctuations in stress during the first few months post-hatch due to rapid neurodevelopment. In many songbirds, these first few months mark a critical learning window after which the individual cannot learn song from a male tutor. Past literature has found that elevated corticosterone (CORT, one of the major stress hormones in birds) and nutritional deprivation during this sensitive post-hatch period is correlated with decreased song quality as a support of Developmental Stress Hypothesis. To date, few studies have examined mechanisms behind this phenotypic modification due to changes in the developmental environment. One possible mechanism behind this change in song quality is the epigenetic status of the neural glucocorticoid receptor (GR) via DNA methylation. Changes in methylation status may alter gene expression, thus leading to downstream changes in both neuroanatomy and phenotypic expression. Here, we propose that one cause for this change in song quality is due to altered methylation status of the neural GR. The neural GR has been selected because of the presence of multiple CpG islands and transcription factor binding sites, as well as previous findings in the mammalian literature suggesting that GR is vulnerable to epigenetic changes. This proposed change in epigenetic status is the first time a mechanism behind the Developmental Stress Hypothesis has been proposed. To test this hypothesis, oral CORT was administered to wild

Eastern Bluebird (*Sialia sialis*) nestlings. We have identified a putative GR promoter sequence for this species, and are in the process of investigating changes in the methylation status as well as area and volume of the song nuclei through the use of bisulfite sequencing. In this presentation, we discuss how this putative GR promoter sequence may be used to determine whether epigenetic modification is involved in reduced song quality after exposure to various developmental stressors and how birdsong can be used as a model system for ecological epigenetics.

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### **Comparative Genomics of Budding and Fission Yeasts Extends the Chromosomal Theory of Speciation**

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Chromosomal rearrangement, which changes the gene order and possibly also the gene content of a chromosome by inversion, translocation, duplication or deletion, may create a gene flow barrier. Indeed, both theoretical and empirical studies have suggested that chromosomal rearrangement facilitates speciation. Chromosomal rearrangement has also been shown to contribute to organisms' adaptation to different climates or habitats. However, it is still unclear whether, at the genome scale, higher rates of chromosomal rearrangement contribute to faster speciation and better adaptability. In this study, we examined the patterns of genome evolution between two distantly related yeast classes, the budding yeasts (*Saccharomycetes*) and the fission yeasts (*Schizosaccharomycetes*), which diverged about 500 million years ago. Compared to fission yeast class, the budding yeast class display much higher species richness (~1000 vs. 4 species) and a wider range of ecological niches. In consistent, we found a much lower rate of chromosomal rearrangement in fission yeasts than in budding yeasts, revealing much less variation in chromosome number and genome size, a higher degree of synteny conservation, a lower rate of gene order change, and more conserved gene content in fission yeasts. We speculated that these differences were due to a low recombination rate and low abundance of transposons in fission yeasts. Our findings extend the chromosomal theory of speciation to a genome scale, and provide a plausible explanation for the mysterious disparities in species richness and ecological niches between the budding and fission yeasts.

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### **Cell cycle regulation and the evolution of multicellularity: Identification of candidate multicellularity genes through evolutionary transcriptomics**

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Multicellularity has evolved at least 25 times throughout the history of life on earth, yet despite its common occurrence, no genetic mechanism exists to explain its origin. The volvocine algae are a model system to study the evolution of multicellularity because they consist of a recently evolved (approximately 200 million years ago) monophyletic group of organisms that morphologically span from unicellular (*Chlamydomonas reinhardtii*) to colonial multicellular (*Gonium pectorale*) to multicellular with germa and soma differentiation (*Volvox carteri*). *C. reinhardtii* and *G. pectorale* grow and divide similarly. However, when *G. pectorale* cells under go multiple fission, the daughter cells remain attached to each other; whereas in *C. reinhardtii*, the daughter cells separate. This, along with other experimental evidence, suggests that the colonial multicellular phenotype of *G. pectorale* is under cell-cycle regulation. In this experiment, we used RNA-Seq analysis to investigate changes in gene expression across the 24-hour cell cycle of *G. pectorale*. Five strong candidate *G. pectorale* multicellularity genes were identified through a two-step process. One of these candidate multicellularity genes is a cell adhesion gene, that when transformed into the unicellular *Chlamydomonas*, results in a multicellular phenotype with cytoplasmic connections. Here, I present data on the functional verification of this cell adhesion gene in the gain of function *C. reinhardtii* transformants.

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### **Divergence genomic pattern in a case of incipient speciation from *Heliconius* butterflies**

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Genomic Speciation predicts that incipient divergence under conditions of gene flow show heterogeneity patterns in the

genome. The wing colour pattern of *Heliconius* butterflies seems to have played a main role in the adaptive radiation of this genus in the Neotropic and make it an ideal model to test predictions of genomic speciation. We analysed the role and interaction of 12 candidate genes of the colour pattern in a bimodal hybrid zone between the incipient species: *Heliconius erato chestertonii* and *H. e. venus* from Colombia. A total of 136 SNPs were found in the 5 candidate genes associated to red elements in the forewing and the 7 genes associated to yellow elements in the hindwing. These colour pattern elements are controlled by the mendelian loci *D* and *Cr* in *H. erato*. Our results show a reduction of nucleotide diversity (mean  $\pi = 0.008$ ), high estimates of genetic structure for an unlinked gene and all the candidate genes (mean  $F_{ST} > 0.6$ ), evidence of strong natural selection on the candidate colour pattern genes ( $P < 0.05$ ) and absence of evidence of selection on unlinked genes ( $P > 0.05$ ). Our analyses confirmed that the bimodality of the hybrid zone is also reflected in a molecular level. More even, the pattern of genomic differentiation among *H. e. chestertonii* and *H. e. venus* support the thesis of a heterogeneous pattern of genomic differentiation in natural populations where incipient speciation occurs.

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### **Transcriptional response of the diatom *Cyclotella cryptica* grown across a salinity gradient**

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Salinity is a primary ecological barrier to the distribution of protists. Like most other protists, diatoms are ancestrally marine. Colonizations of freshwater, however, have seeded a flora as diverse as that in their ancestral habitat. The physiological and genetic mechanisms of adaptation to freshwater are largely unexplored. Diatoms with distributions that span the salinity gradient ('euryhaline') are prime candidates for understanding the underlying genetic bases of salinity acclimation and adaptation. We grew a euryhaline species in conditions spanning the full natural range of salinity. We measured growth reaction norms and transcriptional responses to altered salinity using RNA-seq. The data will show which genes are involved in the salinity stress response and how that response changes across ambient salinity. We plan to expand these experiments to include more distantly related taxa to determine whether independent freshwater colonizations followed the same, or different, genetic routes into freshwaters.

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### **Uncovering the mechanism behind cold-sweetening in potato tubers using transcriptomics**

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Cold temperature (4°C) is routinely used in potato agriculture to prevent budding during storage. However, this leads to a phenomenon known as cold-sweetening where sugars accumulate in tuber tissue. The presence of reducing sugars in tuber tissue during frying results in an undesirable browning and production of toxic substances, such as acrylamide. Potato varieties exhibit different levels of cold-sweetening, suggesting an underlying genetic mechanism controlling sugar production during cold storage. To untangle the mechanism behind cold-sweetening we measured glucose concentration and sequenced the transcriptomes of 10 different potato varieties that show different levels of glucose accumulation before and 3 months after cold storage. Many of the genes that exhibit a transcriptome expression profile similar to differences observed in glucose levels appear to be involved in regulating the response to cold stress as well as desiccation stress. This suggests that pathways regulating the response to cold are shared with water loss and that post-translational regulation is involved in control of cold-sweetening.

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### **The Island Rule from a population genetic perspective: mitochondrial sequence variation in an insular population of Soemmerring's gazelle (*Nanger soemmerringii*).**

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Insular dwarfism, the evolutionary change toward smaller body size in large mammals when their range is limited to isolated islands, has been reported in numerous extant and extinct mammal species. The phenomenon, referred to as the Island Rule when it includes the reverse trend of gigantism in small mammals, is of significant biological interest. In this study, we focus on the evolutionary and conservation implications of a striking reduction in body size observed in an island population of the gazelle species *Nanger Soemmerringii* (Cretzschmar, 1828), by comparing the population genetic structure of island and mainland gazelles. Mitochondrial control region sequences showed that a thriving population of Soemmerring's gazelles on Dahlak Kebir, one of the over 300 islands and islets of the Dahlak archipelago on the Red Sea, has genetic structure significantly divergent from mainland populations. This genetic distinctiveness of the island gazelles and their striking conformity to the Island Rule are discussed from conservation and evolutionary perspectives. Furthermore, we use a simulation based analysis of the sequence data to estimate the date and route of the colonization of Dahlak Kebir by Soemmerring's gazelles.

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### **Transcriptomic responses to hydrogen sulfide exposure in replicated susceptible and tolerant populations of *Poecilia mexicana***

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Physiochemical stressors can drive evolutionary divergence among populations and lead to local adaptation. Simultaneously, such stressors elicit the expression of plastic coping mechanisms that mitigate adverse effects. Understanding how plasticity and evolved population differences interact to shape organismal responses is critical for the prediction of ecological and evolutionary consequences of environmental stress. We studied genome-wide expression responses in genetically distinct and locally adapted populations of extremophile fishes (*Poecilia mexicana*) living in toxic, hydrogen sulfide (H<sub>2</sub>S) -rich springs to identify potentially adaptive population differences in gene regulation. To do so, we exposed common-garden raised individuals from two evolutionarily independent population pairs from sulfidic and non-sulfidic environments to varying concentrations of hydrogen sulfide (0, 0.5, 3, or 6 mM) for 22 hours. After exposure, we immediately collected gill tissues from a total of 87 individuals and used RNA-sequencing to quantify gene expression patterns. Transcripts were mapped to the *P. mexicana* reference genome, and variation in transcript abundance among treatments and populations was analyzed with the EdgeR package in R. The goal of this research is to test how gene expression response to H<sub>2</sub>S exposure differs between sulfidic and non-sulfidic populations. Accordingly, we predict that genetic background plays a larger role than plasticity in response to H<sub>2</sub>S exposure. In other words, origin of ecotype will be a strong predictor of response to H<sub>2</sub>S. Overall, this study will begin to disentangle how H<sub>2</sub>S-induced plasticity and genetic population differences contribute to phenotypic variation in nature.

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### **Empty niches after extinctions increase population sizes of modern corals**

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Estimates of population size through time can show how history has shaped the evolution of current species, provide accurate models to interpret contemporary genetic data and generate a framework to implement successful conservation strategies. This is relevant when paleontological evidence is rich and provides direct ecological evidence on what triggers demographic variations. We studied how variations in abiotic and biotic environments since the late Pliocene shaped historical changes in the population sizes of corals of the genus *Orbicella*, the major current reef builders in the Caribbean. Our results suggest populations of all three *Orbicella* species share patterns of population decline around 1-2 Ma coincident with the extinction of 80 % of Caribbean coral species. The change in population size is consistent for the three species despite their ecological difference. Most recently, the two shallower specialist, *O. faveolata* and *O. annularis* expanded their population sizes by at least two fold. The expansion of these two species coincides with the recent disappearance of their sister competitor species *Orbicella nancyi* (organ pipe) [1]. Our study suggests populations of modern *Orbicella* species are capable of rebounding from reductions in population size under suitable conditions and the evolutionary potential for corals to adapt to climate change may be higher if one accounts for the recent erosion of genetic diversity during the recent past.

## **Molecular evolution of the American pika (*Ochotona princeps*): in consideration of adaptive substitutions in an alpine mammal**

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American pika (*Ochotona princeps*) populations are assumed to be restricted to their high-elevation distribution due to poor heat tolerance, while their persistence is facilitated by adaptation to hypoxia. However, there has been limited genetic evidence to support the latter assumption. Resolving this gap between apparent phenotypic evolution and molecular signals of adaptation thus represents a challenge to the field of ecological genetics. This challenge may be approached by first identifying the selective force and subsequently investigating the molecular targets of selection. When considering pika populations, it is reasonable to assume that they experienced strong selective pressure to develop adaptations to hypoxia as distributions shifted upslope and became progressively fragmented as climate warmed following the Last Glacial Maximum. We would thus expect to find molecular signals of this adaptive event in the genome. We then must identify a small number of genes with signals of positive selection among the many thousands of genes in the genome. This issue can be somewhat alleviated, however, if we have prior reason to believe that a particular gene has been a target of selection. That is, if a gene's product is an important component of metabolic regulation, then perhaps a new high-fitness mutant allele arose and was strongly selected for in recent evolutionary time. Following this assumption, a reasonable approach would be to select genes that have previously shown evidence of contributing to pika-specific adaptation and then investigating those markers for adaptive evolution. Here, we present evidence for positive selection acting at two putatively adaptive loci within isolated lineages of *O. princeps*. These loci were identified by first assessing levels of genetic variability at candidate genes among two subspecies of *O. princeps* using draft genomes available from public databases in order to identify useful loci for tests of positive selection. Then, after identifying 54 loci in association with pika adaptation, we tested the possibility that positive selection acted on the *Ochotona* lineage relative to the Euarchontoglires clade using the CODEML program in the PAML package. These analyses indicated six putative adaptive loci, which we then used to sequence multiple individuals from a broad geographic range. We subjected the resulting data to multiple tests of non-neutral evolution using Tajima's D, Fu's F, and Ramos-Onsins and Rozas's R2 test statistics. These tests have provided additional support for positive selection acting on two of the six loci. We also report a summary of molecular diversity for these two putatively adaptive loci.

## **Genomic background of diapause pathways of a European spruce bark beetle**

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The European spruce bark beetle, *Ips typographus*, is an important forest pest in Norway spruce stands. Due to chemical communication with pheromones, polygyny, voltinism, fecundity and its ability to produce sister broods, *I. typographus* is very efficient in colonizing its host tree. Abundance of this species rises rapidly after abiotic disturbances, like storm, snow or drought. This may lead to heavy economical losses and ecological impacts in forest ecosystems.

In winter, *I. typographus* enters a reproductive diapause in the adult stage when the critical day length falls below 14.7 hours. Diapause is terminated in December/January and is followed by a temperature-dependent quiescence. In Europe we find populations with facultative (multivoltine) and obligate (univoltine) diapausing beetles. Diapause phenotypes can be distinguished by physiological traits, i.e. gonad development, emergence rates, and respiration rates under different photoperiodic and thermal conditions.

Diapause phenotypes of Central European (low elevations and high elevations in Austria) and Scandinavian (northern Sweden) populations have previously been determined in physiological studies. We will apply ddRADSeq to identify loci linked to diapause phenotypes by comparing facultative and obligate diapausing individuals across several populations.

Thereafter, we will screen various European populations for their ratio of facultative and obligate diapausing individuals to obtain data on the genetic structure, emphasizing functional genetic variation associated with the diapause phenotype.

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### **Target enriched Double Digest RADseq: A novel approach for the high resolution characterization of the endosymbiont *Wolbachia***

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*Wolbachia* is probably the most widespread endosymbiont bacterium in arthropods. This symbiont can influence the reproduction of its host by inducing cytoplasmic incompatibility, male killing, parthenogenesis or feminization. Therefore *Wolbachia* is an important factor in the biology and evolution of insect species.

Characterization of *Wolbachia* is not trivial, since the bacterium cannot be cultivated and are often present in low abundance in their hosts. Therefore Sanger Sequencing of multiple *Wolbachia* genes is the most common way to characterize this bacterium. However, this method has some limitations: 1) since multiple *Wolbachia* strains can be present within one host, it is difficult to characterize less common strains 2) certain *Wolbachia* strains are present in low abundance and can be easily overlooked by conventional Sanger Sequencing and 3) Sanger Sequencing of multiple populations and characterization of multiple genes is inefficient and costly.

Here we present a modification of the current ddRADseq protocol commonly utilized in population genetic studies. The enrichment of specific *Wolbachia* fragments, followed by ddRADseq and Illumina MiSeq sequencing enables the high-resolution characterization of *Wolbachia* in multiple individuals. Thus our approach represents a novel, efficient and costeffective technique to characterize this and potentially other endosymbionts.

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### **Effect of heat stress duration on root growth and development in *Arabidopsis thaliana***

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Exposure to elevated temperature can trigger a variety of changes in gene expression, including increased production of heat shock proteins, which stabilize and protect other cellular proteins but are costly to produce. The pathways through which heat stress influences root development are not fully understood, but the transcription factor SCARECROW (SCR), which maintains stem cell identity during root growth, may play a role in this process. We tested the effect of 9 different durations of heat stress exposure on root growth and SCR expression in 5-day-old *Arabidopsis thaliana* seedlings. Periods of exposure lasting 4 hours or less had little effect, but root growth decreased significantly after 6, 12, and 24 hours at an elevated temperature. Oddly, an 8-hour heat stress treatment did not seem to affect root growth at all. Expression of SCR protein in the cortex of the root tip appeared to decrease after 4 hours of heat exposure. These results suggest that a period of between 4 and 6 hours of heat stress is necessary to reduce root growth, and that lower levels of SCR protein in the tips of elongating roots may be involved in this response.

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### **Biodiversity and nutritional mutualism of *Wolbachia* in Bat flies (Streblidae, Nycteribiidae)**

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This research aims to investigate the biodiversity and mutualistic function of *Wolbachia* in bat flies (Streblidae, Nycteribiidae). *Wolbachia* symbionts are one of the most prevalent facultative symbiotic bacteria in arthropods and so far have been classified in 16 supergroups (A to Q). They are generally described as maternally inheritable reproductive parasites that may have a negative impact on host fitness. However, they are related to a wide range of associations with host organisms which are still largely unknown. The association between bat flies and *Wolbachia* is one of the unknown host-*Wolbachia* relationships. Bat flies are diverse group of obligate blood-sucking Diptera, and regarded as a part of the Hippoboscoidea superfamily that includes tse-tse flies (Glossinidae). As hematophagous insects, they are expected to

have endosymbiotic interactions for their nutritional demands. Previous research has found that bat flies have symbiont associations including Gammaproteobacteria and Alphaproteobacteria, including *Wolbachia*. Based on Multi Locus Sequence Typing (MLST), we obtained Maximum Likelihood phylogenetic trees and examined the biodiversity of *Wolbachia* in diverse species of bat flies. We found supergroups A, B, F and unassigned *Wolbachia* strains. This result is interesting because even though the *Wolbachia* phylogeny is generally congruent with the host phylogeny, *Wolbachia* strains found in bat flies are very diverse and some strains are not closely related to *Wolbachia* found in tsetse flies (supergroup A) despite their close evolutionary relationship to bat flies. Although in each host population *Wolbachia* are usually acquired vertically, our result suggests relatively recent environmental acquisitions within bat fly clades. Moreover, we found the supergroup F *Wolbachia* strain to have very high infection rates (94.4% to 58.3%) in some species of bat flies compared with the general infection rate across other insects (20%). Interestingly, bat fly *Wolbachia* are closely related to wCle, a nutritional mutualistic *Wolbachia* strain found in *Cimex lectularius* (bed bugs). We performed diagnostic PCR to test whether the *Wolbachia* strains found in bat flies also contain the biotin synthetic pathway that only exists in wCle, and confirmed that only the F supergroup *Wolbachia* strain have the pathway. Considering the high infection rate and presence of the intact biotin pathway, the *Wolbachia* may be in evolutionary transition point from facultative symbionts to obligate mutualists in bat flies. This study may contribute to uncover transmission routes of *Wolbachia* as well as evolutionary transition of symbiosis to obligate mutualism.

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### **Crosstalk, Adaptation and Plant Range Limits.**

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Mustard plants (Brassicaceae) include approximately 3,700 species, several crop species (cabbage, radish, canola, etc.) and the model for molecular plant biology, *Arabidopsis thaliana*. Despite this diversity, mustards generally inhabit high altitude temperate regions where populations have patchy distributions. Mustard species are also characterized by the production of glucosinolate defense toxins. Our hypothesis is that regulation of glucosinolates could underlie the evolution of the spatially-restricted distributions. This is counter to previous hypotheses on defense evolution that have argued that variation in defensive chemistry is the consequence of spatial distributions, life history patterns, growth rates etc. The molecular mechanism underlying the hypothesized evolutionary constraint is the co-option of the well-documented antagonistic crosstalk between defense and stress tolerance signaling pathways in *Arabidopsis* and other species. Adaptation to abiotic stress tolerance and defense against generalist herbivores is needed to expand range to lower elevations or for the persistence of low elevation trailing edge populations under climate change. In support of this hypothesis, previous studies using family-structured analysis with *Boechera stricta*, a close North American perennial relative of *Arabidopsis*, indicate a negative genetic correlation between chemical defense and drought-tolerance levels. Here we present experimental genetic support from extended generation crosses of *B. stricta* showing non-independent segregation of the traits. In a cross, we also found a marker associated with change in the genetic correlation. The marker is linked to the candidate gene MYC2, a transcription factor involved in the crosstalk. Molecular evolution analysis of the MYC2 sequence is currently underway for species and population comparisons and will also be presented. We are also examining gene expression levels of the candidate TF between populations.

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### **Multifaceted DNA metabarcoding to address critical data gaps for threatened bat species**

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The standard approach to obtain population data on bats involves capture and tagging, which causes stress to the bats and requires extensive amounts of expertise, time, and expense. With White-nose Syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans*, leading to declines of up to 90% in some species, new methods are being explored to obtain critical data on bat populations that minimize both the stress on bat populations and the spread of WNS. DNA metabarcoding is a high-throughput, DNA-based identification technique used to obtain information about environmental or fecal samples. Here, we introduce Multifaceted DNA metabarcoding (MDM), which is a technique that combines the use of next-generation DNA sequencing, DNA barcodes, and bioinformatic analysis to simultaneously collect data on multiple points of interest. We plan to utilize this approach to obtain critical data about bat populations from fecal samples collected in a non-invasive manner; assays include species identity, sex, individual genotype, presence of *P. destructans*, diet (nectarivorous and insectivorous), and endo- and ecto- parasite load. Samples would be collected by placing tarps under flyways (e.g. cave exits) of active roosts and periodically collecting individual scat samples. DNA is extracted from the samples and used in a number of PCR reactions containing different primers designed to enrich target data. These PCR products are then uniquely barcoded such that all samples can be pooled into a single library for a single run on a next-generation sequencing platform. Bioinformatic analyses involve sorting samples by barcode, sorting

sequences by assay, assembly into contigs, and annotation using BLAST searches of NCBI's nr/nt database. We are currently testing this technology to determine its accuracy for quantifying each population parameter.

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### **Analysis of Alternative Storage Conditions for DNA Recovery from Field Samples**

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As ecologists increasingly employ molecular methods, they find that tried and true preservation solutions (e.g. ethanol or formalin) may not be optimal when samples are targeted for genetic analyses. Before traveling to remote sample sites, researchers need to consider which preservation methods are likely to yield the largest quantity and highest quality of DNA based on their travel times and field conditions. They also need to consider whether they will have access to preservatives at remote sites and whether those preservatives can be safely transported. To determine which preservation methods would most reliably preserve tissue for genetic analysis under a range of field conditions, we examined total DNA recovery from female fruit flies (*Drosophila melanogaster*) individually held in various solutions (70% ethanol; 2% SDS, 100 mM EDTA; 1% SDS, 50 mM EDTA; 0.66% SDS, 33 mM EDTA; Zymo© lysis buffer; Zymo Expedition© lysis buffer) at three different temperatures (22oC, 4oC and -20oC) for varying lengths of time (1 day, 4 weeks, and 8 weeks). We predicted that insects held in Zymo Expedition© buffer would yield the overall highest DNA recovery since this buffer was designed for field collected animal tissue. We also predicted that variation in DNA recovery from insects held in different solutions would increase with preservation time and holding temperature. Although we observed significant differences in total DNA recovery from our samples, no trends were identified. Our next step is to use these DNA samples as templates for amplification of the mCOI gene by PCR to compare the quality of the DNA we isolated from the insects stored under various conditions.

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### **Population differentiation in New York populations of the plant pathogen *Fusarium graminearum***

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*Fusarium graminearum* (Fg), a haploid filamentous fungus, is a causal agent of Fusarium head blight (FHB) disease on wheat and barley. The disease leads to decreased yield, economic losses, and the contamination of grain with toxic secondary metabolites, such as trichothecenes. In North America, FHB is caused mostly by Fg isolates with the 15-acetyl(A) deoxynivalenol (DON) trichothecene genotype. Recent population shifts in Canada and the northern US have led to the decrease in frequency of 15-ADON isolates as isolates carrying the 3-ADON genotype have increased in frequency. In this study, we examined the genetic variation within and between Fg collections isolated from wheat heads from two locations in New York state. These locations are known to differ in climatic patterns, cropping systems, and the frequency of the 3-ADON trichothecene genotype. Although this species is capable of self-fertilization, patterns of genetic variability also provide clear evidence for frequent outcrossing. Previous studies have revealed Fg isolates cluster primarily based on trichothecene genotypes. We used a genotyping by sequencing (GBS) approach to collect genetic variation data from loci spread evenly across this species' 4 chromosomes. Here we report on our investigation of genetic differentiation between geographical regions and between trichothecene genotype clusters and what they can tell us about Fg population history and adaptation.

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### **Eye Size and Opsin Expression Variations in *Daphnia***

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Vision provides a means by which organisms can construct a representation of their environment to attain information from. However, it is not always clear what information is useful for that particular organism. The genus *Daphnia* is comprised of small planktonic crustaceans that have a single compound eye. The size of this eye is potentially determined by a tradeoff of the benefits of information acquisition against energetic and predation risk costs. *Daphnia* also contain a variety of opsins, G-protein coupled receptors with roles in vision, many of which contain unknown functions or whose function is unknown in *Daphnia*. *Daphnia* vision might be used in predator avoidance, locating mates, or foraging. However, the *Daphnia* eye has evolved to allow for a higher spatial resolution than expected and the variety and number

of opsins their eyes contain also appears unjustified based on their behavior. Comparisons of eye size between females and males in 34 species of North American *Daphnia* were made to determine if there are consistent patterns of eye size variation that might offer insight about the function of *Daphnia* vision. *Daphnia* opsin expression levels were also analyzed, in order to ascertain function.

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### **Investigation into the Genetic Basis of Leaf Shape in Grapevines**

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Leaf shape is a distinguishing and diverse taxonomic characteristic in grape (*Vitis spp.*). In addition to acting as a reliable indicator for species and cultivar identification, leaves also provide information about plant function in an environmentally adaptive context. However, little is known about the genetic basis of leaf shape morphology in grapes. In this project, phenotypic analysis using morphometrics, and genotypic analysis using single-nucleotide polymorphism (SNP) markers have been combined to better define the genetic basis of grape leaf shape. The population consists of parents, *V. aestivalis* 'Norton' and *V. vinifera* 'Cabernet Sauvignon', and 182 F<sub>1</sub> progeny, at Missouri State University Fruit Experiment Station. For phenotyping, important leaf shape characteristics (n=17) were identified based on venation pattern, lobes, and sinuses. Morphometric analysis has been completed to quantify leaf shape variation. Approximately 43,000 SNP markers generated by genotyping-by-sequencing (GBS) have been identified in this population. Statistical analysis has been performed using a General Procrustes Analysis (GPA) to produce trait measurements in the form of Principal Component (PC) scores. Correlation analysis of genotyping and phenotyping data has been calculated to identify chromosomes containing possible quantitative trait loci (QTLs) responsible for leaf shape.

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### **Ecological field studies and copy number variation (CNV) analyses shed light on the selective mechanisms maintaining North American white clover cyanogenesis clines**

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White clover (*Trifolium repens*) is a widespread, perennial legume that is native to Europe and was introduced to North America within the last 500 years, where it has repeatedly evolved climate-associated clines in cyanogenesis, the ability to produce hydrogen cyanide upon tissue damage. In these clines, higher proportions of cyanogenic plants occur in warmer and more arid climates. Two genes, *Ac* and *Li*, encode the two biochemical components required for cyanogenesis, and both loci have evolved gene deletion alleles, thus creating four possible cyanotypes: *Ac+Li+* (cyanogenic), and *Ac+Li-*, *ac-Li+*, and *ac-Li-* (acyanogenic). Additionally, both *Ac* and *Li* exhibit copy number variation (CNV; from 0-7 copies). We used field experiments, wild population sampling, and genetic analyses to assess the selective and genetic factors that shape cyanogenesis cline evolution. Field experiments were conducted at Washington University's Tyson Research Center, located near St. Louis, MO. In a germination experiment utilizing 218 wild samples of seeds collected from 20 sites across North America, we found that a higher proportion of cyanogenic seedlings survived in a field plot at Tyson vs. greenhouse facilities, resulting in increased frequency of the *Li+* allele in the field at early life stages. Using replicate cuttings from 161 adult genotypes that were sampled across 15 sites from Florida to British Columbia and grown from seed in the Washington University greenhouse facilities, we performed a common garden experiment at Tyson; plants were assessed for herbivory and fitness traits throughout the 2015 field season. We found that cyanogenic plants experienced significantly less herbivore leaf damage than acyanogenic plants. Fitness measurements for these 161 genotypes began on April 11 and are ongoing. Once completed, we will assess relationships between herbivory, fitness, CNVs, and seasonal climatic fluctuations. Lastly, we collected ~50-60 wild samples from each of four climatically distinct North American locations (Gainesville, FL; Ardmore, OK; St. Louis, MO; and Duluth, MN). We found a positive correlation between *Ac* CNV and aridity, as well as a positive correlation between *Li* CNV and minimum winter temperature; these results suggest local climatic adaptation through selection on CNVs. Future work will utilize genome-wide SNP data, population genomics with more extensive wild sampling across North America, and QTL

mapping of fitness traits in reciprocal common gardens to identify SNPs associated with local adaptation and to further assess the adaptive role of CNVs in North American white clover.