



Ecological Genomics Institute

Kansas State University

POSTER ABSTRACTS

1

Diversity of Thermophilic Microorganisms within Hawaiian Fumaroles

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Fumarolic environments provide heat and moisture suitable for thermophilic microorganisms to live, but display environmental gradients responsible for diversity. Fumaroles are scattered across the southeastern portion of the Island of Hawaii as a result of the volcanic activity from Kilauea Crater and Pu'u' O'o vent. Each fumarole differs from the next in terms of its temperature, pH, moisture, and elevation gradients, forming microenvironments that create different microbial communities with respect to the number and diversity of thermophilic organisms. We used metagenomics to detect 16S rDNA from archaeal and bacterial thermophilic microorganisms present in fumaroles. To determine the effects of environmental gradients (including temperature, pH, elevation, and precipitation) on microbial diversity within and among fumaroles, we obtained 22 samples from 7 fumaroles over a three-day period in February of 2007. Elevation differed among fumaroles from sea level to 4,012 ft and precipitation varied from less than 20in/yr to greater than 80in/yr. Temperature variations within individual fumaroles vary from 2.3°C to 35°C and the pH variances range from 0.4 to 2.0. Temperatures of the different fumaroles range from 29.9°C to greater than 105°C, with pH values that range from 2.55 to 6.93. Further data on the microbial diversity within fumaroles and among fumaroles will be determined once the sequencing of the microbial 16S rDNA regions is completed. We are currently assembling and sequencing clone libraries of bacterial and archaeal 16S rDNA fragments from these fumaroles.

2

Genotypic Variation in Soybean Molecular Responses to Elevated Carbon Dioxide Concentration

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A critical step in maximizing crop yield in a future of elevated atmospheric carbon dioxide concentration (CO₂) is identifying genotypic variability in response to elevated CO₂ and understanding the molecular basis for the variation. We compared photosynthesis, leaf metabolites and global gene expression of three soybean cultivars grown at ambient CO₂ (380 ppm) and elevated CO₂ (550 ppm) at the Soybean Free Air Concentration Enrichment (SoyFACE) facility. The cultivars were selected because in previous years of the SoyFACE experiment Clark showed no yield enhancement at elevated CO₂ (+1%), Pioneer 93B15 showed moderate yield enhancement at elevated CO₂ (+13%) and Holt showed a large yield enhancement (+33%). While midday stimulation

of photosynthesis did not differ substantially among cultivars, acclimation of photosynthesis measured as decreased maximum Rubisco activity, was only apparent in Pioneer 93B15 and Holt. All cultivars showed substantial increases in soluble sugars and starch at elevated CO₂. However, there was significant variation among cultivars in investment of carbon in antioxidant metabolites and total antioxidant capacity. The molecular basis for these changes in photosynthetic capacity and antioxidant metabolism are being investigated by expression profiling using Affymetrix gene chips. The aim of this research is to identify physiological, biochemical or molecular targets for improving the performance of soybean cultivars at elevated CO₂.

3

Combining field experiments and genomic analysis yields evidence for local adaptation in Swedish populations of *Arabidopsis thaliana*

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For decades, local adaptation has been studied at the phenotypic level through reciprocal transplant experiments, where the differential survival of individuals is measured in various habitats. However, what is far from being substantiated is the genetic basis of this differential survival. In order to investigate the genomic basis of local adaptation, we crossed *Arabidopsis thaliana* lines from genetically and geographically differentiated populations, planted seeds of parents and their hybrid offspring reciprocally in the field, and tracked temporal changes in allele frequencies at multiple genetic markers. In July 2006, nearly 600,000 total seeds from parents and F₂ offspring of 6 crosses were sown in a block design at three sites each in northern and southern Sweden. Plots of each parental and hybrid type were subsampled for leaf tissue three times throughout a single generation of these annual plants, and seed set was estimated to assess lifetime fitness. Genotyping is currently underway using a panel of 149 SNP markers from across the genome. Preliminary genotype data from a single timepoint (November 2006) that seedling survival of parental lines in southern Sweden tends to be biased towards southern parents. Further, instead of the expected ratio of 50:50 (the proportion at which parental lines were sown), a few lines show a very significant deviation in the direction of southern parental lines. These preliminary results support the hypothesis that native populations in Sweden may be locally adapted, and suggest that natural selection is an important force in maintaining the genetic differentiation between them.

4

Adaptive divergence in host plant use and historical demography in the grasshopper *Hesperotettix viridis*

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The broad distribution and specialized feeding habits of the oligophagous grasshopper *Hesperotettix viridis* present unique opportunities for investigating the process of evolutionary divergence in host plant use by a generalist group of herbivores. Using data from AFLPs and mitochondrial DNA sequences we contrast patterns of adaptive and neutral genetic variation in different host-feeding populations of *H. viridis* to unravel the relative contribution of allopatric/sympatric and neutral/adaptive processes to explain lineage diversification and host race formation. Reciprocal transplant experiments of *H. viridis* populations from the Great Plains indicate that sympatric populations are specializing on different hosts; local adaptation with fitness consequences and observations of assortative mating suggest that ecological divergence of host races is occurring. Using population genomics methods we distinguished neutral loci from loci under divergent selection in contrasting pairs of different host-associated populations. We conducted AFLP analysis of 677 *H. viridis* individuals from 23 populations sampled in 2005-2006 from Kansas, Nebraska, Oklahoma, New Mexico, Colorado, Washington, and Oregon with 240 loci. In 8 pairwise comparisons of *H. viridis* populations feeding on different host plants, we identified significantly more outlier loci (loci with *F_{ST}* values higher than the 0.99 quantile of simulated values generated by assuming neutrality) than in 8 pairwise comparisons of populations that fed on the same host plants. The outlier loci identified in these comparisons had a high degree of identity, suggesting that similar genetic mechanisms were driving divergent selection across a large geographic area. Both AFLP and mitochondrial DNA data suggest a recent common ancestry of alternative host races of *H. viridis*. We sequenced 92 individuals from 25 populations at a 372-bp segment of the COI gene region of mitochondrial DNA. Molecular clock analysis of an mtDNA haplotype network suggests the existence of a single Pleistocene refugium that expanded into the Central Great Plains 71,142 ± 15,918 (SD) years ago. Combining information from the AFLP and mtDNA data sets, we infer that differentiation of host races is a recent, post-Pleistocene phenomenon that cannot be attributed to solely allopatric differentiation, which supports the hypothesis of two-stage speciation in which partial reproductive isolation or ecological divergence formed in allopatry is later reinforced under local sympatric conditions.

5

Genomic analysis of post-mating changes in the honey bee queen

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The molecular mechanisms underlying the post-mating behavioral and physiological transitions undergone by females have not been explored in great detail. Honey bees represent an excellent model system with which to address these questions because they exhibit a range of “mating states,” with two extremes (virgins and egg-laying, mated queens) that differ dramatically in their behavior, pheromone profiles, and physiology. We used an intermediate mating-state in an attempt to understand what underlies the transition from a virgin to a mated, egg-laying queen. We used same-aged virgins, queens that mated once but continued to attempt mating flights, and queens that mated once and initiated egg-laying. Differences in the behavior and physiology between groups correlated with the underlying variance observed in transcript abundance in the brain and the ovaries. These changes were correlated with either a flight-oriented pattern or a mating-oriented pattern. Overall, these results suggest that the brain and the ovaries of queens are uncoupled, allowing the initiation of mating to trigger major changes in the ovaries, but only a small number of changes in the brain. Comparison of our results to previous studies of mating changes in *Drosophila melanogaster* identified common biological processes affected by mating, including stress response and alternative splicing. Furthermore, our data indicates that the genes important in division of labor in worker honey bees are also regulated during the mating process in queens, and support the theory that genes involved in reproduction in queens may have been co-opted for use in division of labor in workers. Studying the underlying molecular mechanisms of post-mating changes in honey bee queens will not only give us insight into how molecular mechanisms regulate physiological and behavioral changes, but may also lead to important insights into the evolution of social behavior.

6

Determining the epigenetic basis for inheritance of trichome density in *Mimulus guttatus* (yellow monkeyflower)

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Trichomes are hair like projections that develop from the plant epidermis, and patterns of trichome development in plants are likely to be ecologically important. Trichomes can deter herbivory, as well as reduce transpiration in dry climates. In the emerging model species *Mimulus guttatus* (yellow monkeyflower) leaf wounding on early leaves can lead to increased trichome density on subsequent leaves. This developmental pattern of increased trichome production can be epigenetically transmitted to progeny, with offspring exhibiting similar post-wounding trichome densities as their wounded parents. We are currently investigating the epigenetic basis for inheritance of trichome patterning in six *M. guttatus* genotypes: four recombinant inbred lines (RILs) and the parental lines that were used to generate these RILs. Using a combined bioinformatic and phylogenetic approach, we have identified orthologs of candidate genes involved in trichome development from the nearly complete *M. guttatus* genome sequence. Several of these candidate genes may exhibit differential expression in the offspring of wounded vs. control plants. To determine the proximate mechanism for epigenetic control, we will determine the methylation state of cis-regulatory sequences associated with each of our candidate genes using a methylation-sensitive PCR approach. By investigating both levels of expression and states of methylation in orthologs of genes known to pattern trichome development, we aim to gain insight into the epigenetic basis for inheritance of trichome patterning in *M. guttatus*.

7

Contrasting the epidemiology of evolutionarily independent strains of rabies in a common host species

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Molecular epidemiological research consists of characterizing patterns of transmission and molecular evolution of pathogens in a single host population. While much has been learned by utilizing this approach, comparative approaches will further our understanding of evolutionary constraints and ecological underpinnings of disease transmission. The rabies-striped skunk (*Mephitis mephitis*) host-pathogen system provides a useful model to study the relationship between host ecology and molecular epidemiology. Two evolutionarily independent strains of rabies occur in striped skunks, one in the northern and the other in the southern Great Plains. The border between the two strains was stable near the Kansas-Nebraska border until 2003, when the southern strain appeared to shift north during an epizootic outbreak in Nebraska. The apparent movement of the southern skunk strain suggests that there was either a rabies void in Nebraska, or the southern skunk strain is actually displacing the northern skunk strain to its current position in northeastern Nebraska. We characterized portions of both the rabies genome and striped skunk genome from brain samples of infected striped skunks collected during the epizootic outbreak. We sampled 29 infected individuals north of the epizootic region, 28

individuals in the epizootic zone, and 29 individuals south of the outbreak. Sampling occurred in a transect from areas where only the northern strain occurred, through the zone of contact, and into the range of the southern strain. Molecular analysis of the rabies genome indicated that the two viral strains exhibited different epidemiological characteristics. The northern strain had higher nucleotide and haplotype diversity than the southern strain, had no pattern of isolation by distance. In contrast, the southern strain had a star-patterned phylogeny characteristic of epizootic outbreaks and similar variants clustered both in evolutionary and geographic space. These contrasting patterns of evolution suggest that the northern strain was a slow moving endemic with limited transmissibility, while the southern strain expanded into Nebraska in periodic outbreaks. Differences in host contact rate and population density often drive disease transmission rates. Analysis of striped skunk population genetics, however, indicated equivalently high levels of gene flow from South Dakota through Nebraska to Kansas ($F_{ST} = 0.01$), suggesting connectivity between Kansas, Nebraska, and South Dakota striped skunk populations. No molecular evidence of demographic perturbations were found in any skunk populations. Results from the host populations suggest that it is not host population genetics that is affecting rabies boundaries and epidemiology, but rather the rabies strains that are interacting differently with their respective striped skunk hosts. With global climate change altering the environment, many diseases are either newly emerging or expanding their ranges. A greater understanding of how viruses utilize genetic variability to alter their transmission rates is crucial for the development of more effective vaccines. This research is especially important considering the rapidly increasing contact between humans and wildlife hosts of potentially zoonotic diseases.

8

Comparing the developmental genetic basis for bilateral flower symmetry between two model species: snapdragon and monkeyflower

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Bilaterally symmetrical flowers are considered adaptive, and thought to have evolved to enhance the utilization of diverse and reliable pollinators through increased specialization. Based on research in the model species snapdragon (*Antirrhinum majus*), we are beginning to understand the developmental network of genetic interactions that establishes bilateral flower symmetry.

Characterization of snapdragon mutants with reduction in, or complete loss of, bilateral flower symmetry has uncovered multiple interacting gene products responsible for establishing the dorso-ventral axis. These include the TCP family transcription factors CYCLOIDEA (CYC) and DICHOTOMA (DICH), and the MYB-type transcription factors RADIALIS (RAD) and DIVARICATA (DIV). CYC, DICH and RAD are necessary for the establishment of dorsal flower identity, whereas DIV gene products specify ventral flower identity. In order to begin understanding the level of conservation of this developmental network among flowering plants, we are utilizing the emerging model species yellow monkeyflower (*Mimulus guttatus*). We have used a combined bioinformatic and phylogenetic approach to recover all putative orthologs of the symmetry genes, CYC/DICH, RAD and DIV from the nearly complete *M. guttatus* genome sequence. We have identified a number of putative orthologs of these symmetry genes that arose from gene duplication events along the lineage leading to *Mimulus*. To generate an understanding of the role these genes play in *Mimulus* flower development, we are using detailed floral dissections and RT-PCR to assay spatial patterns of gene expression. The results of these expression assays should be quite interesting in light of the multiple gene duplication events we have uncovered. We will follow these RT-PCR experiments with more detailed assessments of gene expression patterns using in situ mRNA hybridization.

In addition, we plan to add a functional component to this research by characterizing CYC/DICH, RAD and DIV protein function using a Virus-Induced Gene Silencing reverse genetic approach in *M. guttatus*.

9

Loss of American and Slippery elm genomes due to cryptic invasion of Siberian elm: evidence from AFLPs

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Siberian elm (*Ulmus pumila*) has recently been recognized as an invasive species, and is known to extensively hybridize with slippery elm (*U. rubra*), but not American elm (*U. americana*). While Siberian elms and some American elm cultivars are Dutch elm disease (DED) resistant, most American and slippery elms are not. In this study, we found support for hybridization among all three of these species, and evidence that some DED resistant "American" elms such as the American Liberty elm may be distant backcrosses with Siberian elm. Amplified fragment length polymorphisms (AFLPs) from Missouri populations of the three species of elms, putative hybrids and their offspring, and American Liberty elms were subject to phylogenetic and non-metric multidimensional scaling analyses (NMDS). Parsimony analysis of presence/absence data revealed four clades: one of each species and one of hybrids.

Distance trees show a grouping of Liberty elms with putative Siberian x slippery elm hybrids and their greenhouse-grown offspring. This suggests that Liberty elms are genetically more similar to Siberian elm hybrids than they are to other American elms. NMDS analysis showed tight clusters of each “pure” elm species except American elm; separated from the “pure” species clusters was a loose association of Liberty elms with Siberian elm hybrids. Genetic evidence of extensive cryptic invasion of Siberian elm in Missouri and in DED resistant “American” elms may signify a long-term gradual loss of genetically “pure” native species. Because Siberian elm has been present in North America since the 1860s, its genome may have been integrated into the genomes of native elm populations long before DED, incidentally producing some resistant American elm backcrosses.

10

Hybrid molecules result from small deletions on the CHD-Z intron, but lead to big errors in avian sexing analyses

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The CHD family of genes is a highly conserved region of the genome found in all eukaryotes. This gene family influences chromosome segregation and chromatin structure, and is associated with regulation of gene activation. Additionally, the CHD family of genes has been linked to chick viability in some avian species. The CHD-1 gene is also sex-linked in birds, and ornithologists employ the use of the gene as a universal sexing marker. The use of the CHD gene is widespread across many fields in avian ecology, from studies of sex chromosome evolution to studies in behavioral ecology. We have optimized laboratory protocols for genetic sexing of the monomorphic Upland Sandpiper (*Bartramia longicauda*) using two independent sets of primers to amplify regions of the sex-linked CHD-Z and CHD-W genes. Blood samples were collected in the field, DNA was extracted, and PCR reactions were carried out with primers P2/P8 and 2550F/2718R. We unexpectedly discovered a polymorphism in the region of the CHD-Z intron amplified by the primers P2/P8 which caused 4 males to be misidentified as females (n=90 mated pairs). We have isolated, cloned and sequenced the three CHD-Z alleles in our population. The Z allele is 335 base pairs (bp) in length, while Z' has a 4 bp deletion and Z'' has a separate 5 bp deletion. Homozygous (ZZ) males show one band in agarose gel analysis and are easily differentiated from females (ZW), which show two bands. However, heterozygous (Z'Z'') males show two bands in the same pattern as that for females due to an interaction between the two Z alleles. While the Z' and Z'' fragments are only 330 and 331 bp long, they anneal together during the PCR process and form hybrid molecules that appear as an approximately 400 bp fragment. Our results indicate that the P2/P8 primers can lead to missexing of monomorphic birds and polymorphisms may be difficult to detect if they occur at low frequency within a population. Errors of this sort are the opposite of what has occurred in the past and could lead to significant errors in studies of population biology and behavioral ecology of birds.

11

Population dynamics and Higher-Level Phylogenetics in the Systematic placement of *H. puera* in order Lepidoptera

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Hyblaea puera is a major pest moth which invades the teak plantations in tropics. It qualifies the name teak defoliator by causing complete defoliation of teak in the early growing season. Teak defoliator outbreaks are regular annual feature in teak plantations in Nilambur, Kerala. It is difficult to predict the exact time and place of these outbreaks. Evidence gathered from the past decade on the population dynamics of *H. puera* indicates habitual, short range movements of emerging moth populations, suggesting that these spread to larger areas, generation after generation, affecting entire teak plantations. In spite of a century of accumulated data on the biology and ecological relationships of the teak defoliator, our knowledge regarding the population dynamics is inadequate. In order to understand the genetic structure and population dynamics of this moth, we devised a novel marker system which has the power to discriminate the individuals from a given population. We coined the name as Random Amplified Genome Encoding Primer (RAGEP) since random amplification was done by employing gene specific primers at lower stringency. The reproducibility, species-specificity, heritability, stringency of the RAGEP marker system was demonstrated. We also generate species-specific tags to further characterise the RAGEP marker system. Our results provide authenticity to accept the novel molecular tool as a marker system to discriminate both inter and intra insect population. Using both mitochondrial and nuclear RAGEP's we were able to address that the endemic insects were not involved in causing major outbreaks.

H. puera is a type genus that represents the family Hyblaeidae and superfamily Hyblaeoidea. *H. puera* was first described in 1794, and was originally included in the family Noctuidae (Hampson, 1894). In 1984 Fletcher and Nye placed the family Hyblaeidae along with the superfamily Pyraloidea based on the morphological characters, currently the family got its own superfamily status Hyblaeoidea.

However, the position of this family is currently uncertain within the group Obectomera. We provide the molecular data for the Hyblaeoidea to support the morphological understanding. The relationships between the smaller superfamilies like Hyblaeoidea with highly diverse superfamilies were still in debate. We try to understand the phylogenetic utility mitochondrial genes in orders level in the class Insecta. We further confirmed our preliminary observation with different dataset at family level. We are able to resolve Hyblaeidae family in lepidopteran cluster using two promising nuclear genes along with the mitochondrial genes. We observed that

superfamily Hyblaeoidea clusters with Pyraloidea with larger dataset in class Insecta. We further analysed the family level clade also confirms that the proximity of Hyblaeidae was with superfamily Pyraloidea in the lepidopteran cluster. We also prove that our phylogenetic data goes with the multivariate statistical analysis in Euclidean space.

12

Prairie soil bacteria induce expression of *C. elegans* genes involved in fitness and innate immunity

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Free-living soil nematode communities at the Konza Prairie Biological Station near Manhattan, Kansas, show taxa specific (genus level or below) differential responses to disturbances including nutrient enrichment by nitrogen addition. These nematode responses could either be due to direct effects of changes in soil chemistry resulting from perturbations, or indirect effects where the bacterial community responds first and effects are seen in the next trophic level, the bacterivorous nematodes. Our recent data show that bacterial communities on Konza prairie respond to disturbance, suggesting indirect effects may play a role in shaping nematode community structure. As we are interested in the genes that may be responsible for community changes, we are using the free-living soil nematode *Caenorhabditis elegans* to model responses of native soil nematodes found at Konza prairie. *C. elegans* has been cultured in the lab for over four decades on agar plates feeding almost exclusively on *E. coli* OP50, which served as their source of nutrition as well as their immediate environment. In order to model a more natural environment for a soil nematode in the lab, we isolated native soil bacteria from Konza Prairie, two of which were isolated in association with native soil nematodes (*Oscheius* sp. and *Pellioditis* sp.).

Microarray analyses identified 204 genes that were differentially expressed by *C. elegans* in response to the different bacterial environments. Loss-of-function mutants were available for 21 of the identified genes, which allowed us to test their contributions to fitness in each bacterial environment. We found specific, significant correlations between expression levels and life history data of mutants affecting multiple genes. Furthermore, a mutant phenotype had not been reported for three of these genes, demonstrating that assessment of *C. elegans* gene functions in more natural environments allows new gene functions to be determined. Interestingly, genes implicated in innate immune response were over represented in the genes identified. We used pathogenicity assays to identify new susceptibility and resistance genes required for defense against different types of native bacteria with the majority of genes found to significantly effect survival. This defense response was not specific to *C. elegans* as we have found that the native bacteria induced a variety of pathogen-associated effects in native nematode taxa. Overall our results suggest that soil nematode fitness in a given natural environment in part depends upon the regulation of metabolic and defense functions that modulate trophic and pathogenic interactions with bacteria.

13

Molecular evolution of cuticle-protein gene families in the malaria vector *Anopheles gambiae* and other insects

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Insect cuticle serves as an environmental interface and is the scaffold for a diverse array of structures that cover and ornament each developmental stage. Cuticle is composed primarily of chitin and numerous structural proteins. Nonetheless, it was unexpected to learn that mosquitoes devote well over 1% of their genes to those coding for cuticular proteins. Why are there so many genes and what functions do the proteins serve? Answers to these questions are coming from our comparative analysis of cuticle-protein gene families, especially the CPR family, in three mosquito species (*Anopheles gambiae*, *Aedes aegypti* and *Culex pipiens*). These mosquitoes have over 50% more CPR genes than *Drosophila*, an increase that is due primarily to the amplification of sets of highly similar paralogs, or 'sequence clusters', that are co-expressed. Patterns of nucleotide variation indicate that extensive concerted evolution is occurring within these sequence clusters, despite the frequently complex organization of these genes within larger tandem arrays. Linked single-copy genes have conserved one-to-one orthologs in other species, whereas sequence clusters vary in gene number and show a strong pattern of reciprocally monophyly. The parallel evolution within different sequence clusters of compact gene architectures and the dearth of pseudogenes despite their age suggest selection for increased production of gene products. We have found that sequence-cluster proteins in the CPR family have complex repeats and extreme amino-acid compositions relative to single-copy CPR proteins in *An. gambiae*. These features appear to be selectively maintained and suggest a functional basis for the preferential amplification of these genes. The molecular mechanisms that have facilitated this unusual and recurrent pattern of molecular evolution in mosquitoes, but not to our knowledge in other insect species, remain to be determined. Further comparative and functional analyses should provide important insights into the ecological significance of cuticle diversification.

14

Transposable elements are profuse and variable in a bacterial symbiont of weevils

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Insertion sequence (IS) elements are the most common transposable elements in bacterial genomes. Although ISs are rare in free-living species, they often proliferate after bacteria transition to an intracellular lifestyle. ISs are an integral source of genomic instability and could substantially contribute to the microevolution of intracellular pathogens and mutualists. However, little is known about the level of IS-mediated genomic variation within and among populations of intracellular bacteria, especially young obligate endosymbionts. Therefore, our goal was to assess the intraspecific IS load variation in the maize weevil endosymbiont, SZPE (*Sitophilus zeamais* primary endosymbiont), which harbors thousands of IS256 and IS903 copies. We collected weevils from four populations across the United States (KS, NE, IN, and PA), and then used qPCR to estimate the IS256 and IS903 quantities within individual weevils. We found that IS256 and IS903 both exhibit >10-fold copy number variation among weevils, and that the PA population harbors significantly more IS256 and IS903 elements than the other populations. Because multicopy IS elements can be loci for intragenomic recombination and deletion, this IS load variation within and among SZPE populations could lead to substantial intraspecific variation in the metabolic capabilities of this obligate symbiont.

15

Biomass for Biofuels: unraveling the genetics of plant architecture in panicoid prairie grasses

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Branching in grasses determines overall biomass, and is thus an important factor in biofuels production. It is under genetic control yet strongly influenced by environmental factors such as shading and external damage. We are studying control of branching in foxtail millet, a panicoid grass that will be sequenced next year by the Department of Energy Joint Genome Institute. Foxtail millet is a model system for switch grass, being closely related, diploid (switchgrass is polyploid), and having a small genome close in size to rice. One of the issues for switchgrass when it is grown as a monoculture for biofuels production will its response to shading, and we have been studying the genetics of branching and shading in foxtail millet in order to gain insight into these phenomena. Quantitative trait loci (QTL) studies show that basal branching (tillering) and branching along the stems are under largely separate genetic control, and that the most likely candidate genes that can be identified from maize and sorghum, such as *teosinte branched1* and *barren stalk1*, may only control tillering. Axillary branching appears to be largely controlled by as yet unidentified genes, the identification of which will be a primary target after the genome has been sequenced. Tillering responds in a predictable manner with shading: a greater degree of shading reduces the number of tillers produced. However, axillary branching shows pronounced gene by environment interactions, suggesting that it is under complex genetic control. We expect that insights gained in foxtail millet will be directly applicable to switchgrass, and that understanding the genetic and environmental control of branching will result in improved strategies for biomass production.

16

The Genetic Basis of Shoot Architectural Variation in Arabidopsis

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Plant shoot architecture, in particular the arrangement of branches on a plant, is one of the most diverse morphological characteristics across plant species. Variation in shoot branching both within and across species may reflect historical adaptation to different environmental conditions (e.g., grazing or wind) or reproductive requirements (e.g., pollen or seed dispersal). Relatively little is known about the molecular mechanisms responsible for evolutionary changes in shoot architecture. We use *Arabidopsis* as a model for shoot evolution and attempt to identify quantitative trait genes (QTGs) for intraspecific variation in shoot branching through a combination of network-scale candidate gene association mapping and linkage mapping. Based on our results, three genes – MORE AXILLARY GROWTH 2 (MAX2), MORE AXILLARY GROWTH 3 (MAX3), and SUPERSHOOT 1 (SPS1) – are strong candidate QTGs. In an attempt to validate and molecularly characterize a shoot branching QTG, we have resequenced an extensive amount of the MAX2 genomic region from 24 accessions and have created MAX2 Nearly Isogenic Lines (NILs) of 11 different natural haplotypes of MAX2 in a common genetic background. Phenotypic and gene expression experiments with the MAX2 NILs are ongoing and will provide insight into if and how MAX2 alleles cause natural variation in shoot branching.

Messages in a Bottle: A Genomics Approach to Investigating Principles of Ecological Stoichiometry in a Self-Sustaining, Enclosed Ecosphere

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Ecological stoichiometry is the study of the relationships among chemical elements and energy balance in biological systems, drawing understanding from principles of molecular and cell biology, physiology, ecology and evolution. The concept encompasses a main objective of physiological genomics, which is to bridge the gap between an organism's genotype and an observed, functional phenotype, and it can address large-scale processes of energy flow among biological compartments within ecosystems. Studies of this breadth require a systems-level approach to address the diversity of processes and mechanisms across multiple scales of biological organization.

Here, we describe our experimental genomic approach to study ecological stoichiometry in a simplified, self-sustaining closed microcosm based on trophic interactions among microbes, microalgae and the small (< 1.5 cm), long-lived atyid shrimp, *Halocaridina rubra*. This system represents a subset of the interactions possible within the shrimp's natural anchialine habitat of the Hawaiian Archipelago and can be supported in hermetically sealed containers without intervention in excess of 15 years. Prior studies of *H. rubra* mitochondrial DNA as well as behavioral and field observations indicate that genetically and ecologically distinct lineages exist.

Currently, we are capitalizing on the ability to maintain distinct lineages under laboratory conditions to provide comparative insight to stoichiometry by comparing bacterial population structure and shrimp physiology among populations and under different nitrogen and phosphorus input regimes. Bacterial population structure is being evaluated by 16S-based automated ribosomal intergenic spacer analysis (ARISA) and restriction fragment length polymorphism (RFLP). Shrimp physiology is being evaluated at whole animal and mitochondrial respiration levels, as well at the transcriptional level by quantifying expression of the mitochondrial genome. Although differences between lineages are not yet apparent (< 1 month of sampling), our preliminary data indicate selective grazing by shrimp on the microbiota and exceptionally low respiratory activity in *H. rubra* for a crustacean of this size and activity level.

Female meiotic drive maintains deleterious polymorphism for male fitness in the yellow monkeyflower, *Mimulus guttatus*

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Evolutionary theory predicts the efficient removal of deleterious genetic variation from populations by natural selection, particularly stabilizing and directional selection. However, most plant and animal populations harbor abundant variation for fitness-related traits. Models invoking only a balance between mutation and selection against deleterious alleles generally fail to explain this paradox. One alternative, which has received little attention in this context, is meiotic drive caused by non-Mendelian chromosomal transmission through asymmetric female meiosis (female meiotic drive). Female meiotic drive is predicted to be a ubiquitous force in higher plants and animals and may necessarily impair male gametogenesis, but almost nothing is known about its consequences in natural populations. Here, we show that selfish evolution at a female meiotic drive locus first identified in interspecific monkeyflower (*Mimulus*) hybrids maintains deleterious variation for male fertility in the outcrossing yellow monkeyflower, *M. guttatus*. Molecular evidence demonstrates a recent partial selective sweep (to >30% of the population) by a driving haplotype (D), consistent with an observed 60:40 transmission advantage over alternative haplotypes through female meiosis. However, the D haplotype also causes severe inbreeding depression of pollen fertility. Thus, antagonism between a selfish chromosomal transmission advantage and male fitness costs maintains polymorphism with unconditionally deleterious effects on individual male fitness and population genetic load. Our results establish a novel and powerful system for understanding selfish chromosome evolution and show that chromosomal drive can be an important contributor to the dynamics of deleterious variation, a central issue in the maintenance of sex, the diversification of mating systems, and the evolution of human disease.

19

Rust and Drought Effects on Gene Expression and Phytohormone Concentration in the Dominant Species of Tallgrass Prairie

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Studies of genome-wide gene expression in wild plant species exposed to multiple stressors are rare. Our objectives were to determine the effects of drought and rust stress on gene expression in *Andropogon gerardii*, the dominant grass in tallgrass prairie, and associated levels of phytohormone production. In a factorial design, plants experienced drought or non-drought conditions and were inoculated with the rust pathogen *Puccinia andropogonis* or not. Gene expression was evaluated using maize microarrays. We have found that plants undergoing drought stress exhibited significantly decreased expression of genes associated with photosynthesis and a hypersensitive reaction, while genes associated with dehydrins and heat shock proteins exhibited increased expression. Ongoing analyses compare the effects of drought and rust. Preliminary results indicate an increase in phytohormone production when both stresses are present compared to a single stress for many phytohormones. This study will help to clarify plant responses to multiple stresses, and so contribute to a better understanding of adaptive strategies and trade-offs in both natural and agricultural systems.

20

Genomic analysis of reversible neurotoxicity in the earthworm *Eisenia fetida* using a custom-designed, genome-wide high density oligo probe array

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The earthworm *Eisenia fetida* is one of the most used species in standardized soil ecotoxicity tests. Endpoints such as survival, growth and reproduction are ecologically relevant but provide little mechanistic insight into the toxicity pathways, especially at the molecular level. To better understand toxicological modes of action and to facilitate the development of molecular biomarkers, we have obtained 30,245 unique EST sequences from *E. fetida* and have designed a novel microarray with 15,119 60-mer oligonucleotide probes. These probes target the unique non-redundant EST sequences identified in *E. fetida*. Using this array we have profiled gene expression of *E. fetida* after exposure to CL-20, a cage cyclic nitramine previously found exhibiting reversible neurotoxicity to worms. Worms were exposed for 6 days to CL-20. Half of the exposed worms were allowed to recover in a clean environment for 7 days.

Electrophysiological analysis showed that the conduction velocity of worm medial giant nerve fiber was significantly decreased after 6-d exposure to CL-20, and that giant nerve fiber function was restored at the end of the 7-d recovery period. Total RNA samples isolated from four treatment groups (6 replicates per group), i.e., 6-d control, 6-d exposed, 13-d control and 6-d exposed with 7-d recovery, were analyzed using the new 15K oligo array. Bioinformatics and statistical analyses have identified specific neurological pathways affected by CL-20 and recovery of these pathways after CL-20 removal. These results provide significant insights on the CL-20 toxic mode of action and how earthworms can recover from chemical stressors.

21

Universal Patterns of Environmental Adaptation in Microbial Genomes

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Adaptations in organisms for specific and diverse environments are encoded within their genomes. As thousands and thousands of microbial genome sequences become available new tools are needed to uncover patterns of evolution, to better understand the ecology of organisms and in general, to perform better comparative genomic analysis. We have quantified the complexity of amino acid usage in 500 genomes. Information content, a function of the diversity and frequency of amino acid motifs in genomes, varies widely in organisms but exhibits statistically significant trends. These data can be exploited in the comparative analysis of genomes, in the analysis of environmental genomes (metagenomes) and in better understanding the evolution of phenotype diversity. Quantifiable measures of selection exist within microbial genomes such that their optimal growth temperatures, oxygen requirement and whether or not they are free-living organisms or host-dependent can be deduced. These analyses can be exploited to better understand microbial ecology and microbial diversity. More specifically, individual genes experiencing the greatest purifying selection can be identified. We identify motifs that are persistent in a genome under certain temperatures of optimal growth, are indicative of adaptation to high pressure and characterize specific environments.

Comparative Metagenomics of Microbial Communities from Pristine and Contaminated Groundwater

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Microbial community DNA isolated from contaminated groundwater located at the US Dept. of Energy Field Research Center (FRC) in Oak Ridge, TN, was analyzed to determine the effects of chronic exposure to contaminants on microbial community structure. The sample was obtained from a site (FW106) experiencing long-term exposure to high levels of uranium and other heavy metals, nitric acid and organic solvents. Analysis indicates a very low species diversity community (~13 OTU) dominated by denitrifying γ - and β -proteobacteria. Metabolic reconstruction of the dominant γ -proteobacterial species reveal adaptations for specific geochemical stresses including the following: denitrification pathways; pathways for degradation of organic compounds such as 1,2-dichloroethene, acetone, butanol, methanol and formaldehyde; accumulation of multiple heavy metal efflux systems (czcABC, czcD, cadA, merACPT, etc.). Analysis indicates that lateral gene transfer is the predominant mechanism of introducing genetic variation in the community, resulting in the lateral acquisition of, for example, an acetone carboxylation pathway and heavy metal efflux systems. The sample was compared to a second groundwater metagenome from a pristine FRC site (FW301) to determine differences between the two communities. In contrast to the low species diversity of FW106, the FW301 community is represented by multiple phyla including all 5 classes of proteobacteria, Planctomycetes, Chloroflexi, Actinobacteria, Acidobacteria, Bacteroidetes and Firmicutes. In contrast to the FW106 sample which resulted in significant read assembly, the FW301 sample is composed largely of single reads that do not assemble into contigs (95%). Abundance profiling of geochemical and cytochrome genes between FW106 and FW301 and between FW106 and the acid mine drainage (AMD) metagenome show distinct environmental signatures between the samples. This analysis verified the previous observation of accumulation of heavy metal and other toxin efflux mechanisms in FW106 as well as an accumulation of specific c-type cytochromes in FW106 that may be important in heavy metal resistance.

Gene x environment interactions and human behavior

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A growing body of research suggests that risk for psychiatric disorders is mediated by gene x environment interactions, frequently implicating a functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR). The purpose of this study was to investigate how genotype influences the associations among environmental stressors and anxiety. A sample of 156 college students was characterized for neurotransmitter genotypes, stress, and behavioral outcomes. Early life stress (i.e. before age 13) was significantly associated ($p=0.015$) with increased anxiety levels in males homozygous for the 5-HTTLPR short (S) allele. No association between 5-HTTLPR genotype and anxiety was found with stress experienced after age 13. It may be that a stressful rearing environment engages a defense strategy, priming the individual for anxiety and a subsequent withdrawal from danger. Conversely, evidence from other studies suggests that the S allele protects against negative behavioral outcomes in a highly supportive rearing environment. Variation in allele frequencies among populations with divergent cultural norms further supports an S allele driven mechanism of adaptive phenotypic plasticity regarding serotonin function, early childhood experiences, and behavioral state.

Plant Adaptation to Elevated Carbon Dioxide: Using a Lipidomic Approach to Identify Alterations in Lipid Metabolism and Signaling in Arabidopsis Thaliana

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From the beginning of the industrial revolution, anthropogenic activities have caused the concentration of atmospheric CO₂ to increase from about 270 parts per million (ppm) to over 370 ppm today. Current estimates suggest that atmospheric CO₂ concentration will reach 550 ppm by the year 2050 and over 700 ppm by the end of the 21st century. Preliminary data indicate that lipids are altered in response to CO₂. Lipid alterations in response to CO₂ may result directly from altered flux of carbon into lipid synthesis, some lipid alterations may be the result of gene expression changes, and some alterations in lipids may in themselves be regulatory. The objective of our work is to study the impact of elevated CO₂ level on the levels of phospholipids and galactolipids in Arabidopsis thaliana plants from varying locations and in a laboratory-selected genotype. Phospholipids and galactolipids from fifteen Arabidopsis thaliana

genotypes grown at 380 ppm and at 700 ppm are being profiled by automated direct infusion electrospray triple quadrupole mass spectrometry. The data will be analyzed using multivariate techniques to determine the effects of CO₂ levels, on lipid molecular species, as well as on gene expression, phenotypic, and physiological data. Multivariate statistical analysis will also help establish relationships between lipid molecular species and the other measured parameters in order to better understand the biological processes underlying the adaptation of plants to elevated CO₂.

25

Dunaliella as a Model of Eukaryotic Microbial Dispersal, Diversification and Biogeography

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The halophilic alga *Dunaliella* is an ideal model for global biogeography of eukaryotic microbes. Most previous global *Dunaliella* isolates are from perennial aquatic habitats. In contrast, *Dunaliella* from the Great Salt Plains (GSP) of Oklahoma and the Nevada/Utah Great Basin are from terrestrial hypersaline habitats. Based on four gene sequences, the latter are more diverse and form a monophyletic clade exclusive of most previous isolates. Some morphological and physiological differences were also evident. The greater diversity may be explained in part by more extensive sampling at the GSP; the separate clade is probably explained by habitat factors (terrestrial vs. aquatic). We speculate that stressful and hypervariable terrestrial saline habitats enhance mutation rates and spatial/temporal niches, promoting adaptive radiation compared to more stable hypersaline aquatic habitats. Present evidence is inadequate to assess geographic barriers to dispersal. We plan a broad geographic sampling of *Dunaliella* from aquatic and terrestrial saline habitats, using cultured isolates and direct environmental sequencing, to address the roles of geographic distance and habitat in microbial phylogeography. Supported by NSF grants MCB-0132097 and 0132083.

26

Combining studies of gene expression and protein function to determine the role of symmetry genes in the evolution of stamen number

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Bilaterally symmetrical flowers have evolved multiple times across angiosperms, and evolutionary transitions from radial to bilateral symmetry are considered to have enhanced the utilization of diverse and reliable pollinators through increased specialization.

Lamiales s.l. is a lineage of flowering plants that is a focal point for evolutionary, ecological and developmental genetic research on the causes and consequences of floral symmetry. Nearly all species of Lamiales develop bilaterally symmetrical flowers with 5 stamens. However, Lamiales forms a large and diverse lineage, and there have been multiple evolutionary modifications to this bilateral floral plan. For example, replacement of the 5th, dorsal stamen with a residual staminode is a common phenotype in Lamiales. Additionally, there have been multiple evolutionary reversals to a 5-stamen condition, as well as continued stamen loss through reduction of one of the two remaining stamen pairs. Multiple evolutionary shifts in stamen number have occurred in the Veronicaceae (Lamiales), the lineage to which the model species, snapdragon (*Antirrhinum majus*), belongs. Specifically, within Veronicaceae there have been independent evolutionary reductions in stamen number (from four to two fertile stamens) in the lineages *Mohavea*, *Gratiola* and *Veronica*. Dorsal flower identity genes, identified through developmental genetic work in the model species snapdragon, are good candidates for explaining these transitions in stamen number. These include the dorsal flower identity gene *CYCLOIDEA* (*CYC*), which is necessary for establishing dorsal flower identity, including dorsal stamen abortion, in snapdragon. We have previously shown that an evolutionary shift in *CYC* expression is correlated with stamen number evolution in *Mohavea*.

Currently, we are using both expression (semi-quantitative RT-PCR and in situ mRNA hybridization) and functional (virus-induced gene silencing, VIGS) analyses, to determine the specific role that *CYC*-like genes have played in shaping variation in stamen number in *Mohavea*, *Veronica* and *Gratiola*. Ultimately, by focusing on taxa exhibiting evolutionary innovations in floral organ number, we aim to determine how changes in the expression and/or function of genes controlling bilateral flower symmetry have contributed to phenotypic novelties.

Molecular analysis of *Metarhizium anisopliae* isolates from Thailand

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Metarhizium anisopliae is a mitosporic entomopathogenic fungus that has been exploited extensively as biological control agent (BCA) against several pests. *Metarhizium anisopliae* isolates from several insect hosts and from various sugar cane growing areas of Thailand, were examined for genetic diversity using polymerase chain reaction (PCR)-based technology, involving amplified fragment length polymorphism (AFLP) was used to assess the genomic variability between 4 isolates of *Metarhizium* spp strains. Amplified fragment length polymorphism (AFLP) analysis of entomopathogenic fungus evidence provides a means of obtaining a reproducible DNA profile in a relatively short period of time in species for which no sequence information is available. Genomic DNA from mycelium of each strain was optimised and the use of cetyltrimethyl ammonium bromide (CTAB) and sodium chloride (NaCl) was incorporated. All strains could be typed in these conditions. DNA were double-digested by two restriction endonucleases (EcoRI and MseI) and ligated to oligonucleotide adapters. Two consecutive PCR reactions (pre-amplification and selective amplification) were performed using a modification of the AFLP protocol described by Gibco (Invitrogen, Rockville, MD). The DNA fragments were separated by electrophoresis using silver staining for band visualisation. Based on 23 AFLP primer combinations, a total of 1504 bands were detected. An average of approx. 65 bands were scored for each primer pair. Among of which 3 polymorphic fragments (obtained from E-AGG/M-CAA, E-AGG/M-CAA, E-AGG/M-CAA) were identified as potentially a strain specific. DNA fragments of between 0.26 and 0.38 kp were obtained. These markers have practical utility for (1) establishing conspiracy in the cultivation and distribution of *Metarhizium* sp (2) identifying geographic sources. The results also suggest that AFLP markers may be useful for the tracking of specific biocontrol strains in the field.

Ecotoxicogenomics of Fecal Bacteria of Deer Mice (*Peromyscus maniculatus*) in a Former Heavy Metal Mine

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More than 100 years of mining contaminated 2500 mi² of land and water with heavy metals in southeast Kansas. Communities have been remediated for lead, zinc and cadmium contamination to within federal standards of safety but lingering contamination is great enough to adversely affect wildlife communities. Previous studies in this area have shown less diverse and less thrifty small mammal communities at remediated sites than reference sites, and associated gastrointestinal microflora also exhibited reduced diversity and altered community composition. Having demonstrated that the environmental contamination has affected biodiversity of both mammal and microbial communities, we now turn our attention to the impacts of contamination at a genomic level. Of increasing concern is the observation that heavy metal resistance in microfloral communities is linked to increased antibiotic resistance. We studied bacterial genes responsible for heavy metal and antibiotic resistance in intestinal microbial communities of *Peromyscus maniculatus* to determine the ability of contaminated landscapes to alter the microbial community gene pool. We collected fecal samples from 5 *P. maniculatus* at 4 sites: 2 remediated sites in Galena, KS, and 2 reference sites never exposed to heavy metals in Neosho Wildlife Area in St. Paul, KS. We hypothesized that the presence of heavy metals at the remediated site would select for a direct increase in heavy metal resistance genes and a subsequent increase in antibiotic resistance genes in gastrointestinal bacteria when compared to the control site, as measured by DNA analysis. DNA extracted from fecal samples was analyzed using microarray and quantitative PCR. The microarray chip and PCR primers were created using known sequences in bacterial genomes for traits such as ampicillin, tetracycline, and cadmium resistance. We found increased presence of both heavy metal and antibiotic resistance genes at remediated sites than at reference sites, suggesting that selection pressure for these traits is occurring in remediated areas. These results suggest that remediation was not sufficient, and may be a catalyst for increased antibiotic resistance within remediated sites. Such an outcome would have serious implications for human health and biomedicine.

Acclimation of soybean to growth at elevated CO₂ under field conditions is dominated by transcriptional up-regulation of carbohydrate metabolism and respiration not down-regulation of photosynthesis

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The impacts of growth at elevated CO₂ on plant performance are recognized to form a continuum from instantaneous, direct responses (e.g. altered Rubisco kinetics) to slower, indirect responses (e.g. reduced stomatal conductance lowering water use and ameliorating drought stress) and acclimation (e.g. reduced expression of Rubisco and reallocation of N to other processes). Acclimation of

photosynthesis to elevated CO₂ has been investigated most thoroughly because: (1) it has a significant impact on the carbon and nitrogen budgets of plants, and (2) it can be studied by targeted molecular and biochemical analysis of photosynthetic enzymes, in conjunction with routine and non-destructive measurements of leaf gas exchange. Other elements of plant function are also likely to acclimate to growth at elevated CO₂ but have traditionally proven more difficult to detect. Microarrays quantify changes in transcript abundance for most, or all, of the genes in the genomes of a number of model species. When transcript profiles in tissue from plants that have grown their entire lifetimes at ambient CO₂ and elevated CO₂ are compared it allows examination of nearly all known metabolic pathways and signaling cascades for evidence of acclimation. This is a uniquely comprehensive analysis of how plants will adjust to optimize their performance under future growing conditions. To better understand plant responses to elevated CO₂ we have combined genomic, biochemical, physiological and ecological investigation of soybean grown in the field at the SOYbean Free-Air Concentration Enrichment (SOYFACE) facility at the University of Illinois. Soybean was grown in four plots at ambient CO₂ (~380 ppm) and four plots at elevated CO₂ (~550 ppm), from sowing until harvest over two growing seasons. This provided a model system, where low genetic and environmental variability between experimental units increased the ability to detect subtle treatment effects. This study tested the hypothesis that soybean grown at elevated CO₂ under field conditions will undergo acclimation that results in greater rates of foliar respiration, in accordance with the greater supply of carbohydrate substrate from enhanced photosynthesis that has been previously observed at the SoyFACE facility. There were 745 transcripts whose abundance was significantly altered by elevated CO₂ in both growing seasons. The principal molecular response of soybean to elevated CO₂ was increased gene expression for many components of respiratory metabolism, including glycolysis, the TCA cycle and mitochondrial electron transport. These molecular responses were reflected in greater pool sizes of key carbon metabolites and greater rates of respiratory oxygen uptake and carbon efflux. The integrated genomic, biochemical and physiological responses provide unique evidence for previously unrecognized acclimation to growth at elevated CO₂. Greater respiration will partially offset the stimulation of carbon gain by elevated CO₂ at whole-plant and ecosystem scales, while also generating additional energy and carbon-skeletons for growth and maintenance.

30

An ecological genomic analysis of species formation in the yellow monkeyflower, *Mimulus guttatus*

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Understanding the mechanisms of species formation is a major goal in evolutionary biology. While there has been much recent work on the determination of genetic mechanisms of intrinsic postzygotic incompatibilities, the genetic basis of ecological reproductive isolating barriers is still largely unknown. Here, we present our research on the genetic basis of species formation through the examination of the coast and inland ecogeographic races of the yellow monkeyflower, *Mimulus guttatus*. We demonstrate that the coast and inland populations of *M. guttatus* constitute two genetically distinct groups through population structure analysis. We then show that adaptations to seasonal drought and ocean salt spray contribute to near complete reproductive isolation between these ecogeographic races using reciprocal transplant field experiments. We determine the genomic architecture of adaptation to these abiotic stresses through Quantitative Trait Loci (QTL) mapping of salt tolerance and flowering time (drought escape). We then establish that the same genomic regions are responsible for flowering time differences between coast and inland populations over a large geographic range (>1200 km), through replicated QTL analysis. Finally, we integrate flowering time and salt tolerance QTL mapping results with the recently assembled *Mimulus* genome to localize candidate genes for adaptation and reproductive isolation.

31

Multi-locus Dobzhansky-Muller interactions cause partial reproductive isolation in an interpopulation cross in the moss *Ceratodon purpureus*

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Post-zygotic reproductive isolation results from the failure of two genomes to function properly when brought together in a hybrid genetic background. Intrinsic hybrid incompatibility may result from Dobzhansky-Muller (DM) incompatibilities, in which improper interactions among gene products from two or more loci leads to developmental problems, or chromosomal rearrangements. These mechanisms can be distinguished by studying the allelic effects of chromosomal regions associated with intrinsic isolation. Here we examine the genomic basis of hybrid breakdown between two geographically distant populations of the moss *Ceratodon purpureus*. Although molecular population data indicated these populations exchange migrants, approximately half of the hybrid progeny exhibited a severely reduced growth rate in early gametophyte development. We identified four unlinked quantitative trait loci (QTLs) that interacted asymmetrically to cause the hybrid breakdown, consistent with predictions based on the DM model. No relationship was found between marker segregation distortion and markers linked to hybrid incompatibility QTLs, suggesting that loci other than those associated with the hybrid breakdown phenotype caused additional hybrid lethality. These observations suggest that complex DM incompatibilities may evolve among populations that are connected by gene flow. Finally, although *C. purpureus* has large, heteromorphic sex chromosomes, the genetic basis of hybrid breakdown was almost entirely autosomal, a significant departure from

studies of hybrid breakdown in animals. We discuss genetic hypotheses to explain this difference, and suggest that bryophytes provide an excellent model system to study the role of sex chromosomes in population divergence.

32

Molecular Evolution of the DREB/CBF transcription factor sub-family in Arabidopsis

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Within species, differences in drought adaptation, including traits involved in stress tolerance and physiology, have been shown to be due to both constitutive and induced differences. Ultimately variation in traits showing constitutive or induced differences among genotypes is due to underlying allelic variation at some loci. Recent expression profiling studies are complementing the extensive data on more traditional phenotypic trait variation with data on within-species variation in gene expression. These studies have revealed that there is variation in the trait of expression level for thousands of loci throughout the genome.

Obvious candidate loci for controlling variation in gene expression are the transcription factor genes. Analysis of the Columbia sequence of *Arabidopsis thaliana* shows that 7,648 loci (25% of all annotated loci in the genome) contain the core DRE (Dehydration Responsive Element) binding site in the promoter region. Here I report on polymorphism and divergence within 57 loci that are part of the DREB/CBF transcription factor family in *A. thaliana*. Data on sequence variation in the DREB transcription factor loci among a diverse sample of 24 accessions of *A. thaliana* show there are some that show no variation in the coding sequence, while other loci harbor amino acid variation and suggest balancing selection. First I report on divergence, analyses to look for adaptive protein evolution between specific DREB family members in the species *A. thaliana* and *A. lyrata*. Second, I report on polymorphism, looking for adaptive evolution, or changes in functional constraint, among closely related DREBs within *A. thaliana*. I am also investigating whether this variation correlates with geography and drought-resistance phenotypes.

33

Ecology of environmental *Enterococcus faecalis*: Characterization of the *fsrABC* operon and antibiotic resistance and virulence determinants

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It is well established that antibiotic resistance and virulence are important in the infection processes, but very little is known about the significance of these determinants in fitness of bacteria in non-clinical environments. Bacteria commonly possess sensory-regulatory systems that respond to environmental cues and control expression of antibiotic resistance and virulence determinants. We investigated the prevalence and expression of antibiotic resistance and virulence genes as well as polymorphism of the *fsrA,B,C/gelE-sprE* operon, a two-component quorum-sensing regulatory system of *Enterococcus faecalis* involved in virulence and biofilm formation via gelatinase (GelE). Enterococci are common in the digestive tract of people and animals as well as various environments such as soil, water, and plants; however, some strains have become important human nosocomial pathogens.

In this study, we assessed the diversity of *E. faecalis* isolated from five environmental sources: swine manure, ready-to-eat food, and house flies collected from swine and cattle farms and restaurants. All isolates were genotyped by pulsed-field gel electrophoresis (PFGE) using the whole chromosomal DNA digested with AluI and the diversity was calculated using Dice indices and UPGMA. The prevalence and distribution of virulence determinants (*gelE*, *esp*, *asa1*, *cylA*) as well as antibiotic resistance determinants to tetracycline (*tetM,A,C,Q,S,O,K,W*) and erythromycin (*ermB*) were correlated to the whole genome fingerprints. Presence of the complete *fsr/gelE-sprE* operon was assessed by PCR and the *fsrC* gene was sequenced to assess its polymorphism.

Cluster analysis of the whole-genome fingerprints exhibited low niche specificity, suggesting that environmental selective pressures are very similar. The *fsrC* analysis of *E. faecalis* from different environments using reticulate and split decomposition method indicating the frequency of intra-species recombination as well as correlation between antibiotic resistance/virulence genes and PFGE genotypes will be presented.

34

The effect of partial asexual reproduction on genetic load: An experimental study of *Mimulus guttatus*

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The reproductive system of a species, which can be defined as the extent to which the species propagates sexually vs. asexually or whether it outcrosses or self-fertilizes, is a primary determinant of genetic variability. The reproductive system is highly variable among populations of *Mimulus guttatus*, the yellow monkeyflower. Genetic load and inbreeding depression are expected to be increasing with increasing asexual reproduction. In this study, I estimate inbreeding depression and several other genetic statistics for a coastal population of *M. guttatus* that exhibits substantial clonal reproduction. Field collected seed were used to establish multiple families, each of which was comprised of inbred and outbred members. Various flower measurements, along with male and female fitness components, were used to obtain estimates of genetic load and heritabilities. These estimates differ from those obtained previously for a different *M. guttatus* that is exclusively sexual in its reproduction.

35

Population transcriptomics of host shifts in the cactophilic *Drosophila mojavensis*

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In the presence of environmental change, natural selection can shape the transcriptome. Those genotypes that are better able to modulate gene expression to maximize fitness will be favored. It is imperative to examine gene expression at the population level as it responds to an environmental shift in order to distinguish random or neutral gene expression variation from the pattern produced by natural selection.

Drosophila mojavensis is a cactophilic fly endemic to the deserts of North America. This species contains four genetically isolated cactus host races each individually specializing in the necrotic tissues of different cactus species. The necrosis of each cactus species provides each of the resident *D. mojavensis* populations with a distinct chemical environment. Using a partial genome cDNA array we previously investigated the role of transcriptional variation in the adaptation of *D. mojavensis* to its hosts using one isofemale line. That dataset produced a set of candidate loci that were differentially expressed in response to host shifts, some of which have a non-neutral pattern of evolution. Using the recently sequenced and annotated genome of *D. mojavensis*, we recently developed a new gene expression array for all the annotated genes. This new microarray consists of 69,997 60-oligonucleotide probes representing 17,504 predicted genes. We now have employed this new array to reveal the effects of genotype, environment and their interaction on gene expression by exposing eight recently collected isofemale lines of *D. mojavensis* from Sonora to organpipe (native host) and agria (alternative host). In addition we quantify the amount of covariation of gene expression between genes.

36

Ecological success of polyploid *Aegilops* (Poaceae) is promoted by multiple origins

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Polyploidy is an important evolutionary factor in plants. The rate of speciation involving polyploidization is estimated to be up to 34%. In addition, estimation of divergence between duplicated genes suggests that nearly all flowering plants underwent a polyploidization event during their evolution emphasizing the high impact of polyploidization on plant evolution. The high evolutionary success of polyploids is also expressed in the finding that established polyloid species often occupy a wider geographical range or variety of habitats than either diploid progenitor. Particularly with allopolyploidy, the combined phenotypic effect of fixed heterozygosity, the existence of duplicated loci, may result in increased ecological amplitude of the allopolyploid relative to diploid progenitors. Multiple origins of polyploid species may further promote their ecological success by preventing a severe reduction in genetic variability which would impede local adaptation and range expansion. Although many studies have found evidence for multiple origins of allopolyploid species, data are lacking on whether these multiple origins increase the ecological breadth of the species.

In our manuscript we show that in a group of allopolyploid species, range size and abundance are correlated with the number of potential origins. We found that allopolyploid *Aegilops* species contain multiple chloroplast haplotypes each shared or closely related to haplotypes of the diploid progenitor species, indicating multiple origins as the major source of variation. The number of estimated origins and of haplotypes in each allopolyploid species was strongly positively correlated to the total area occupied and the tendency for the species to be common. Additionally, we found differences in ecological tolerance among independent origins in *Aegilops*

truncialis, the species with the largest range. Our results suggest that multiple origins could give rise to differentially adapted lines that could represent different ecotypes within one species.

These results strongly indicate that the introduction of genetic variability by multiple origins can increase the ecological success of allopolyploid species. It is feasible that after multiple origins occur in succession, density-dependent selection may favor each additional origin to be ecologically distinct. In addition recombination among these multiple origins may facilitate further expansion of the ecological niche of allopolyploids.

37

Evidence of Novel Viruses by Analysis of Nucleic Acids in Virus-like Particle Fractions from *Ambrosia psilostachya* and Other Plants

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Among the genes playing major roles in ecology are those borne by the microbiota. Successful metagenomic approaches mostly have targeted relatively homogeneous samples such as water and soil. We report a similar approach to the microbiota, particularly viruses, associated with plants. Knowledge of the viruses present in an ecosystem is important to understand better the impact of viruses on plants and the interaction of plants among themselves. The characterization of nucleic acids in a virus-like particle (VLP) fraction of plant extracts will provide information to achieve this aim. We developed and tested a procedure that provides such characterization.

It isolates VLP from homogenates of plant tissue by differential centrifugation. Nucleic acid in the VLP fraction is released after DNase I treatment by digestion with proteinase K, followed by phenol extraction and ethanol precipitation. The nucleic acid (VNA) is amplified randomly and the products are cloned and sequenced. As a test of the procedure we targeted *Ambrosia psilostachya*, western ragweed, which grows in the Tallgrass Prairie Preserve of northeastern Oklahoma. Amplifiable nucleic acid was found in six of twelve specimens and sequences were characterized from four of them. Evidence was obtained for the presence of viruses belonging to two virus families (*Caulimoviridae*, *Flexiviridae*). Multiple viral species were found in two of the four specimens and varied in amount among recovered sequences from less than 1% to 37%. None of the sequences represented those from known viral species. Indeed, one of the sequences appears to represent a member of a yet undescribed genus within the *Flexiviridae*. Retroviral sequences were abundant in all plants. Ribosome-derived sequences from bacteria and fungi also were present in small amounts. The method also was applied to a pool of plant material from a one square meter plot and resulted in the identification of sequences belonging to one or more *Carmovirus* members. Thus, the VLP-VNA method is a useful tool in expanding our knowledge of the universe of viruses and in cataloguing viruses and other microbes present in an ecosystem of interest. It is being applied to survey viruses present in most plant species present in the Tallgrass Prairie Preserve. Research supported by the US National Science Foundation (NSF) EPSCoR program, the Oklahoma Agricultural Experiment Station, and the SR Noble Foundation. The NSF is also supporting a Plant Virus Ecology Research Coordination Network for interested scientists.

38

Molecular Analysis of Grosbeak (*Cardinalidae*: *Pheucticus*) Hybridization in South Dakota

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Black-headed Grosbeaks (*Pheucticus melanocephalus*) and Rose-breasted Grosbeaks (*Pheucticus ludovicianus*) are passerine birds known to hybridize in the Great Plains - Rocky Mountain suture zone of North America. Past work on avian hybrid zones within this suture zone credits exogenous factors, such as temperature and precipitation, for the generation and maintenance of hybrid zones, but little is known about the endogenous factors acting within these zones. The objective of this study is to genetically characterize the zone of hybridization using modern genomic techniques to determine if postzygotic reproductive isolating mechanisms are acting within the grosbeak hybrid zone, as apposed to prezygotic reproductive isolation. Mitochondrial (ND2 gene) and nuclear (neutral marker) sequencing analyses are currently being conducted on Grosbeak DNA samples collected across the hybrid zone in South Dakota. The impact of postzygotic isolation will be tested by exploring and contrasting patterns of genetic differentiation and gene flow across the Grosbeak hybrid zone. Haldane's Rule, which predicts avian female hybrids to have lower fitness compared to male hybrids, will be tested by contrasting patterns of gene flow between maternally inherited mitochondrial DNA and bi-parentally inherited nuclear DNA. Longitudinal clines comparing the mitochondrial haplotypes (ND2 gene) of current and historical samples suggest this Grosbeak hybrid zone has been narrowing over the past 40 years. These preliminary results suggest reinforcement of reproductive barriers between these Grosbeak species is occurring and nuclear data will be used to test these conclusions. Nuclear

DNA will also be used to assess linkage across the hybrid zone. Linkage disequilibrium in hybrids is predicted to decay with increased backcrossed generations, and elevated levels of linkage disequilibrium in the hybrid zone may indicate a preponderance of F1 hybrids.

39 **Investigating Bacterial Diversity Along Hot Spring Thermal Gradients by rRNA-Tag Pyrosequencing**

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Central goals of the study of microbial diversity are to reveal how communities are organized and to understand how abiotic factors and biotic interactions combine to shape the distribution patterns of community members. However, simply describing patterns of diversity has historically been a challenge for microbial ecosystems. With recent advances in high-throughput DNA sequencing technologies, however, the first goal has become achievable, at least for comparatively simple microbial communities. Here, we used an rRNA-tag pyrosequencing approach to document the distribution patterns of bacterial community members for photosynthetic microbial mats along the thermal gradients of two alkaline hot spring channels in Yellowstone National Park. Such communities have been the object of intense study for more than four decades and therefore make excellent model systems for investigating microbial diversity. These mats are particularly notable for their abundance of ecologically diverse groups of the cyanobacterium *Synechococcus* and photosynthetic green non-sulfur bacteria (*Chloroflexi*) at temperatures between approximately 50-73 °C.

Duplicate samples were collected from ten locations along each channel at temperatures ranging from 38 °C to the upper boundary for photosynthetic biofilm. For each sample, the V3 hypervariable region of the 16S rRNA gene was amplified from community DNA with bacteria-specific, barcoded primer sets that enabled parallel pyrosequencing of samples. The filtered data set included a total of approximately 34,000 sequences, with nearly 400 identified unique V3 sequence tags. Community diversity and richness decreased with increasing temperature at the same rate along both gradients. Together, cyanobacteria and photosynthetic *Chloroflexi* account for approximately 60% of the total sequence reads for the combined data set. The respective relative abundances of these two groups are strongly negatively correlated, irrespective of the specific identities of the community members themselves. This result contradicts the conventional view that *Synechococcus* and *Chloroflexi* maintain tightly co-occurring distributions resulting from a producer-consumer relationship, in which photoheterotrophic *Chloroflexi* consume low molecular weight organic compounds excreted by cyanobacterial primary producers.

40 **Development of Microfluidic Chip-Technology for Real-Time Detection of Invasive Species within Ships' Ballast Water**

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Biological invasions of exotic species are considered to be among the most serious threats to local biodiversity and ecosystem functioning. In freshwater and coastal ecosystems, passive transport within the ballast water of ships is the primary vector for long-distance dispersal of aquatic species and has been the source of introduction for some of the most harmful pest species present in North America, including Zebra Mussel (*Dreissena polymorpha*) or European Green Crab (*Carcinus maenas*). Despite its unequivocal importance, little is known about species composition and frequencies in ballast water tanks. Many freshwater and marine invertebrates, for instance, form microscopic pelagic larval stages which are likely to be carried in ballast tanks in large numbers. However, detection and species-level identification of planktonic invertebrate larvae is notoriously difficult and in many cases impossible due to high morphological similarity and lack of differentiating traits among species. Here we present the outline and first results of a recently initiated project with the aim to develop a fast, chip-based device for the detection of invasive species in plankton samples from ballast water tanks. The technological concept is based on silica beads functionalized with species specific oligomer probes. Functionalized silica beads hybridize to 18S RNA molecules of target species. In order to expedite the time consuming hybridization process, the reaction takes place within a microfluidic system containing microchannels and a reaction chamber. The integrated reaction chamber is packed with functionalized silica beads. Target RNA solution is then fed in a continuous flow through the chamber, where the RNA is brought into close contact with the oligomer probes due to the nature of the bead packing within the chamber. Our initial results document the feasibility of this approach using beads functionalized with oligos specific for Green Crab DNA. After optimization of the technology using the Green Crab model, chips will be designed for species specific detection of selected species posing a potential threat to North American freshwater ecosystems. The system will comprise 4-5 species of molluscs and crustaceans, but will be extendable to a wider scale in the future.

41

A Gene Presence/Absence Polymorphism Controls Adaptive Variation for Cyanogenic Glucoside Biosynthesis in White Clover (*Trifolium repens* L.)

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White clover is polymorphic for cyanogenesis, with both cyanogenic and acyanogenic plants occurring in nature. The ecological genetics of this chemical defense polymorphism has been studied for over 90 years. It is controlled by two independently segregating genes: *Ac/ac* controls the presence/absence of cyanogenic glucosides; and *Li/li* controls the presence/absence of their hydrolyzing enzyme, linamarase. Here we report that *Ac* corresponds to a gene encoding a cytochrome P450 of the CYP79D protein subfamily (CYP79D15), and that the *Ac/ac* polymorphism arises through the presence/absence of CYP79D15 from the white clover genome. In other cyanogenic plant species, CYP79D orthologs catalyze the first step in cyanogenic glucoside biosynthesis. The molecular basis of the *Ac/ac* polymorphism in white clover parallels our previous findings for the *Li/li* polymorphism, which also arises through the presence/absence of a single-copy gene. The nature of these polymorphisms may reflect white clover's evolutionary origin as an allotetraploid derived from cyanogenic and acyanogenic diploid progenitors.

42

Quantitative Trait Loci Affecting Flowering Time in *Arabidopsis thaliana* Grown at Elevated CO₂

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Flowering is a critical milestone in the life cycle of plants because it influences pollination, biomass accumulation, and reproductive success and therefore may alter processes at the species, community, and ecosystem levels. Thus, understanding flowering time responses to global change drivers, such as elevated atmospheric carbon dioxide concentrations (CO₂), is necessary for predicting the impacts of global change on natural and agricultural ecosystems. Recent studies suggest that CO₂ plays an important role in determining flowering time. For example, in experiments reporting the response of flowering time in plants grown at CO₂ levels predicted for 50-80 years into the future, 57% of wild species and 62% of crop species exhibited altered flowering times in response to elevated CO₂. Furthermore, several studies have reported intra-specific variation in the response of flowering time to growth at elevated CO₂ indicating the potential for selection to act on this trait under future atmospheric conditions. The objective of the current study was to examine the genetic basis of elevated CO₂-induced changes in the flowering time of *Arabidopsis thaliana* using quantitative trait locus (QTL) mapping. To do so we grew 151 recombinant inbred lines (RILs) generated from a cross between Landsberg erecta (*Ler*) and Cape Verde Islands (*Cvi*) ecotypes at current (380 ppm) and elevated (700 ppm) CO₂. We measured flowering time, biomass at flowering, as well as leaf area and leaf number at flowering. We found significant genetic variation in the response of these traits to growth at elevated CO₂. In addition, we identified several genomic regions associated with changes in flowering time with growth at elevated CO₂.

43

Temperature-dependent sex determination in the snapping turtle: molecular genetic analysis of thermal sensitivity

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Temperature-dependent sex determination (TSD) was first reported 40 years ago in an African lizard. It has since been shown that temperature determines sex in many vertebrates, including some fish and amphibians, several lizards, numerous turtles, and all crocodylians. Even so, two basic questions are as salient today as they were when TSD was discovered. While the first question concerns the adaptive significance of this mode of sex determination, the second focuses on the genetic, physiological, and developmental mechanism(s) underlying TSD. We are taking a multifaceted approach to identify the mechanism(s) involved in the evolution of temperature sensitivity in the common snapping turtle, *Chelydra serpentina*. For instance, we are cloning and characterizing expression of candidate genes previously shown to play a role in sex determination and/or sexual differentiation in other amniotes. More importantly, we are using methods that allow unbiased discovery of genes that are differentially expressed between incipient testes and ovaries in embryos incubated under male and female thermal regimes. To date, we have used DD-PCR to clone and sequence approximately 50 ESTs that are differentially expressed in turtle gonads during the temperature sensitive period of development. We compare expression of one of these genes (*Obt1*, ovary biased transcript 1) to expression of candidate sex-determining genes. This novel gene is differentially expressed very early in the temperature sensitive period but in opposition to *Dmrt1*, a gene that plays a highly conserved role in the development of male vertebrates, male *D. melanogaster*, and male *C. elegans*. Temperature effects on expression of *Obt1* and *Dmrt1* precede differences in expression of *Sox9*, *FoxL2*, aromatase, and the androgen receptor. By focusing on the very brief temperature sensitive period in the snapping turtle, we expect to identify genes involved in the earliest stages of sex determination and discover novel candidates that may be involved in transducing temperature into the genetic cascade that determines sex.

44

Interaction of atmospheric CO₂ concentration and temperature affects flowering phenology of *Arabidopsis thaliana* ecotypes post-industrialization

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Atmospheric carbon dioxide concentration (CO₂) and mean global temperature have rapidly increased since industrialization and this rising trend is expected to continue through the end of the 21st century. Increases in CO₂ and temperature that are likely to occur in future may alter trajectories of plant adaptation. We may improve our predictions about the future impacts of these environmental changes on plant fitness and productivity by understanding plant phenological responses to climatic changes that have already occurred. Therefore, we examined how increases in CO₂ and temperature from the pre-industrial period (270 ppm/ 25°C) to the present period (380 ppm/ 26.3°C) have jointly affected the flowering phenology of *Arabidopsis thaliana* ecotypes. We grew thirteen naturally occurring ecotypes (n=10) under four treatments to simulate pre-industrial and present environments: 270 ppm CO₂ + 25/ 18°C day/ night temperature; 270 ppm + 26.3/ 19.3°C; 380 ppm + 25/ 18°C and 380 ppm + 26.3/ 19.3°C. CO₂ and temperature interactively affected both flowering time and biomass (at flowering) in these ecotypes. Consequently, at the lower temperature (25/ 18°C), an increase in CO₂ from 270 ppm to 380 ppm caused significant acceleration in flowering, but no significant changes in biomass. This CO₂-mediated acceleration in flowering was lost at the higher temperature (26.3/ 19.3°C); resulting in no significant changes in flowering time between 270 ppm and 380 ppm. Many ecotypes however, flowered at a significantly greater biomass at 380 ppm compared to 270 ppm at the higher temperature (26.3/ 19.3°C) and ecotypic variation in plant responsiveness to increase in CO₂ was present. Interestingly, all ecotypes exhibited a striking similarity in their flowering time responses to the CO₂ increase within each temperature environment. Our results suggest that no significant change in flowering time occurred between the pre-industrial and current period in these ecotypes; although many of these ecotypes did flower at a significantly higher biomass when grown under current conditions than under past conditions.

45

Template for Evolutionary and Ecological Functional Genomics: Population Genetic Structure within a Population

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Natural selection is thought to operate most frequently among individuals within a population; however, the process is more often studied at larger scales of genetic variation. Needed are model systems where different genotypes can be identified and replicated for experimentation. Plants of *Boechera stricta* are perennial close wild relatives of *Arabidopsis* in North America. We used 9 microsatellite loci to genotype 237 individuals of *B. stricta* within a population in the Black Hills. Our goal was to identify different self-fertilizing lineages (lines). The model-based software STRUCTURE was used to determine the number of lines and to assign individuals to lines. Simulations for 6 lines had highest posterior probabilities, and individuals were often assigned to lines with relatively high confidence. In growth room experiments, we have also found statistically significant quantitative genetic variation among lines for ecologically important traits. Our goals are to plant a common garden experiment of lines in the field and examine the ecological consequences of molecular genetic variation for candidate genes.

46

Selection on trichome production in *Arabidopsis lyrata* subsp. *kamchatica*

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Understanding the mechanisms maintaining morphological variation is a major focus of evolutionary biology, and environmental heterogeneity is thought to be one of the primary factors in the maintenance of this variation. *Arabidopsis lyrata* subsp. *kamchatica* shows variation in both the number of trichomes produced per leaf and the existence of trichomes within and among Alaskan populations. In surveys of both wild populations and herbarium specimens, we found that trichome-producing plants were more common in drier habitats, while glabrous plants were more abundant in wetter environments. In addition, using phenotypic selection analysis, we found divergent selection on trichome production in populations that differ in soil moisture; in the driest population there was direct linear selection for increased trichome production on the upper (adaxial) leaf surface, whereas in a relatively wet population the opposite pattern was found. Further, in a wet population containing both glabrous and trichome-producing plants, glabrous plants produced significantly more fruits than trichome-bearing individuals, indicating selection against the trichome morph. Overall, our

results demonstrate that heterogeneity in selection among populations could be responsible for the maintenance of trichome variation. Further, we propose that soil moisture may serve as a selective agent on trichome production.

47

Ontological Analysis of Five cDNA libraries of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

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Termites (Isoptera) are eusocial insects that are separated into morphologically and behaviorally distinct castes. They are hemimetabolous and exhibit unique polyphenisms (one genotype developing into several phenotypes), have a complex social structure. Every termite within a colony shares an identical genetic code within their DNA, we therefore hypothesized that the differences between castes (morphology, physiology, behavior) are due to differential gene expression. To identify the differentially expressed genes, we have assembled an initial EST library from five cDNA libraries. The cDNA libraries were constructed for two larval stages and three castes (workers, soldiers, and alates) of the termite *Reticulitermes flavipes*. To date, we have sequenced ~17,000 clones. The average insert size in each library was about 800 for the soldier and alate libraries, but there was no clear pattern of the frequency of insert sizes for the worker library. The worker and soldier library had a similar proportion of around 24% bacterial sequences, while the alate library had about 9% bacterial sequences. Indicating either a lower bacterial load within alates or a greater level of bacterial contamination within the worker and soldier libraries. Each respective EST library had about 22% of its sequences that were assigned a putative function (e-value <1E-10) within each library. This sequence data obtained the EST libraries allows us to infer possible candidate genes involved in caste differentiation whose gene expression levels we can test using a microarray approach for each caste.

48

Predicting Prokaryotic Ecological Niches Using Genome Sequence Analysis

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In the very near future we will be in possession of thousands of prokaryotic genome sequences. This collection will include many microbes that have not been previously identified, isolated, or observed. We hypothesize that the evolutionary pressure exerted by an ecological niche selects for a similar genetic repertoire in the genomes of prokaryotes that occupy the same niche, and that this is due to both vertical and horizontal transmission. To address this hypothesis, we developed a method to classify prokaryotes by calculating their Protein Family (Pfam) domain distributions and clustering them with all other sequenced prokaryotes. Clusters of prokaryotes are visualized as mountains on a topographical map which we term a "niche map". When compared to a phylogenetic map based on 16S rRNA, this niche map more accurately groups prokaryotes according to functional and environmental attributes. This approach can thus be used to associate uncharacterized prokaryotes with their ecological niche as a means of predicting their functional role directly from their genome sequence. As a further comparison, we have created a second niche map using the Clusters of Orthologous Groups of proteins (COGs) metric. We find that both Pfam and COG metrics result in the clustering of many of the same niche associations, in addition to the formation of different niche groups. It is therefore likely that multiple metrics are required to describe the correlation between a prokaryote's genome and its niche.

49

Analyses of Boreal Forest Fungal Communities in Soil Through High Throughput Clone Library Sequencing

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We are characterizing fungal diversity and community structure in soil within the Bonanza Creek LTER site in Interior Alaska using high throughput sequencing of PCR clone libraries in collaboration with the MIT-Broad Institute genome center. We have tested for phylogenetic biases by comparing fungal species abundances obtained using standard TOPO TA cloning methods versus a low bias, blunt-ended cloning system. We have also developed and validated a tagging approach allowing pooling of samples for high-throughput library sequencing followed by bioinformatic segregation of the sequences according to source sample. We find little bias in our methods based upon phylogenetic as well as OTU-level community similarity measures. To date, the project has generated roughly 70,000 bidirectional clone sequences which reveal that boreal forest soil fungal communities are remarkably diverse in species numbers and in deep phylogenetic breadth. For example, previously undocumented clades of fungi with divergence at the class to phylum level have been recovered. The boreal forest soils we have sampled are dominated by plant associated (e.g.

mycorrhizal) fungi, and are strongly structured with respect to time (season), space, and plant successional stage. However, the factor correlated with the strongest discrimination of fungal communities is soil horizon. This factor appears to operate at shallow phylogenetic scales; in other words all deep lineages occur in all horizons, but closely related taxa often show no overlap in occurrence among horizons. The implications for assembly rules in fungal communities will be discussed.

50

Spatial and temporal variation of plant transcription profiles in a field simulation of climate change

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The application of genomic techniques to answer ecological questions has created a need for experimental manipulations of non-model species in a field setting. We measured transcription profiles of a dominant tallgrass prairie plant (*Andropogon gerardii*) in field populations exposed to environmental variation reflective of global climate change. Our goal was to compare relationships between gene expression and environmental variables across time (the growing season) and space (replicate field plots). Despite relatively low differences in mean temperature of experimental and control plots, gene expression was much more likely to be a function of temperature than water availability both over time and space. There was little overlap in the functional groups of genes that demonstrated a relationship between expression and temperature through time and through space. In addition, we found that physiological variables reflective of photosynthetic activity (WUE, FvFm) were strongly related to the expression level of many genes both over time and space indicating that higher-level phenotypic changes accompany transcriptional changes. These data provide insight into how this dominant species is likely to respond to climate change in the Great Plains and suggests gene level characteristics that ultimately can lead to replacement by other species under dryer, hotter conditions. Moreover, this experiment demonstrates that genomic studies of non-model species in the field can identify specific genes and functional groups of genes that are ecologically important despite extensive variation in environmental conditions and genetic background.

51

The evolutionary and functional significance of variation in a primate malaria gene

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Recent evidence suggests that different primate species may exhibit parallel changes in the same genes that result in similar phenotypic consequences. However, it is unknown whether the evolutionary histories of these variants and the mechanisms through which they act are also similar in different species. Old World primate species, including humans, are frequently affected by closely related species of pathogens, and pathogen pressure is a major engine of adaptive evolutionary change. Hence, genes involved in immune function, especially those that are well-documented targets of selection in humans, may harbor evolutionarily and functionally parallel genetic variation segregating within different primate species. Here we show that genetic variation in the cis-regulatory region of the malaria-related FY gene in a wild population of yellow baboons (*Papio cynocephalus*) bears strong functional and evolutionary similarities to segregating variation in the human FY gene. Specifically, variation in this region is associated with infection by a malaria-like parasite, influences expression of the FY gene, and is characterized by a signature of selection both within this population and between East African baboon populations. However, while in humans a single cis-regulatory variant controls the presence or absence of both FY gene expression and parasite infection, the relationship between the baboon FY cis-regulatory region and these traits involves multiple genetic variants and environmental effects. These results suggest that similar evolutionary pressures may produce convergent genetic solutions and convergent phenotypic outcomes within different branches of the primate lineage.

A systems biology approach for discovery and characterization of genes involved in adaptive physiological traits

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Scientists are increasingly expected to predict the future state of terrestrial ecosystems and the effects of environmental perturbations, such as climate change, on the goods and services they provide to society. One of the more formidable challenges associated with this expectation is predicting species composition of future ecosystems without having the knowledge to identify the mechanisms or potential for rapid evolution. This is especially evident with physiological traits, which are difficult to score based on visible phenotype, thereby complicating efforts to discern trait variance within and among populations. Given advances in genomic technologies, there is now an unprecedented opportunity to provide a mechanistic understanding of the processes by which genetic change translates to phenotypic variation and the resultant manifestation of distinct physiological traits. In essence, there is now an opportunity –although currently unrealized– to evaluate the genetic potential of individuals within a population to express adaptive physiological traits to future environmental pressures.

To address this need and opportunity, we devised an approach that integrates functional genomic information (transcriptome, metabolome, and enzyme activity) with phenotypic and genetic characteristics. Specifically, a physiological state is classified by comparing expression signatures from individuals within a population to our compendium of plant stress expression phenotypes. Signatures are integrated with network analysis to define highly co-regulated gene modules, which are then correlated with additional forms of genomic information using novel systems-based statistics and linked with genetic information (QTL, SNPs, etc.).

To test this approach, we initiated a multi-generation competition study within a simplified model plant terrestrial ecosystem using both a wild type and a genotypic variant lacking an important gene for nitrate reduction. By scanning expression signatures against our expression compendium, we independently determined that the mutant was characterized by an N-limiting phenotype. The associated signature was integrated with network analysis and highly correlated expression patterns, termed modules, were significantly correlated with metabolites and enzymes participating in N metabolism. Upregulation of this module was therefore associated with greater N-reduction and assimilation, higher seed production, and increased genotypic frequencies for wild type plants.

This study demonstrates a strategy for using an integrative approach to classify the physiological state of a given genotype and then determine the underlying molecular mechanisms governing the expression of that phenotype. Such information is critical to current and future investigations in evolutionary ecology, where a mechanistic understanding of adaptive physiological traits may enhance predictions of species distributions to future climate scenarios.

Population Divergence and Selection on Pigment and Pattern in Garter Snakes

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Genetic correlation is predicted to exert evolutionary constraints on individual traits. Few studies, however, have explicitly sought to test the microevolutionary outcome of genetic correlation in wild populations. Common garter snakes (*Thamnophis sirtalis*) exhibit varying degrees of genetic correlation between patterning and pigmentation traits in different regions within their range. Covariation of trait means over multiple populations within regions is expected to follow a trajectory equivalent to the slope of the genetic correlation observed in each region. We explored the relationship between genetic correlation and population differentiation of color traits in two regions within the range of *T. sirtalis*. We conducted a breeding study using litters from California and Manitoba to estimate the genetic correlation between pigment saturation and pattern size in each region. We found California *T. sirtalis* to exhibit a correlation of 1.0 between the traits, whereas Manitoba *T. sirtalis* showed a correlation of ~0.7. We then collected phenotypic and microsatellite data from numerous populations in each region in order to compare divergence of the quantitative phenotypic color traits (Q_{st}) with divergence at neutral markers (F_{st}). In ten California populations, trait means differed among populations but the traits retained their proportionality such that each trait changed in lockstep with the other as predicted by their high genetic correlation. Furthermore, Q_{st} was equivalent to F_{st} in California, suggesting that trait divergence was an outcome of drift alone. In Manitoba, trait means differed among populations but no correlation between the two traits was observed over the fifteen populations studied. Q_{st} for pigment saturation was significantly greater than F_{st} , indicating the action of selection, whereas Q_{st} for patterning was equivalent to F_{st} , indicating that drift alone was the mechanism causing divergence in that trait. Our study supports the hypothesis that genetic correlation exerts evolutionary constraint on individual traits in wild populations and provides evidence for the role of selection in decoupling the phenotypic expression of color traits in ectothermic vertebrates.

54

Local Adaptation and Life History Evolution in *Arabidopsis thaliana*

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The evolution of reproductive timing in plants plays a key role in adaptation to climate. In widespread species such as *Arabidopsis thaliana* in which different populations experience wildly different season lengths and growing conditions, local adaptation may involve the evolution of distinct life history tactics. *A. thaliana* is perhaps uniquely suited to integrative studies of local adaptation as the developmental pathways underlying flowering time are well known, and there is extensive natural variation in flowering time genes. We are exploring genetic and phenotypic associations with fitness using a set of 360 accessions comprising both ecotypes from widely dispersed natural populations as well as lines carrying mutations in key flowering time loci. These accessions were grown in five European common gardens that span the native geographic and climatic range of *A. thaliana*, with the timing and number of yearly plantings chosen to coincide with the phenology of local *A. thaliana* populations. The effects of several candidate genes on life history and fitness vary across seasons and sites, as do selection pressures on phenotypic traits related to the timing of flowering.

Populations of *A. thaliana* with naturally occurring null alleles of *FRIGIDA* -- a major flowering time gene whose loss is associated with a rapid-cycling phenotype -- can give rise to multiple cohorts per year, but this is only the case at sites with a mild climate. Thus across the native range of *Arabidopsis* we see variation in the relationship between candidate genes and phenotype as well as shifting selection on life history traits.

55

Life is hard then you die: Age, flight behavior and senescence in the honey bee

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Foraging bees, those collecting nectar and pollen at flowers, produce the highest mass-specific metabolic rate ever measured while flying. Conservative estimates suggest these hard working flight muscles contract over 4 million times per day during an average 5 hours of flight per day and 240 wing beats per second. Thus foragers' flight muscles may experience high levels of oxidative stress during normal daily activities. In contrast, bees working in the hive take only 1-2 flights per day. Bees that shift from hive work to foraging at a younger age die sooner resulting in an average lifespan of 3 -4 weeks for bees born in summer vs. 5-6 months for bees born in fall that will over winter within the hive. To test the effects of age and increased flight behavior on functional capacity, senescence and lifespan we tested the flight capacity of bees at different ages and different levels of experience by flying the bees in variable gas mixtures. Flight capacity is greater in foragers than hive bees. Additionally foragers show an initial increase in capacity with the onset of foraging behavior, followed by a plateau and a decreased capacity with increased age. We also measured multiple indicators of flight muscle stress response and damage including: hsp70, catalase, total antioxidant activity and protein carbonyls in young and old bees either working in the hive or foraging, and in bees of both ages with varying amounts of flight experience. Hsp70 levels in flight muscles increase in flown foragers regardless of age but do not increase with exposure to high temperature. Total antioxidant activity increase by over 60% and catalase levels nearly triple in young foragers (8-10 d post eclosion; foraging for only 1-2 days) flight muscles after one day's worth of foraging. However, there is no significant difference between these two measures in old forager's flight muscle (30-32 d post eclosion; foraging for at least 12 days). There was no difference in flight muscle carbonyl content regardless of age or activity. The reduced levels of catalase and total antioxidant capacity in old bees after one day's foraging activity corresponds to reduced flight capacity and decreased foraging efficiency observed in aged bees suggesting that a decreased response to muscular stress may lead to a rapid onset and/or increased pace of whole organism senescence and ultimately shorten lifespan.

56

Fitness and Fecundity Connect Genes to Ecosystems in Model *Arabidopsis* Mesocosms

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Studies that span the gene to ecosystem continuum can provide a unique perspective on the mechanisms responsible for how individuals within and among populations respond to one another (i.e., competition). Few investigations, however, have identified critical phenotypes that explicitly link genes to populations, and beyond to ecosystems. Therefore, we initiated an experiment to

explore how mechanisms at one level of biological organization can be understood in terms of consequences at a different level of organization. Our approach involves growing wild type and mutant *Arabidopsis* plants that lack an important gene for nitrate reductase activity (i.e., *nia2*) at ambient and elevated CO₂ in model greenhouse mesocosms. Changes in the relative abundance of plants are tracked over multiple generations, while a suite of measurements characterize treatment and genotypic effects on gene expression, enzyme activities, metabolite concentrations, leaf gas-exchange, vegetative and reproductive biomass, seed production, and seed germination, and subsequent effects on population and ecosystem-scale processes.

Now in their fourth generation, wild type and mutant plants show distinct patterns of behavior that can be traced to deletion of the *nia2* gene and to CO₂ treatments. Plants grown at elevated compared to ambient CO₂ show altered patterns of gene expression. Analysis of these differentially-expressed genes using MAPMAN indicates that carbohydrate metabolism is preferentially impacted by the CO₂ treatments. Effects on carbon metabolism were confirmed with enzyme and metabolite profiling that we were able to integrate into the MAPMAN framework. Deletion of the *nia2* gene also impacted patterns of gene expression compared to wild type plants, reflecting effects on amino acid synthesis, Calvin cycle intermediates, and carbon metabolism.

Perturbations to the carbon and nitrogen metabolism had consequences that extended beyond the physiology of individual plants. In particular, *nia2* mutants produced less reproductive biomass, produced fewer seeds, and had lower rates of seed germination than did wild type plants. These effects on fitness and fecundity contributed to differential competitive behavior between the wild type and mutants, with wild type plants increasing in abundance over successive generations compared to the *nia2* mutants.

Our hypothesis that deletion of the *nia2* gene would alter competitive interactions between wild type and mutant plants was confirmed. While we originally proposed that these interactions would be mediated by competition among plants for available soil nutrients, we now believe that the effects of a single gene deletion in our experiment propagate through multiple levels of biological organization via impacts on fitness and fecundity. Thus, seed production and seed germination are critical processes that connect genes to populations, and beyond to ecosystems.

57

Genomic Response to Climate Changes in the Ecological System of the Tall Grass Prairie

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With the changing of the climate, including altered rainfall patterns and increased temperature, how does individual plant respond at the level of genetic and regulatory mechanism? In order to answer this question, we conducted field study on two closely related species in the tall grass prairie, *Andropogon gerardii* and *Sorghastrum nutans* with treatments of altered precipitation and warming in year 2006. Responses at other levels such as tissue, individual plant, community, and ecosystem were also investigated. Gene regulation was studied using cDNA microarray from maize.

Through statistical analysis of the gene expression data of year 2006, we identified a set of genes that were differentially expressed under climate changes, which further tested by the results of quantitative real-time PCR. We also elucidated the relationships between gene expressions and other physiological and environmental factors (like soil properties, precipitation, temperature et al.) using high throughput regression.

58

Clinal variation in freezing tolerance among natural accessions of *Arabidopsis thaliana*

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Freezing temperatures represent a significant abiotic challenge to plants given their sessile lifestyle and inability to escape ambient atmospheric conditions. We investigated intra-specific variation in freezing tolerance in natural accessions of *A. thaliana* from across a broad geographic range. Considerable variation in freezing tolerance was observed among accessions both with and without a prior cold-acclimation treatment, suggesting that differences among accessions in cold-acclimation capacity as well as differences in intrinsic physiology contribute to variation in this phenotype. A highly significant positive relationship was observed between freezing tolerance and latitude of origin, indicating a major role for natural selection in shaping variation in this phenotype. To investigate the possible mechanistic basis of freezing tolerance variation among these accessions, we examined expression and nucleotide variation in

the CBF/DREB1 transcriptional activators (genes that play a key role in the ability of plants to undergo cold-acclimation), and expression variation in downstream targets of these genes. Mutations in both regulatory and coding regions of the CBF/DREB1 genes were revealed and likely account for some of the observed patterns of freezing tolerance variation among accessions.

59

Analysis of Expressed Sequence Tags from the Aquatic Midge (*Chironomus tentans*)

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This research aims to examine cellular and molecular responses of the aquatic midge (*Chironomus tentans*) to environmental stressors and to develop stress-specific marker genes for assessing environmental quality in aquatic systems. Preliminary analysis of 4,908 high-quality expressed sequence tags (ESTs) resulted in a total of 2,301 putative transcripts (506 contigs and 1,795 singlets). Putative transcripts were functionally annotated and sorted into 10 biological function categories, including binding (42.1%), catalytic (25.7), translation regulator (2.2), transporter (16.8), structural molecule (10.3), signal transduction (0.8), antioxidant (0.7), enzyme regulator (0.7), transcription regulator (0.6), and motor (0.1) activities. Relatively large number of the sequences (158) appeared to putatively encode proteins involved in oxygen binding, which is consistent with the biology of this insect species that uses the hemoglobin for oxygen storage and/or transport. This work will lead to the development of a midge DNA microarray for examining gene expression changes in response to environmental stressors.