



Ecological Genomics Symposium

Kansas State University

POSTER ABSTRACTS

1

Linking gene expression to phenotype: Effects of four insect herbivores on microarray and metabolite profiles

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A major goal of ecological genomics is to link gene expression with phenotypes of ecological significance. We conducted a whole-genome microarray study of *Arabidopsis thaliana* responses to four insect species and profiled glucosinolates, a major class of inducible, sulfur-containing defensive metabolites we have previously shown to influence the performance of these herbivores. The herbivore that induced glucosinolates caused a coordinate upregulation of genes involved in sulfur uptake, storage, and glucosinolate biosynthesis not seen for the herbivores that did not induce. One herbivore species suppressed glucosinolate levels, a presumed stealth associated with downregulation of genes involved in photosynthesis. This combined approach of genomics and metabolomics provides clues to the basis of specificity of plant responses to different kinds of insect herbivores and target genes for future studies.

2

Adaptive divergence, genetic drift, and incipient speciation in the grasshopper *Hesperotettix viridis*

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The specialized feeding habits of the oligophagous grasshopper *Hesperotettix viridis* present a unique opportunity for investigating the process of differentiation in host plant use in the context of a generalist group of herbivores. We are using AFLPs, microsatellites, and mitochondrial DNA sequences to contrast patterns of adaptive and neutral genetic variation in *Solidago*- and *Gutierrezia*-feeding populations of *H. viridis*. With these molecular data, we will distinguish between three alternative scenarios explaining the historical development of divergence in host plant association. Results of reciprocal transplant experiments are consistent with an interpretation of local adaptation leading to incipient host race formation in sympatry, while preliminary data from AFLP markers suggest that divergence occurred in allopatry, perhaps during the late Pleistocene or early Holocene while populations were isolated in regional refugia. A third possibility is that initial divergence in host plant association occurred in allopatry, but complete reproductive isolation, if achieved, is the result of reinforcement in sympatry after secondary contact. Preliminary mitochondrial sequence data suggest significant geographic structure in genetic variation. Using population genomics methods we distinguished neutral loci from loci under selection by comparing the distribution of *F_{st}* estimates for individual AFLP loci in pairs of populations to simulated values assuming neutrality. In comparing two sympatric *Hesperotettix* populations with different host plants, 6 of 222 (2.7%) loci

exhibited greater differentiation than expected (“outlier” loci), while no outlier loci were detected when comparing two allopatric populations using the same host plant. These outlier loci may contain genes associated with adaptive divergence with respect to host plant use. Comparisons of *Fst* distributions of AFLP loci in additional same-host and different-host population pairs will reveal the degree to which adaptive loci contribute to genetic differentiation among different host-associated populations both within and across geographic regions. The broad geographical and historical scope of our study may illuminate alternative and previously unappreciated mechanisms for host race development and subsequent speciation.

3

Genetic diversity in Striped Skunk and Rabies Virus: Evidence for Host-Pathogen Coevolution?

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Historically, the majority of rabies research consists of epidemiology, pathology, and etiology. Scientists are now realizing the importance of host ecology (patterns of migration, dispersal and sociality) in rabies transmission and evolution. The rabies-striped skunk (*Mephitis mephitis*) model system is useful in studying host-pathogen coevolution because the striped skunk is a major reservoir for two genetically distinct variants of rabies, one in the northern and one in the southern Great Plains. Prior to 2003 the southern variant was not known north of the Kansas/Nebraska border, but is now found as far north as the Nebraska/South Dakota border.

We investigated genetic diversity in the two rabies variants and in corresponding striped skunk populations located in South Dakota and Kansas using 400 bases of the rabies N gene and striped skunk microsatellites. Rabies virus RNA and striped skunk DNA were isolated from 24 skunks from Riley, Pottawatomie, and Geary counties in Kansas, and 22 skunks from Hamlin, Clark, Codington, Deuel, and Grant counties in South Dakota. Sequence analysis of the N gene indicated that the northern variant had 3 times the haplotypic diversity of the southern variant (13 and 4 haplotypes, respectively). Nucleotide diversity among haplotypes from South Dakota skunk populations (0.025 ± 0.013) was one order of magnitude greater than southern haplotypes from Kansas skunk populations (0.0026 ± 0.002). The low diversity in the Kansas population is suggestive of a population expansion in the rabies virus which corresponds with its geographic expansion. Understanding the genetic diversity and structure in striped skunk populations in the central Great Plains and in corresponding rabies variants will provide further insight into the epidemiological patterns of this host-pathogen system.

4

A molecular approach to understanding plant response to global climate change in a Californian grassland ecosystem

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Global warming affects the climate in multiple ways from increased temperature to altered rainfall pattern. Understanding the effect of these climatic changes on different ecosystems is paramount. We are currently investigating the coordinated responses to climate change of soil microorganisms and plants from a Californian grassland ecosystem through a multidisciplinary project. Our aim is to link the different responses, scaling from gene expression to ecosystem function. Here we discuss some aspects of the plants response at the molecular level.

Our experimental setup reproduced a Californian annual grassland ecosystem in climate-controlled greenhouses. A total of 152 mesocosms were filled with three horizons of natural soil packed to specific bulk density and instrumented to follow precisely the plant growth conditions. Seeds, collected from the grassland, were dispersed on the mesocosms to generate monocultures of *Avena barbata* (the dominant species in many California grasslands), or mixed communities including five additional grasses and two forbs. Leaf and root samples were collected at the peak of the growing season from *A. barbata* plants grown under low, ambient and high precipitation treatments and in two different soil types.

The availability of genomic sequences for *A. barbata* was limited and only a few cDNA have been cloned and sequenced previously. We used genomic data from other grass species to design PCR primers in order to amplify specific sequences in our focal species. Using the PCR cloning approach we have sequenced target genes and subsequently studied their expression using real-time RT-PCR. The target genes were selected for their key role in both nitrogen and carbon metabolism. Preliminary data suggests that, at the time of sample collection, plants grown under low precipitation treatments were subjected to mild water stress. Under these conditions, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) gene expression was shown to decline by 40%. The expression of nitrate reductase (Nia) and chloroplastic glutamine synthetase (GS2) was maintained suggesting that nitrate was still available to the plants for uptake. The decline in ADP-glucose pyrophosphorylase (AGPS) expression may indicate that less carbon was available for storage in the form of starch. At high precipitation, the levels of Nia and Rubisco mRNAs decreased while those of GS2 and AGPS were maintained. Under these conditions, the soil pools of nitrate and ammonium were lower compared to the ambient conditions, which could explain the pattern of expression seen for Nia and Rubisco. Furthermore, the up-regulation of the cytosolic GS isoform may indicate a greater need for nitrogen remobilisation in the leaf, which is consistent with a lower nitrogen supply. These results will be integrated with ongoing studies of leaf metabolite levels, plant physiology and ecosystem function to understand the potential significance of genomic responses to climate change.

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Capturing genomic signatures with an anonymous DNA microarray

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Comparative genomics, using the model organism approach, has provided powerful insights into the structure and evolution of whole genomes. Unfortunately, only a small fraction of Earth's biodiversity will have its genome sequenced in the foreseeable future. Most wild organisms have radically different life histories and evolutionary genomics than current model systems. Here, we describe a novel approach using an anonymous DNA microarray platform that gathers genomic samples of sequence variation from any organism. Oligonucleotide probe sequences printed on a custom 44K array were 25 bp long and designed using a simple set of criteria to maximize their complexity and dispersion in sequence probability space. Using whole genomic samples from three known genomes (mouse, rat, and human) and one unknown (*Gonystylus bancanus*), we demonstrate and validate its power, reliability, transitivity, and sensitivity. Using two separate statistical analyses, a large numbers of genomic 'indicator' probes were discovered. The construction of a genomic signature database based upon this technique would allow virtual comparisons and simple queries could generate optimal subsets of markers to be used in large scale assays, using simple downstream techniques. Biologists from a wide range of fields, studying almost any organism, could efficiently perform genomic comparisons, at potentially any phylogenetic level after performing a small number of standardized DNA microarray hybridizations. Possibilities for refining and expanding the approach are discussed.

6

Characterization of CHD-Z polymorphism and associated fitness costs in the Upland Sandpiper.

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The CHD family of genes is widely recognized as a highly conserved region of the genome that influences early development. In avian molecular ecology, this gene has been used as a universal sexing marker. Previous studies have linked an insertion in the intron of CHD-Z with decreased chick viability in moorhens. We have optimized laboratory protocols for genetic sexing of the monomorphic Upland Sandpiper (*Bartramia longicauda*) using the primers P2/P8 to amplify regions of the CHD-Z and CHD-W genes. In the process of sexing individuals in our population, we discovered a polymorphism in the CHD-Z intron. Intensive nest monitoring over 5 field seasons at Konza Prairie Biological Station has revealed low egg viability (mean= 0.86, n = 53 nests). Overall, 35.8% of clutches that survive incubation contain at least one egg that fails to hatch. We are testing for a possible link between the CHD-Z polymorphism and low egg viability in Upland Sandpipers. We have isolated, cloned and sequenced the two CHD-Z alleles and have developed a rapid screening process for Upland Sandpipers. Using this molecular technique, we will be able to rapidly genotype nesting parents and monitor egg viability in their nests.

7

Abundant, Diverse, and Consequential P elements Segregate in Promoters of Small Heat-shock Genes in *Drosophila* Populations

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To test whether the previously-described insertions of transposable elements into Hsp70 proximal promoter regions is restricted to this multicopy gene or a general feature of heat-shock genes sharing Hsp70's distinctive chromatin structure, we surveyed single-copy small heat-shock genes (Hsp22, Hsp23, Hsp26, and Hsp27) in 47 natural populations and discovered 44 novel transposable element insertions in their proximal promoter regions. All are P elements, and none is autonomous. P elements were not randomly inserted in the host genes' proximal promoter regions, but were clustered, most commonly proximal to the transcription start site. In two focal populations, 5-7 P element-containing Hsp26 alleles segregate, as do 7 Hsp27 alleles in the second of these. In lines derived from the former population and subjected to experimental evolution, the allelic frequency of the most common P element varies considerably and is at lower frequencies in lines selected for accelerated development than in controls. These transposons reduce or eliminate Hsp26 gene expression and, depending upon the sex and transposon, increase or decrease inducible tolerance of high temperature, development rate, and fecundity. These phenotypes imply or document that transposable element insertions into small Hsp genes are (a) related to the chromatin structure of the host promoter, and (b) can have dramatic fitness consequences, and therefore create variation on which selection can act.

8

Phytohormone Signaling in Herbivore Responses: Insights from Microarrays

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Plants respond to herbivore attack with dynamic, species-specific defenses coordinated by multiple phytohormones. A whole-genome microarray study discovered approximately 3000 genes which respond to herbivory by four insect species. We compared the abundance of up- and down-regulated genes known to be responsive to various phytohormones in our insect-response set to their frequencies in the Arabidopsis genome to determine whether any were non-randomly elicited by insects. We found that responses to caterpillars involved significantly greater jasmonic acid and nitric oxide signaling than expected, while aphids seemed to have no significant impact on these signaling pathways. Of all species examined, only the generalist aphid *Myzus persicae* elicited a significant salicylate-associated response, and responses to the specialist aphid *Brevicoryne brassicae* did not involve any non-random signaling events. Most interestingly, *Myzus persicae* and the two caterpillar species (*Pieris rapae* and *Spodoptera exigua*) induced many fewer cytokinin-associated responses than would be expected by chance. This seems to indicate that these species have a negative interaction with the cytokinin signaling system, and may indicate a method in which insects may manipulate plant responses. This analytical technique suggests specific hypotheses about the roles of plant signals in insect-responses.

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Genetics and Ecology of Floral Pigment Patterning in Chilean *Mimulus*

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Floral color variation between closely related plant species is ubiquitous. However, the genetic basis and ecological relevance of such variation have been deciphered in relatively few cases. Here we discuss the genetics and pollination biology of color patterning in four closely related species of the wildflower genus *Mimulus* (Phrymaceae, ex. Scrophulariaceae). The parent species differ in flower color but, when crossed, reveal complex spatial patterning of floral pigments that are not seen in the parental species. Analysis of F2 and backcross populations suggests that these complex patterns are in part under modular control, with several patterning elements behaving as simple Mendelian traits. This study is one of the few analyses of floral color patterning to date, and suggests similarities to color pattern control in animal systems including mice and some species of butterflies.

Genetic mapping is now underway to confirm the single-locus nature of several patterning elements, and to progress towards identifying and characterizing these loci. Pollination biology field work suggests that three of the study species are pollinated by a single generalist bumblebee, while the fourth (*Mimulus cupreus*) is strongly reproductively isolated by a lack of pollinator visitation. Morphological analyses, however, suggest that flower shape rather than color are likely to be responsible for pollinator discrimination against *M. cupreus*. This indicates that the species-specific color variation observed in natural populations is probably maintained by non-pollinator sources of selection.

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Eat or be eaten: the life of a soil nematode

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We are interested in the dynamics of belowground community responses to environmental change. As nematodes make up a large component of these communities, we have chosen to specifically investigate their role in community-level processes. Nematode communities at the Konza Prairie Biological Station near Manhattan, Kansas, show trophic level responses to disturbances including nitrogen addition, altered soil moisture and varied burning régime. Bactivorous nematode community composition has been shown to have great potential as a biological indicator of disturbance and recent data show taxa specific (genus level or below) differential responses to perturbations in the field. These nematode responses could either be due to direct effects of the changes in soil chemistry resulting from perturbations (see abstract by Jones et al.), or indirect effects where the bacterial community responds first and the effects are seen in the next trophic level, the bactivorous nematodes. Recent data show that bacterial communities on Konza prairie respond to disturbance suggesting indirect effects may play a large role. As we are interested in the genes that may be responsible for community changes, studies of bactivorous nematodes are particularly attractive, as we can use laboratory studies of the free-living soil nematode *Caenorhabditis elegans* to model the native soil nematode taxa found at Konza. The wealth of genetic and genomic information available for *C. elegans* in conjunction with detailed field studies on Konza will allow us to make connections between individual genes and their role in community dynamics.

C. elegans is cultured in the lab on agar plates seeded with *E. coli*, which serves as the source of nutrition and immediate environment. In order to model a more natural environment in the lab, we isolated cultureable native soil bacterial species from Konza prairie, of which *Micrococcus luteus* was the most abundant. In addition, *Bacillus megaterium* and *Pseudomonas* sp. were isolated in association with Rhabditid nematodes from Konza prairie. We have used cDNA microarrays to identify genes that are differentially expressed in response to altered food resources/bacterial environments. Pair-wise comparisons of *C. elegans* populations fed one of the four aforementioned bacteria species were made in quadruplicate. Statistical analyses revealed 204 genes differentially expressed in response to bacterial treatment after multiple testing correction ($q < 0.01$). As one might expect, many of these genes (19) appear to have metabolic functions. Interestingly, many genes (20) shown to be involved in innate immune responses were also identified suggesting nematodes may need to defend themselves from attack in addition to consuming bacteria as a food source.

Viable loss-of-function mutants were available for 22 of the 204 genes identified by microarray. Currently, life history data has been collected for seven of these mutant strains across all four bacterial environments. Some of the mutants tested showed significant, specific reductions in multiple fitness traits, corresponding with our microarray results. Furthermore, a mutant phenotype had not been reported for at least one of these genes. Thus it seems that not only is the expression of specific genes induced in response to a change in environment, but some of these genes contribute to fitness traits. These results demonstrate that assessing *C. elegans* gene functions in more natural environments can allow new functions to be assigned to genes of unknown function. Overall our results suggest that life in the “real world” for a soil nematode is tough, eat or be eaten, with the outcome in part depending upon the relative expression of metabolic and defense functions.

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Microbial Diversity in Intestine of *P. maniculatus* and *P. leucopus* and Soil of the Tri-State Mining District

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Lead and other heavy metal contamination is known to reduce diversity in environmental bacterial populations. Exposure to these contaminants results in microbial populations dominated by bacteria characteristically able to process heavy metals. Assessment of mouse populations will help determine whether sufficient remediation has occurred at the treatment site. We examined bacterial populations in the soil and in the gut and feces of mice in order to assess changes to microbial diversity in an area contaminated by lead. Trapping was conducted at Neosho Wildlife Area (control site), a man-made waterfowl refuge, and two locations within the city limits of Galena, KS (treatment site). The treatment site was in the Tri-State Mining District, an area remediated for human habitation due to accumulation of high levels of lead from mining waste. Mice were trapped using Sherman Live Traps over a 2-week period. Trap-side field necropsies allowed acquisition of fecal samples from the descending colon. Soil was collected near each trap set where mice were trapped. A total of 16 mice were collected comprising 10 *Peromyscus maniculatus* and 6 *P. leucopus*. DNA extracted from feces and soil were submitted for Massively Parallel Sequencing, allowing comprehensive identification of bacteria present to the genus level. This technology uses a highly-conserved, known DNA sequence common to all bacteria which distinguishes between bacterial genus based on minor base-pair differences in the sequence. Microbes in the gut were also visualized in tissue cross-section on Hematoxylin and Eosin stained slides. Because areas free of contamination are able to support a greater variety of microbes, we hypothesized the control site bacterial populations would be more diverse. Heavy metal resistance has been shown to facilitate antibiotic resistance, therefore, we further hypothesized that the few species of bacteria occurring on the contaminated site will be unusually resistant to heavy metals and also antibiotics. We will present the results of our sequence analysis and discuss the relevance to possible soil and mouse intestinal microflora at the control and previously contaminated sites.

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Global change impacts on poplar tree gene expression: Genotypic variability in carbon sequestration potential

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The best-known commodity produced by the tree vascular system is wood—a valuable, renewable resource for biomass-based products including lumber and paper as well as an excellent biological source for energy production. Atmospheric CO₂ concentrations have risen approximately 35-40% since pre-industrial times. Thus, the development of tree-based technologies to sequester CO₂ within usable wood biomass and feed-stocks has been proposed as one of the best ways to address problems associated with the changing global environment. At the Aspen FACE experiment in northern Wisconsin, groups of 5 distinct quaking aspen clones (*Populus tremuloides*) have been exposed to elevated CO₂ (ambient plus 200 ppb) for the past eight years. The responses of these clones have ranged from little (0-10%) to large (30-40%) enhancement in photosynthesis, height and diameter growth, and late season foliar retention. Here we report on the global gene expression patterns of two aspen clones (216 = non-responsive to elevated CO₂ and 271 = very-responsive to elevated CO₂) as a means of determining why these two clones have such different capabilities to take up and sequester carbon under elevated CO₂. During the past two years (2004 and 2005), we have examined the transcriptional level activity for some 25,000 poplar ESTs via microarray analyses for three time points (early, mid, and later summer) under elevated CO₂ and control treatments. In response to elevated CO₂, transcriptional activity was distinctly different between the two clones. For the CO₂-responsive clone, genes inhibiting protein turnover were up-regulated, while protein degradation genes, stress response genes, and senescence-associated genes were down-regulated. For the CO₂-responsive clone, the carbon taken in was largely allocated toward growth, while in the non-responsive clone more carbon was directed towards defense compounds, phenylpropanoid biosynthesis, and cell wall thickening.

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Using Changes in Small Mammal Population Genomics in the Tri-State Mining District to Assess Remediation Efficacy

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The Tri-State Mining District (KS, OK, MO) flourished from 1850-1970 because of its profitable lead and zinc production. Mine waste such as rock, chat and tailings exposed surrounding populations and habitats to high levels of lead, zinc, and cadmium. Galena, Cherokee County, KS had unacceptably high levels of contamination, and was remediated for human habitation by the Cherokee County Superfund Clean Up Program. Ecotoxicological assessment of the small mammal community will help evaluate if remediation was sufficient to restore healthy habitats. *Peromyscus maniculatus* and *P. leucopus* were trapped in the Neosho Wildlife Area (control) and Galena, KS (treatment). We trapped at each site for six nights using Sherman Live Traps. Eight-six animals were trapped, including *P. leucopus*, *P. maniculatus*, and *Neotoma floridana*. A Shannon Diversity index of 1.4 was calculated for the control site, compared to an index of 1.1 for the treatment site. *P. maniculatus* collected in Galena had an average shorter left hind foot length (18.13 +/- 0.24 mm (average +/- SE)) than those found at the control site (19.57 +/- 0.75 mm) (t-value=2.39, df=21, p-value=0.026). *P. maniculatus* in Galena also had a lower average body weight (14.75 +/- 0.61 g) than *P. maniculatus* collected at the control site (20.29 +/- 2.11 g) (t-value=2.519, df=7.015, p-value=0.04). These data suggest that the contamination affects the growth of *P. maniculatus* leading to a lower population fitness. Liver, kidney and testes samples were collected for histopathological analysis and RNA extraction. We hypothesized that exposed mice would be more prone to abnormal pathology in the liver, kidney and testes. Samples will be analyzed with light microscopy to assess abnormal pathology. To assess the effects of heavy metal toxicity at a genetic level, we are using Affymetrix microarrays to measure changes in RNA expression in the liver, kidney and testes. We will use changes in RNA expression of specific genes to see how the contamination is influencing the health of the animals. This project will help determine if the habitats in the Cherokee County Superfund Site have been sufficiently restored for healthy wildlife communities.

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Gene duplication and the evolution of environmental sensitivity: The case example of phytochrome genes and germination in *Arabidopsis thaliana*

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Phytochromes are members of a duplicated gene family that act as photoreceptors of red and far-red light in plants. During the single process of germination, different phytochromes exhibit extensive functional redundancy but also impressive diversity of function. We have found evidence that all five phytochromes in *Arabidopsis thaliana* contribute to germination, but some are most important for promoting germination at warm temperatures, others at cool temperatures. Some are most important for promoting germination after seeds experience warm-wet conditions, others after seeds experience cold-wet conditions. Some promote the germination of seeds that matured under long days, others promote germination of seeds matured under short days. Some are necessary for germination of seeds matured at cool temperatures, others are not involved in the breakage of maternally cold-induced dormancy. Finally some contribute to dormancy maintenance (or suppression of germination) as well as to germination. Thus the different duplicated phytochromes have evolved different sensitivities to seasonal cues and different environment-dependent functions. Even the most recently duplicated phytochrome, PHYD, has diverged extensively and has evolved novel activities during germination. This diversity of function offers a mechanism whereby seeds can evolve responses to different combinations of seasonal cues and thereby potentially regulate the seasonal timing of germination.

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Microsatellite marker development for *Phlox*: towards understanding the functional significance of reproductive character position

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Phlox is a diverse plant genus with several major lineages that have radiated in the North American flora. Its species exhibit intriguing diversity in relative placement of reproductive organs, and particular morphologies have multiple evolutionary origins (i.e., characters relating to reproductive organs are homoplasious on the phylogenies). We aim ultimately to understand the functional significance of this variation with regard to reproductive biology and pollination ecology. In our current ecological genomics research, we are developing highly variable, co-dominant microsatellite markers that will enable us to 1) construct a genetic linkage map for *Phlox*, and 2) ultimately, conduct QTL mapping focused on traits of interest. Development of genomic libraries enriched for microsatellites, library screening, and testing of markers on individuals from natural populations is described.

16

Drought and Rust Effects on Gene Expression in the Dominant Plant Species of Tallgrass Prairie, *Andropogon gerardii*

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There have been numerous studies examining how plants respond to a single stress. However, plants routinely experience multiple stresses in the field and the response to the combination of stresses is often different than the response to a single stress. In our experiment, big bluestem (*Andropogon gerardii*) plants experienced drought or non-drought conditions and were either inoculated with the rust pathogen *Puccinia andropogonis* or not. Drought and pathogen treatments were combined in a factorial design with each treatment combination present in at least four blocks. Gene expression was analyzed using maize microarrays. Soil moisture measurements were taken daily throughout the experiment as an estimate of drought stress. Additionally, measures of disease severity, leaf water potential, and stomatal resistance were collected for each plant. We 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. 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.

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High-throughput regression analysis as a tool for understanding the relationship between gene expression and other variables

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Gene expression measured using microarrays is typically evaluated in terms of responses to categorical treatments, such as the presence or absence of a stressor, in an analysis of variance. But other variables predictive of gene expression, as well as variables that may be responses to gene expression, are continuous rather than categorical. For example, we have analyzed continuous environmental variables as predictors of gene expression and gene expression as a predictor of continuous physiological response variables. For such regression analyses, the magnitude of the slope or other regression coefficient takes the place of fold-change as the response of interest. Genes in high-throughput regression analysis can be placed in five categories. First, some genes may not exhibit hybridization. Second, some genes may hybridize too infrequently or exhibit too much variability in expression to provide adequate statistical power for analysis, resulting in an inconclusive result. Third, for some genes there may be adequate statistical power to detect a trend, but no trend is detected; for these genes, the lack of a relationship may be fairly conclusive. Fourth, for some genes a significant relationship may be detected in the absence of

problems revealed through regression diagnostics. Fifth, for some genes a significant relationship may be observed, but regression diagnostics may indicate potential problems. For example, a single outlier may be highly influential, yielding an inconclusive result. We illustrate an application of this approach to the analysis of gene expression in big bluestem, *Andropogon gerardii*.

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Evolution of alpha-globin and cytochrome b among three subspecies of the deer mouse, *Peromyscus maniculatus*

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Among subspecies of deer mice, allelic variation at two duplicated alpha-globin genes is associated with physiological adaptations to hypoxic vs. normoxic environments. We conducted a survey of alpha-globin variation among physiologically distinct subspecies of deer mice that occupy different altitudinal ranges. By analyzing nucleotide sequences and structural models of encoded polypeptides, we identified the specific substitutions that differentiate subspecies of deer mice with different blood-oxygen affinities. Functional divergence between physiologically differentiated subspecies is primarily associated with mutations affecting intersubunit contacts between the alpha- and beta-chains of hemoglobin. A large fraction of recovered alleles show evidence of inter-paralog gene conversion, and most conversion tracts alter the amino acid sequences of encoded polypeptides. These findings document the importance of gene conversion in generating functional variation in naturally occurring populations.

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Heritable cis- and trans-regulation of *Plasmodium falciparum* transcripts.

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Although the *Plasmodium falciparum* genome is fully sequenced, the determinants of malaria gene expression remain largely unknown. More than 60% of identified ORFs share no homology with known genes and recognizable transcription factors are rare. Earlier attempts to alter the *P. falciparum* transcriptome with defined antimalarials point to a rigid transcriptional program that is largely insensitive to external perturbations; the parasite has novel regulatory mechanisms with diminished sensory functions. To obtain a global view of segregating transcripts and an opportunity to locate determinants of gene regulation, fluorescence signals from individual elements of microarrays were treated as a heritable trait and analyzed, en masse, using quantitative trait loci (eQTL). Specifically, using RNA from HB3 × Dd2 trophozoites, we cluster transcript variation over a genetic dimension to group gene products that are regulated by common determinants. For several such clusters, it was also possible to provide genomic locations of putative regulatory determinants. Approximately 11% of over 3,000 expressed genes represented on our microarray show a significant eQTL detected by permutation analysis. Both cis and trans effects were ubiquitous. In addition to providing a global view of the regulation of transcription and potential functions for uncharacterized genes, this approach reveals gene expression adaptations resulting from past drug selection histories.

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Ecological Genomics: Transcript profiling of defense and competition

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Plants in the wild that are attacked by herbivores and pathogens often grow next to other plants that represent potential competitors. Therefore, in some cases one would expect the simultaneous evolution of defense and competitive ability. However, the optimal defense hypothesis predicts a tradeoff between these factors. In two transcript profile experiments with *Boechera stricta* a close wild relative of *Arabidopsis thaliana*, we examined gene expression using microarrays under competition and herbivory to see what genes might help or hinder plants to compete and defend. We also measured growth rates and glucosinolate toxin concentrations. We used native goldenrod *Solidago missouriensis* and non-native dandelion *Taraxicum officinale* competitors and a specialist herbivore diamondback moth *Plutella xylostella*. Plants of a single inbred family of *B. stricta* growing with either

goldenrod or dandelion in the lab showed reduced growth, but an increased glucosinolate response only next to the non-native dandelion. Competition mainly caused the down regulation of at least 108 genes, but there were differences between native and non-native competition treatments. In a separate experiment feeding by specialist larvae elicited a glucosinolate response. Transcript profiles in response to herbivory are now being analyzed and will be presented in relation to the genes affected by competition.

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Functional Diversification of the Alpha-Globin Gene Family in Mammals

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The duplication of protein-coding genes, followed by functional changes in one or both daughter copies, is thought to play a fundamental role in adaptive evolution. However, the role of positive directional selection in driving the functional divergence of duplicated genes is highly controversial. Here we report a phylogenetic analysis of sequence variation among functionally distinct members of the alpha-globin gene family. We focus specifically on duplicate copies of alpha-like globin genes in mammals that are expressed during different stages of development. The objectives of the study are (1) to test whether positive selection has played a role in the functional diversification of the alpha-globin gene family; and (2) to test for variation in functional constraint among different members of the gene family, especially as it relates to developmental timing of expression. The globin gene family provides an especially promising system for addressing questions about the evolution of duplicated genes because patterns of sequence divergence can be related to functional properties of the encoded proteins.

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The genetics and fitness consequences of trichome variation in *Mimulus guttatus*

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The evolution of geographical variation is a precursor to speciation and a critical link between micro- and macro-evolutionary processes. Most studies of geographical variation have focused on single trait values, which is not sufficient for characters that exhibit substantial plasticity. Here, I discuss the results from experiments investigating population-level variation in trichome density, a phenotypically plastic trait in the model plant *Mimulus guttatus* (yellow monkeyflower) that may act in resistance to insect herbivores.

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Assessment of the direct effects of pH and ionic concentration on native nematode taxa

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Prior studies from our group have demonstrated that the soil nematode community in tallgrass prairie responds strongly to nitrogen addition, increased soil moisture, and different experimental fire regimes. As nitrogen addition is correlated to a decrease in pH and changes in ionic concentration, we investigated the possibility that changes in the nematode community are controlled by changes in soil chemistry. As pH and the ionic concentration of the water in the soil (i.e., osmolarity) cannot be easily decoupled, we used independent manipulation of pH and osmolarity in laboratory cultures to determine which parameters illicit the greatest response in native Konza taxa. We focused on the native taxa *Oscheious* sp. and *Pellioiditus* sp. from the Rhabdidiidae family, as well as, *Acrobelloides* sp., a nematode from a different family (Cephalobidae) that responded similarly in the field experiments. As a control for laboratory conditions, we also included two strains of *Caenorhabditis elegans*, a model organism which is in the same family as the native Rhabdids. Fecundity was measured across taxa cultured on agar plates where pH, ion species, and ionic concentration were varied. The 36 treatments included the factorial combinations of three pH levels (5, 6, and 7), four ion species (calcium, magnesium, potassium, and sodium), and three ion concentrations (50mM, 100mM, and 200mM). pH elicited a consistent response across all species and strains, showing a significant inverse effect on fecundity (i.e., fecundity was reduced as pH increased). Although ion concentration also had an overall negative effect on fecundity for all taxa, the magnitude of the effect varied with taxon and ion species. Significant differences in taxonomic response were seen across ion species, however there were consistent differential responses across all taxa to monovalent versus divalent ions. Specifically, the fecundity of all taxa declined drastically in response to even small increases in concentration of monovalent ions (calcium and magnesium).

As Konza soils are rich in calcium it is interesting to note that while Konza native taxa responded negatively to increases in calcium concentration, the severity of effect was most severe in *C. elegans*, a non-Konza native. Although the divalent ions potassium and sodium also had an overall inverse effect on fecundity, the effect at the lowest concentration (50mM) was beneficial to most taxa. These results are consistent with the field data that illustrate a negative correlation to both pH and ion concentration. Thus the responses of the nematode community seen previously are likely driven, at least partially, by the influences of nitrogen addition on the pH and ionic environment. Additionally, as increased calcium concentration decreased fecundity in native Konza nematodes, research into the interaction of nitrogen addition, pH, and calcium liberation in the soil may provide new insight to the response of soil nematode communities.

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Effects of global change phenomenon on microbial communities

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Grasslands are among the most endangered ecosystems on Earth and are known to be highly sensitive to an array of global change phenomenon. Although the effects of natural and anthropogenic disturbances have been the focus of long-term study, the research to date has primarily focused on the effects to aboveground processes. Due to difficulty in assessing the extreme levels of diversity in belowground ecosystems, little is known about how global change affects belowground communities. In the last year new methodologies have been discovered that allow assessments of belowground communities with far greater resolution than has ever been available before. To further our knowledge on the effects of global change on belowground ecosystems, a novel mass parallel sequencing methodology was used to determine whether bacterial communities respond to the effects of global change. We utilized four existing field experiments on the Konza LTER that were designed to assess the effects of global warming and altered rainfall patterns, land use conversion, restoration of prairie from old field plots, fire suppression, and nutrient additions. DNA was extracted independently from 126 soil cores and used to generate 197,608 bacterial 16S sequences. To determine the frequency of occurrence of bacterial taxa in each plot, we clustered the DNA sequences using a highly conservative cut off value of 90% similarity, which loosely corresponds to genus. With a total of 23,134 different MOTU's (Molecularly-derived Operational Taxonomic Units) at the 90% level, these projects were shown to maintain a high bacterial diversity and, as expected, this diversity is primarily comprised of bacteria with rare abundance. A mixed-model ANOVA was used to determine if the effects of global change altered the bacterial community structure. With the exception of phosphorous additions, all environmental perturbations led to significant alterations of bacterial communities. Further, with the taxonomic resolution gained from the unprecedentedly large dataset of sequences produced, we were able to differentiate two distinct types of bacterial community change. Nitrogen addition had a highly significant effect ($P < 0.0001$) on bacterial communities; however that effect was limited to a select set of taxa whose abundances were greatly altered. In comparison to this type of taxon specific effect, the broader changes brought about by warming, altered rainfall, and land use change elicited a more ubiquitous effect on community structure. Rather than altering the community structure for a select few taxa, these treatments effected a significant change ($P < 0.04$) in the overall structure and diversity of the community. These results confirm that, similar to the aboveground communities, the belowground communities are highly responsive to environmental change. As the bacterial communities form an important basal component of the soil food web, it is likely that much of the belowground ecosystem that is dependent on bacterial resources is also affected.

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Using Affymetrix Arabidopsis ATH1 genome microarrays to understand the endophytic function of *Periconia macrospinos*

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Fungal root endophytes are common in natural ecosystems yet little is known about their function. We isolated *Periconia macrospinos* from a tallgrass prairie ecosystem at Konza Prairie Biological Station in northeast Kansas and found that it readily colonizes roots of *Arabidopsis thaliana*. Preliminary data indicates that inoculation with *P. macrospinos* substantially increases *A. thaliana* biomass, reduces its susceptibility to pathogens, and increases its drought tolerance. We are in the early stages of dissecting the *A. thaliana* genome with the Affymetrix ATH1 microarray to understand the host responses to *Periconia macrospinos* root endophyte colonization. Preliminary data suggest that genes whose expression is altered likely include those related to pathogen resistance or its induction, photosynthesis and water use efficiency.

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Relationship between Forest Soil Microbial Functional Potential and Soil Biogeochemistry

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The relationship between microbial communities and environmental variables has been investigated quite a lot lately owing to technical improvement in molecular biology and related areas. However, microbial community functions, which are the responsible domain of the relationship, have hardly been studied in a comprehensive fashion as microbial community structures have been studied. A Functional Gene Array (FGA) with 10,498 various microbial functional genes was used to assess the relationship between microbial functional potential and forest soil geochemical measurements. From 25 samples, 23 different soil geochemical variables were measured and derived including C/N ratio, soil respiration rate and N fixation rate and so on. FGA data were prepared for functional genes (FGs) (all individual genes) and functional gene categories (FGCs) (groups of same environmental functions). Series of multivariate statistics were applied for general comparison (Mantel test and Procrustes test) and selection of important geochemical variables and functional genes (Bio-Env and vector fitting). Canonical correspondence analysis (CCA) was performed to analyze between two domains based on selected geochemical variables for CCA model building. General association between microbial functional potential and geochemical variables was significant by the Mantel test ($P < 0.046$). Soil respiration rate was determined to be the common environmental variable selected by several methods with whole and three subgroups of FGA data., and was the sole defining variable of major CCA axis. Tight coupling between C and N cycles is thought to contribute mixed selection of significant geochemical variables between two subgroups. The Mantel test with geographic distance was fairly insignificant suggesting much less heterogeneous distribution of microbial functions than species.

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The comparison of pesticide-pyriethroid-resistance in horn fly (*Haematobia irritans irritans*) among two distant locations in Midwestern United States

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Horn fly has been known for its economic damage to the cattle industry through sucking bloods, irritation, and ruining cattle hide. This study was focused on the assessment of pesticide - pyrethroid - resistance among selected populations in Midwestern United States (Kansas and Oklahoma). An allele specific polymerase chain reaction (ASPCR) was used to isolate specific segments of DNA that are located in the coding regions of a voltage-gated sodium channel of the horn fly. Mutations of this region were recognized for different level of pyrethroid resistance in horn fly. We tested for two types of resistance to the pesticide, *kdr* and *superkdr*. The result reflects two years and three seasons of horn fly collection from 2002 to 2005. The total number of horn flies used for the study was 455, and seven different locations were included. Overall, more than 70% of tested flies showed homozygous *kdr* resistant phenotypes and 5.6% of horn flies showed homozygous *superkdr* resistance. Allele frequencies of *kdr* resistance and susceptible were .75 and .25, and that of *superkdr* resistance and susceptible were .18 and .85 respectively.

Our findings were summarized into three areas. First, no regional difference was shown between States. Second, summer population showed slight increases in *kdr* and *superkdr* resistance which concurred with other studies. Third, there was no phenotypic difference across the gender.

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Environmental and ecological controls on gene expression of root processes in prairie plants

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Thirty-seven percent of the U.S. and ¼ of the earth's land surface is grassland. The tall grass prairie occupies the most mesic areas in the Central Plains of US and is among the most productive grassland. In order to insure the future sustainability of these important grasslands, we must understand how highly productive tall grass prairie responds and adapts to present-day environmental perturbations such as changes in water and nitrogen (N) and be able to predict effects of future scenarios of environmental changes on grassland productivity. Despite the important contribution of roots to plant growth and productivity in prairies, the response and adaptation of roots to stress has

largely been ignored. Comparative studies on the impact of nitrogen availability on gene expression in roots and shoots of the model plant *Arabidopsis thaliana* have shown that the response of roots was far more extensive than that of the shoots. These genes included those encoding proteins involved in nitrate uptake and assimilation, uptake and transport of other elements and water, sugar metabolism, maintaining source-sink relationships, hormone biosynthesis, signal transduction and plant defense to pathogens. However, our knowledge of the molecular response of roots to environmental changes in natural ecosystems is minimal.

The overall goal of this project is to understand the molecular response of roots to environmental stress in prairie grasses and to link environmental factors controlling root productivity in natural systems to temporal and spatial changes in gene expression in the roots of the dominant prairie grass big bluestem (*Andropogon gerardii*). We have taken a genomic approach, which involves identifying genes that are differentially expressed in proliferating roots in response to nitrogen and water availability. The specific objectives of the project are to: 1) construct subtractive libraries of big bluestem roots subjected to nitrogen and water stress and validate the differential expression of these genes in greenhouse experiments, 2) analyze the genome-wide expression in *Andropogon* experiencing nitrogen and water stress, and, 3) extend these growth chamber studies to field grown plants growing in long- and short-term experimental plots at Konza Prairie.

We have constructed subtractive cDNA libraries from the roots of greenhouse-cultivated plants subjected to N and water stress. cDNA libraries from these plants as well from N- plus water-supplemented plants were constructed to isolate and identify the genes differentially induced by nitrogen and water stress. 480 clones from each of the 4 libraries were sequenced and homology with known sequences determined. We have obtained genes involved in amino acid metabolism, stress/defense signaling, transcription/translation, protein processing, cell wall metabolism, secondary metabolism, and respiration/redox reactions. We are continuing to validate expression pattern of these genes with Real-time PCR and reverse northern approaches as well as to continue to sequence another 5000 clones identified in these four libraries. A major new complementary approach to our root specific subtractive libraries is the genome-wide (microarray) study of *Andropogon* gene expression in response to water and nitrogen limitation. Our preliminary experiments have shown promising results using heterologous hybridization of *Andropogon* cDNA with *Zea mays* spotted EST arrays. This microarray approach could be developed into a valuable tool to study the effects of diverse abiotic stresses on native tall grasses.

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Is There Sexual Selection for Plumage Colouration in Pied Flycatchers?

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The conspicuous colouration observed in many animals is thought to have evolved as a response to sexual selection. Theories of speciation, geographic differentiation and the evolution, maintenance and adaptive significance of polymorphism have often deduced information from observed patterns of colour variation. In species that exhibit distinct colour morphs, colouration has been found to correlate with a number of fitness-related traits implying that it is affected by a plethora of varying selection pressures.

The pied flycatcher (*Ficedula hypoleuca*) is a migratory bird that breeds in most of Europe and over-winters in Africa. Pied flycatcher males vary in their dorsal colouration along a continuum that ranges from brown to black. The frequency distribution of the different colour variants appears to be connected to the presence of the collared flycatcher (*Ficedula albicollis*). In areas where the two species co-occur light brown males are found at high frequencies. The frequency of darkly coloured pied flycatcher males increases with increasing distance from the nearest collared flycatcher population. The observed pattern is hypothesised to have arisen as a consequence of fitness advantages that are gained from character displacement in areas of sympatry. In areas where the collared flycatcher does not occur, sexual selection is thought to be the driving force that favours darkly coloured males leading to their higher occurrence. It seems highly unlikely that such a consistent pattern has evolved solely as a consequence of genetic drift, but there is currently little evidence supporting the sexual selection hypothesis. Though the pied flycatcher exhibits colour variation along a continuum (as opposed to distinct morphs) there is presumably a connection to some fitness-related traits among the differently coloured individuals, leading to females favouring darkly coloured males over lighter ones. No conclusive evidence of a concrete fitness advantage has however been presented to date.

The aim of this study is to examine the sexual selection hypothesis within a pied flycatcher population. I am currently in the process of determining whether dark males truly sire more offspring than lighter ones by forming a second pair bond more often or via attaining more extra-pair copulations (and paternities) that do not involve a social bond. If female pied flycatchers breeding in areas of allopatry prefer darkly coloured males over lighter ones, this would be expected to be the case. The amount and distribution of paternities gained outside the initial pair bond is being assessed with microsatellite markers.

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Population Genetic Variation in Flowering time under Seasonal and Geographic Conditions in *Arabidopsis thaliana*

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Flowering time is an important and ecologically relevant trait, which is controlled by a large-number of genes and various environmental factors such as light and temperature. Large genetic variation for the flowering time has been observed in *Arabidopsis thaliana* accessions. Over 5000 lines collected from various geographic locations across different times of year are being genotyped for 149 common SNPs to determine relatedness and rough population structure. Samples containing maximal diversity will be selected for high density genotyping with a custom 250kSNP/tiling array and subsequently phenotyped for flowering time in simulated seasonal conditions representing Spain and Sweden. This data set will be used for whole genome association mapping. Our growth chambers contain far-red, red, true-white, and blue fluorescent bulbs on electronically dimmable ballasts that recreate daily sunrise, bright midday, and sunset conditions. The light color, intensity and day length, as well as temperature and relative humidity changes throughout the seasons and is more dramatic in Sweden vs Spain.

We have tested these conditions by mapping QTL responsible for spring flowering time in the Kas-1/Col-g11 recombinant inbred lines (RILs) across two replicate blocks. A linkage map from 96 RILs was established using 119 markers (64 new SNPs added to 55 existing markers). One major QTL, mapping to the FRIGIDA (FRI) locus, was detected on the top of chromosome 4 that showed significant gene x environment interaction. One QTL near FLM (a homologue of FLC) epistatically interacted with the FRI QTL. Four other minor QTL were also detected in different environments. These QTL x environment interactions suggest that subtle changes in light, temperature, and relative humidity are differentially felt by known alleles controlling flowering time and may be responsible for adaptation to regional environments.

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What for? Linking foraging behavior to an orthologue of the foraging (for) gene in the predatory mite *Phytoseiulus persimilis*

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The predatory mite *Phytoseiulus persimilis* (Acari:Phytoseiidae) is a specialist predator widely used in biological control of spider mites. Using quantitative genetics techniques we have previously measured the genetic contribution to phenotypic variation in several predator foraging behaviors, and associated this variation with possible tradeoffs which may constrain the evolution of foraging. We are developing molecular tools that can be used for studying these well-defined inheritable biological characters. Thus far we have optimized whole genome amplification techniques to obtain a microgram level of amplified genomic DNA from a single mite for subsequent genetic studies using molecular markers. We were then able to develop three different molecular markers from *P. persimilis*: 12S, ITS, and for gene. The for gene has been shown to be involved in the natural variation in foraging behaviors in fruit flies, honeybees, and red harvester ants. The molecular marker on the for gene, cyclic GMP- dependent protein kinase, can now used to study allele-specific association mapping of the foraging behavior to this locus. The for gene marker, spanning ~700 bp including two introns, is being developed by using DGGE (denaturing gradient gel electrophoresis). In order to develop multiple molecular markers on the coding genes of the mite, we also have made a cDNA library and sequenced 960 random clones. We have recently selected predator lines which exhibit different levels of the traits of interest and are testing them with the molecular markers that we developed. We also envision that the markers can be used for establishing linkage mapping for studying genetics of mite in the long-term.

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Estimation of microsatellite mutation rates in *Arabidopsis thaliana*

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Mutations are the ultimate source of genetic variation and estimates of mutation rates are important for many evolutionary theories. Microsatellites are hyper-variable markers comprised of many short tandem nucleotide repeats, such as di-, tri-, and tetra- nucleotide repeats. These markers are distributed throughout eukaryotic genomes and have high mutation rates. Despite the proliferation of *Arabidopsis thaliana* as a model system and microsatellites as widely used markers in plant genetics, there are no estimates of microsatellite mutation rates for *A. thaliana*. I will present preliminary mutation rate estimates for several microsatellite di-nucleotide repeat locus in this plant species.

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The *Allonemobius-Wolbachia* host-endosymbiont system: evidence that *Wolbachia* infections can modify patterns of host gene expression

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The intracellular, alpha-proteobacteria *Wolbachia* has been shown to induce a wide range of phenotypes in its eukaryotic host, including cytoplasmic incompatibility and male killing. Despite much work on these phenotypic effects, there has been little evidence to suggest that *Wolbachia* can alter patterns of gene expression in its host.

Here, we used a curing experiment in conjunction with mRNA differential display (DD-PCR) to determine if the wCon strain of *Wolbachia* alters patterns of gene expression in its cricket host, *Allonemobius socius*. In screening approximately 28% of the cricket transcriptome, *Wolbachia* infections were found to modify the expression of sixteen mRNA fragments. Following DD-PCR, these differentially expressed mRNA fragments were excised, re-amplified, cloned, sequenced, and blasted to known nucleotide and protein databases. One fragment in particular, the fused gene complex Ubiquitin-RpL40, was consistently and significantly underexpressed (i.e., ~2 fold suppression) in *Wolbachia* infected individuals, as confirmed by quantitative real-time PCR. It is noteworthy that the selectively maintained, highly conserved Ubiquitin is targeted by *Wolbachia* in these crickets, as this protein is integrally involved in numerous cellular functions including cell cycle regulation, maintenance of chromatin structure, and tagging abnormal and foreign cellular proteins for degradation. Although the role of all of these differentially expressed genes in host-endosymbiont biology is currently unknown, our results do suggest that *Wolbachia* infections can modify patterns of host gene expression.

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Tardigrade Biology and Ecology: Biodiversity, Distribution and Ecosystem Functioning

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Tardigrade Biodiversity:

The water bear has long been a curiosity of evolution but sidelined because of its lack of economic importance. Of 750 known limno-terrestrial species, only 32 are considered cosmopolitan while the others are regional or endemic. Yet, we find it in great numbers and diversity at many locations around the globe. But our knowledge of the group and our ability to work it into the ecology of an area is limited by outdated monographs and few experts. Born from the frustration of poor resources for identification we have begun a project to be able to identify tardigrades to species by the development and standardization of a "bar code" series of DNA markers as a process that will enable rapid and accurate identification.

Tardigrade Distribution:

With an accurate method of identifying and verifying species we will be able to undertake diversity assessments of tardigrade communities. Our work to pin down species identifications with DNA markers will enable investigators to use biodiversity to analyze ecosystem functions. Preliminary studies suggest tardigrades to be more susceptible to air pollution than the lichens and moss they inhabit. Thus, they should be better bio-indicators of change. We wish

to look at communities of tardigrades and/or assemblages of invertebrates, and do experimental work on the links between their diversity and ecosystem stability. Such data may lead to a method of monitoring the changes in the associations between animal and habitat and then extrapolate patterns and impact of pollution sources on a local, regional, and world ecological basis.

Ecosystem function:

Tardigrades are ubiquitous and abundant yet little is known about their role in ecosystem functioning. Preliminary work on respiration rates suggest they could play a large role in carbon cycling in some ecosystems. Tardigrades changing to and from cryptobiosis might predict temporal functioning in habitats and ecosystems. We are currently investigating comparative trophic phylogeography relative to geophysical/chemical habitat suitability models. Eventually we would like to be able to understand the interaction between gene expression, cryptobiosis, respiration and the physical parameters that drive it.

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The microevolution of Interleukin-6 in an immunocompromised species, the black-footed ferret.

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Inbreeding depression as a result of population bottlenecks heightens the extinction risk with lower reproduction and survival rates and the loss of the ability to resist disease. The highly endangered black-footed ferret (*Mustela nigripes*) experienced an extreme population bottleneck ($n < 10$) that has had both genotypic and phenotypic consequences. In addition to the loss of >95% of their genetic diversity, this species exhibit signs of immunosuppression. Black-footed ferrets are much more susceptible to coccidia and cryptosporidia infections than their congeners and recent studies indicated that under ambient conditions, black-footed ferrets produced 75% less Interleukin-6 (IL-6) than two of their closest relatives, the Siberian polecat (*Mustela eversmanni*) or the domestic ferret (*Mustela putorius furo*). IL-6 is a pleiotrophic cytokine vital for acute phase reactions, inflammatory responses and hematopoietic activities. It is unclear if lowered production of IL-6 in *M. nigripes* is a characteristic of the species and a result of speciation events that occurred ~800,000 years ago or the result of the dramatic population bottleneck that occurred 20 years ago. As a first step to uncover the genomic processes that have produced this phenotype, we compared among 3 ferret species the ratios of synonymous to nonsynonymous base pair substitutions and the resulting predicted amino acid sequences. To compare population processes that produced these genotypes, we compared coalescence times of the IL-6 gene to neutral genomic markers. To produce IL-6 sequences from the three species of ferret, we designed primers (based on the already known *Canis familiaris* IL-6 DNA sequence) around each of the 5 exons in the IL-6 gene and then amplified these segments on the *M. nigripes* genome. A 500 bp sequence of *M. nigripes* DNA was then used to probe a BAC DNA library of domestic ferrets to find the gene. Using gene walking techniques, we sequenced the 5000bp IL-6 gene from the domestic ferret BAC library. These sequences were then used to design primers to sequence IL-6 in other ferret species.

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The genetic architecture of development rate in rainbow trout: QTL, QTL x maternal environment, and candidate gene expression

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Embryonic development rate is associated with optimal emergence timing in salmonid fishes, and has important implications for the survivability and performance of juveniles as they emerge from the gravel for feeding.

Furthermore, embryonic development rate has been associated with both juvenile growth performance and adult age at sexual maturity. We have identified a major embryonic development rate quantitative trait locus (QTL), accounting for greater than 20% of the variation in this trait, in line crosses of rainbow and steelhead trout (*Oncorhynchus mykiss*). We tested whether this and other detected QTL exhibit significantly different additive effects in different maternal cytoplasmic environments, and have begun to identify candidate genes and their expression in this region. For QTL mapping, doubled haploid mapping progeny were produced by androgenesis using eggs from nine different females (or maternal cytoplasmic environments). Line crosses used include an Oregon State University (OSU) female Shasta-type rainbow trout line crossed with the Clearwater River (CW)

steelhead. Briefly, unfertilized eggs were irradiated to destroy the maternal nuclear DNA component, and then fertilized with sperm from one OSU x CW F1 individual. After fertilization, diploidy is restored by preventing the first embryonic cleavage. Time from fertilization to hatch was recorded for each individual reared in a constant 11°C environment. Six QTL were associated with development rate, and both maternal cytoplasmic environment (MCE) and QTL x MCE significantly contributed to variation in development rate. Additive effects of the major QTL, *tth-OC-8a*, were not significantly different among MCE, but some minor effect QTL (*tth-OC-b* and *tth-OC-9*) did exhibit significant QTL x MCE. In a separate study to identify candidate genes in the region of the major development rate QTL, we have mapped two genes in the region and followed the expression of these genes during embryonic development. These genes, growth hormone receptor (GHR) and a negative growth and differentiation gene (GADD45-beta) are duplicated, and one duplicate maps to the region of the development rate QTL.

Embryonic expression of the duplicates is quite different early in development for GADD45-beta, but very similar for GHR. Additional fine mapping, molecular evolution, and expression analyses are underway to further define the identity of and variation within genes functionally associated with this trait. This QTL region is also associated with spawn timing in rainbow trout, and shows greatly reduced recombination rates in both male and female maps.

Further dissection of this region can have important implications in understanding the genetic and evolutionary mechanisms regulating life history differences within *O. mykiss*.

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Molecular Population Genetics of a Chemical Defense Polymorphism in White Clover (*Trifolium repens* L., Fabaceae)

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White clover (*Trifolium repens*) is naturally polymorphic for cyanogenesis (HCN release following tissue damage).

The ecological factors favoring cyanogenic and acyanogenic plants have been studied for over 50 years, making this one of the best documented examples of an adaptive polymorphism in plants. Cyanogenic plants are strongly favored in the presence of small, generalist herbivores; however, frequencies of cyanogenic plants decrease dramatically with cooler climatic conditions (possibly reflecting autotoxicity in areas with frequent frost-induced cell rupture). The cyanogenesis polymorphism is controlled by two, independently segregating Mendelian genes: *Ac/ac* controls the presence/absence of cyanogenic glucosides; and *Li/li* controls the presence/absence of their hydrolyzing enzyme, linamarase. In this study, we have examined the molecular evolution and population genetics of *Li* as it relates to the cyanogenesis polymorphism. We report here that *Li* exists as a single-copy gene in plants possessing linamarase activity, and that the absence of enzyme activity in *li/li* plants is correlated with the absence of much or all of the *Li* gene from the genomes of these plants. Consistent with this finding, we confirm by RT-PCR that *Li* gene expression is absent in plants lacking enzyme activity. In a molecular population genetic analysis, we find a striking absence of nucleotide variation at *Li* in a worldwide sampling of cyanogenic plants, as well as statistically significant evidence for positive directional selection; these findings are consistent with a species-wide selective sweep at *Li* associated with the cyanogenesis adaptive polymorphism.

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Exploring species boundaries with phylogeography and morphometrics using the polytypic minnow *Notropis stramineus*

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Notropis stramineus, a species of North American minnow (Family Cyprinidae), is common in sandy-bottomed streams and lakes shores throughout Eastern North America. Across its wide distribution, the species has much morphological variation. This variation has led to two subspecies being described (one eastern, one western).

Additionally, this species has two disjunct populations; one small disjunct population occurs in Tennessee along the Mississippi River and another large disjunct population occurs in Southern Texas. Though morphologically distinct, the disjunct population in southern Texas has been left without a subspecies description because of problems with nomenclature and lack of study. We will present work on both the morphological and mtDNA divergence of the disjunct population of *N. stramineus* in comparison to populations in the species' contiguous distribution. Specifically, we will examine hypotheses concerning the origin of this disjunct population. We will also address the hypothesis that this disjunct population is actually a cryptic species.

Transcription Factor Profiles and cis-Regulatory Motif Distributions in Arabidopsis Genes Regulated by Herbivory

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Plant responses to insects and other environmental stresses are complex, involving differential perception, multiple signaling pathways, and the transcription of appropriate defense-responsive genes. In this study, we examined genes in *Arabidopsis* differentially regulated by wounding or by specialist or generalist insects from two feeding guilds using a whole genome oligo microarray. Of the 3000+ differentially regulated genes, we identified 198 unique transcription factors from 34 different families that were up- or down-regulated in local or systemic tissues at different time points after herbivore feeding. A large percentage of these transcription factors were members of the AP2/ERF, MYB, Homeobox, C2H2, bHLH, and WRKY gene families. The various treatments elicited the accumulation of different transcription factor transcripts. A bioinformatics analysis revealed that the distributions of enriched motifs involved in water stress responses, as well as ABA-, ethylene-, JA- and SA- signaling, in the promoters of co-regulated genes also differed among insect treatments. Our results suggest that differences in gene expression patterns by wounding and differently feeding insects are partly controlled at the transcription factor level.

Although transcription factor profiles differed among insect treatments, some are found in several treatments and could be part of a generalized response to insect herbivory. In many, but not all cases, the expression patterns of specific transcription factors correspond to enrichments of their compatible binding sites in down-stream affected genes, suggesting that more complex protein-motif or protein-protein combinations may be necessary to activate or repress the transcription of genes after herbivore attack.

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Fish and Chips: Uncovering “Behavior Genes” via Heterologous Competitive Genomic Hybridization

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The rapid recent and repeated radiation of African Cichlid fishes has produced a wealth of behavioral, ecological and morphological diversity among closely related species. The low level of genomic divergence allows us to use one microarray in order to study the molecular mechanisms that underlie the evolution of such great diversity. I have adapted traditional cDNA microarray techniques to use heterologous competitive genomic hybridization (HCGH) toward the identification of genes that have undergone significant sequence divergence. The increase sequence divergence may be the result of selective pressures of natural selection suggesting that these genes play a role in adaptation to the environment, including behavior. In order to estimate the effect of sequence divergence on apparent hybridization ratios, I have also applied this technique to different species of *Drosophila* for which genomic sequence data is available. In addition to its value in identification of adaptive sequence divergence, HCGH offers a necessary control for inter-species heterologous hybridization techniques in expression profiling.

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Natural Patterns of Disease Resistance Genes across a Precipitation Gradient

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The dominant tallgrass prairie grass *Andropogon gerardii* is found across a precipitation gradient from the relatively moist Eastern United States to the relatively dry Western United States. Since moisture facilitates infection by foliar bacterial pathogens, this precipitation gradient acts as a putative selection gradient for *A. gerardii* *R* (resistance) genes. The maize locus *Rxo1* is responsible for resistance to the pathogenic bacteria *Burkholderia andropogonis* and homologs have been identified in *A. gerardii*. Sequence analysis of *Rxo1* genes in *A. gerardii* have identified polymorphisms both within and among individuals. Populations of *A. gerardii* have been sampled from Ohio to Colorado for analysis of *Rxo1* homolog variation both within and among populations. Results may elucidate the relationship between *R* gene composition and ecological pressures.

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Wheat Plant Gene Expression in Response to Russian Wheat Aphid Feeding

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The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is a serious pest of world wheat and barley crops, causing losses of ~00 million (U.S.) annually. Eleven *D. noxia* resistance genes in cereal crop plants have been mapped and their phenotypic effects categorized as reduced aphid growth rate and/or increased compensatory plant growth. *Dnx*, a member of a wheat *Dn* gene family, is located on the short arm of wheat chromosome 7D and is resistant to *D. noxia* biotypes 1 and 2 via antibiosis and tolerance. A SSH EST library constructed with *Dnx* and *Dn0* (susceptible) tissues revealed 47 highly up-regulated *Dnx* sequence clots elicited by biotype 1. Using microarray technology, results of recent studies indicate that *Dnx* plants up-regulate >500 ESTs for defense signaling, aphid toxicity and tolerance of tissue damage or water loss; and down-regulate ~50 ESTs for membrane secretion, translation, transcription and cell division. *Dnx* plants up-regulate biotype-specific response motifs varying in type and number of defense response.

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Elevated CO₂ disrupts Arabidopsis developmental timing through alterations in the autonomous floral pathway

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We examined the molecular, physiological, growth, and developmental responses of an experimental system of closely-related genotypes of *Arabidopsis thaliana* to growth at elevated atmospheric carbon dioxide (CO₂). The genotypes included: SG, a line with the highest selection response for increased seed yield at elevated CO₂; CG, a control line randomly selected at elevated CO₂; and two parental genotypes (P1 and P2) from which SG and CG were derived. We observed a disruption of the developmental program of SG when grown at elevated CO₂. Specifically, this disruption was characterized by both a 12 day delay in the time to floral initiation and a larger plant size at floral initiation for plants grown at 700 versus 380 ppm CO₂. In contrast, unselected CG, P1, and P2 showed similar time to floral initiation and size at floral initiation between both CO₂ treatments. To date the molecular mechanisms responsible for delayed floral initiation of elevated CO₂-grown plants has yet to be elucidated. Therefore, we used real-time RT-PCR to examine the expression patterns of floral development genes through the development of CG and SG plants grown at current (380 ppm) and elevated (700 ppm) CO₂. These genes were: FLC, a floral repressor gene found in both the autonomous and vernalization floral pathways; SOC1, a gene that integrates floral signals from several flowering pathways; and LEAFY, a floral meristem identity gene. We found similar patterns and timing of expression of FLC, SOC1, and LFY between CG plants grown at current and elevated CO₂. The expression of FLC in current CO₂-grown SG plants decreased dramatically prior to flowering but remained very high in elevated CO₂-grown SG plants. The expression of both SOC1 and LFY up-regulated prior to visible floral initiation in both current and elevated CO₂-grown SG plants; however, this up-regulation was delayed in plants grown at elevated CO₂. In conclusion, delayed floral initiation of SG plants grown at elevated CO₂ is mitigated through alterations in the autonomous floral signaling pathway and is likely related to changes in carbon metabolism associated with growth at elevated CO₂.

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Ecological Genomics of Termites (Isoptera)

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Termites (Isoptera) are hemimetabolous eusocial insects that live in complex societies. Termites exhibit eusociality not unlike that found in ants and some bees and wasps: presence of castes (reproductives, soldiers, workers), polyphenisms (one genotype developing into several specialized phenotypes), multiple developmental pathways, complex communication, symbiosis with protozoans and bacteria, construction and maintenance of complex nests, and use of "agriculture." Whereas Kin Selection explains the maintenance of eusociality in haplodiploid Hymenoptera, a comparable theory for termites, in which both sexes are diploid, is not available. Since all extant termites are eusocial, comparisons between non-eusocial and eusocial taxa are not possible. Finally, termites differ

in substantial ways from honey bees. Thus, genomic studies on termites will provide information on the genetic basis of cooperation and social living and lead to comparative studies with evolutionarily divergent honey bees, ants, and some wasps. We have initiated studies on the ecological genomics of eusociality in termites. To this end, we have constructed five caste- and life stage-specific cDNA libraries for the termite *Reticulitermes flavipes*. We are in the process of sequencing 15,000 ESTs and characterizing the genes. A consortium of termite researchers has been assembled to collaboratively and synergistically utilize the resources being developed. The ongoing studies and the resources being developed are expected to significantly advance our knowledge of ecological genomics of termite eusociality. They will also help elucidate the interactions between genes, behavior, and environment that together result in a highly complex society encompassing many traits also observed in human societies.

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The Genetic Basis of High-Altitude Adaptation in Deer Mice

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Elucidating genetic mechanisms of adaptation is a goal of central importance in evolutionary biology, yet few empirical studies have succeeded in documenting causal links between molecular variation and organismal fitness in natural populations. Here we report a population genetic analysis of a two-locus alpha-globin polymorphism that underlies physiological adaptation to high-altitude hypoxia in natural populations of deer mice, *Peromyscus maniculatus*. This system provides a rare opportunity to examine the molecular underpinnings of fitness-related variation in protein function that can be related to a well-defined selection pressure. We surveyed DNA sequence variation in the duplicated alpha-globin genes of *P. maniculatus* from high- and low-altitude localities (i) to identify the specific mutations that are responsible for the divergent fine-tuning of hemoglobin function, (ii) to assess the functional consequences of these mutations with respect to hemoglobin-oxygen affinity, and (iii) to test whether the genes exhibit the expected signature of diversifying selection between populations that inhabit different elevational zones. Results demonstrate that functionally distinct protein alleles are maintained as a long-term balanced polymorphism, and that adaptive modifications of hemoglobin function are produced by the independent or joint effects of five amino acid mutations that modulate oxygen binding affinity.

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Assessing the spatial diversity of alder-associated *Frankia* along a high latitude elevation gradient.

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In Alaska, the expansion of woody shrubs into arctic tundra in response to climate warming represents one of the most significant recent changes to the high latitude terrestrial biosphere. Alders (e.g. *Alnus fruticosa*) are unique among arctic shrubs because of their symbiotic association with *Frankia*, a gram positive diazotroph responsible for a substantial portion of ecosystem-level N inputs. Though some views in microbial community ecology suggest that bacteria are universal in their distribution and functionally redundant, we hypothesize that differences amongst alder-infective strains of *Frankia* may play an important role in the expansion of alder within tundra communities. As part of a larger research effort aimed at exploring the effects of genotype-genotype variation in the *Alnus-Frankia* symbiosis on N-fixation potential, we characterized the spatial structure of a population of alder-associated *Frankia* along an expanding toposequence of *A. fruticosa*. Specifically, our interests were threefold: 1) the overall diversity of nodulated *Frankia* 2) the genetics of *Frankia* in well established plants versus colonizing plants at the top of the toposequence, and 3) the spatial scale at which *Frankia* symbionts are structured. Our field site was E-SE facing alpine tundra community ranging in elevation from ~700-1200m. *A. fruticosa* exists in discreet patches throughout this community with established plants at the bottom of the slope and relatively isolated saplings at the leading edge. In late July 2003 we collected root nodules along the toposequence from 14 individual alder genets ranging in size from saplings to mature plants. Our sampling effort employed a hierarchical approach that included: 1) multiple lobes on the same nodule, 2) multiple nodules positioned throughout the same root length, and 3) nodules from root lengths collected at 4 sampling points (upslope, downslope, left, and right) surrounding each tree. Nodules (n = 142) were surfaced sterilized, lyophilized, and ground with a mixer mill. We isolated amplifiable DNA from nodules using a Qiagen Plant DNeasy kit modified for gram-positive bacteria by the addition of lysozyme and selectively amplified *Frankia* DNA at three loci: the glutamine synthetase gene, the 16S-23S spacer, and the *NifD-K* spacer using redesigned and published primer sets. A subset of amplicons from each loci were sequenced in both directions.

Based on the polymorphisms seen in those sequences, we carried out targeted PCR-RFLP analyses of 188 DNA extracts (142 nodules plus replicate extractions). Though overall diversity amongst the Frankia was low, we discovered three haplotypes at each locus. Two of the haplotypes (Hap1 and Hap2) were separated by only a SNP while the third haplotype (Hap3) was quite divergent. There was no evidence of recombination for Hap3, but we found all possible combinations of Hap1 and Hap2 at each locus. In most instances, however, RFLP analysis revealed primarily 1:1 and 2:2 pairings suggesting multilocus linkage disequilibrium. Variation within a root length and within a tree was minimal; however, we did detect a clumping of multilocus haplotypes amongst trees, indicating a strong spatial structure across the population of alders investigated. These clumps were oriented parallel to the slope suggesting a localized spread of infective strains, possibly through water tracks. Interestingly, saplings at the top of the toposequence were colonized by the more divergent third lineage while trees and saplings further downslope were not. While nothing is known of the physiological aspects of these host-symbiont pairings, our results are consistent with the hypothesis that novel lineages of Frankia may play a role in facilitating the expansion alder in arctic and subarctic populations.

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Ecological Genomics and Climate Change in the Tallgrass Prairie

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In a previous study (Travers et al., in press) we found that field manipulations of precipitation variability predicted from climate change models resulted in consistent changes in gene expression of the dominant grass in the tallgrass prairie, *Andropogon gerardii*. However, it is unknown if these changes in transcription ultimately translate into phenotypic differences that may influence survival and reproduction (e.g. photosynthesis, growth). We conducted a second field study in order to correlate physiological measures of photosynthetic activity with expression levels of over 12,000 genes using a microarray analysis of the transcriptome of *Andropogon gerardii* across a range of environmental conditions. We also simultaneously sampled a closely related species of the tallgrass prairie, *Sorghastrum nutans*, in order to compare the transcriptional profiles of a less common species of grass to the keystone species, *A. gerardii*.

Across a range of environmental conditions predicted from climate change models, there were statistically significant relationships between gene transcription levels at eleven loci and all but one of the five physiological variables. Despite similar environmental conditions, the two species did not share any genes that were significantly related to the physiological traits. Decreased photosynthetic activity associated with heat shock protein production may indicate a cost of stress response in *A. gerardii* that is absent in *S. nutans*.

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Phylogenetic Implications of mtDNA Sequence Variation in Deer Mice from California

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Walker et al. (2006) revealed low levels of mtDNA sequence divergence indicating that the populations of *P. sejugis* are insular isolates of the *P. maniculatus* from Baja California and suggesting the existence of a distinct *P. maniculatus* (Baja)/*P. sejugis* haplogroup. To identify the northern extent of this haplogroup, we compared sequence variation in the ND3/ND4L/ND4 region of the mtDNA for 99 individuals of *P. maniculatus* from California and compared these to reference sequences for *P. maniculatus* from Baja California, Colorado, Oregon and Washington, and to *P. sejugis* and *P. keeni*. The level of sequence divergence (0.825%) reveals a close association between the *P. maniculatus* from Baja California and those from southern and central California. However, comparison of the southern California and Baja populations to those from northern and eastern California resulted in substantially higher sequence divergences (3.92 and 3.76%, respectively). Comparison of the *P. maniculatus* from Colorado, Oregon and Washington to those from northern and eastern California yielded low percent sequence divergences (0.80 and 1.75); comparisons to the populations from southern and central California

yielded values suggestive of interspecific divergence (4.17% and 3.99%). These data suggest that the Baja *maniculatus/sejugis* haplogroup extends west from the Sierra Nevada Mountains and from Baja California north to include southern and central California. Consistent with biogeographic and taxonomic data for other southwestern sigmodontine rodents, we hypothesize that the Baja *maniculatus/sejugis* haplogroup represents a distinct Pleistocene peripheral isolate of *P. maniculatus* and that *P. maniculatus* as currently recognized is polyphyletic.

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Genetic Network Modeling at the Ecological Population Level

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Systems biologists are developing genetic network models of an increasing number of biological processes at the individual or lower levels. However, models at higher levels of organization are needed to understand how gene-by-gene and gene-by-environment interactions contribute to evolutionary fitness. Elevating network models to the population level presents significant theoretical challenges in terms of (1) the proper mathematical formulation to use, (2) integrating submodels of processes whose functioning is understood to variable levels of refinement, (3) computational intractability. We are constructing a gene-based, life-cycle model of *Arabidopsis thaliana* with submodels for (1) germination, (2) development to flowering, and (3) reproduction. The respective submodels include (1) temperature, light quality, hormonal, and moisture effects on seed dormancy release and events leading to radicle emergence, (2) vernalization and photothermal effects on the phenology of floral commitment, (3) temperature effects on the development of reproductive structures and seeds. Plant developmental and survivorship data is currently being taken from field studies in Europe, along with in situ microclimatological measurements.

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Exploring the links between plants, viruses, vectors, and their environment

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Arthropod vectors play an essential role in dissemination of viruses; more than 70% of plant-infecting viruses are transmitted from one host to another by arthropod vectors. Our research is devoted to investigating plant-virus-vector interactions with the goal of developing a better understanding of the complex sequence of events leading to virus acquisition and transmission by vectors. Our long-range goal is to use arthropod genomics tools to develop novel and biologically-based strategies that specifically target and control insect pest populations without compromising the health of non-target organisms. We are working with the following arthropod vector and plant virus combinations: 1) *Frankliniella occidentalis* (Western flower thrips) and Tomato spotted wilt virus (TSWV); 2) *Peregrinus maidis* (corn planthopper) and Maize mosaic rhabdovirus (MMV); and 3) *Aceria tosichella* (wheat curl mite) and High Plains virus (HPV). Our research goals are to identify insect genes that are important for virus infection of the arthropod vectors using a functional genomics-based approach and to develop diagnostic tools for virus detection and monitoring in the plant and vector hosts. At the ecological level, we are interested in identifying key environmental factors that influence the geographical distribution of arthropod vectors and their relationships with the viruses they transmit.

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Genetics of drought adaptation in *Mimulus*

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Local adaptation has been widely documented in plants, but far less is known about the traits conferring this adaptation to specific environments. Water availability is a fundamental determinant of plant distributions, and likely has been an important selective factor in shaping the evolution of numerous species complexes.

Consequently, plants have evolved several strategies to cope with water limitation, which involve morphological, physiological, and life history traits. Here we use a quantitative trait locus (QTL) mapping approach to examine the genetic architecture of putatively adaptive drought response traits that differ between *Mimulus guttatus* and *M. nasutus*, two closely related flowering plants with contrasting mating systems. We constructed a linkage map from a

large F2 mapping population with >150 EST markers that is largely consistent with other maps in *Mimulus* based on shared collinear markers. Analyses have identified several regions with putatively strong effects on water-use efficiency (d13C) and flowering time, including one co-localized on LG13 that maps to approximately the same location as floral QTL detected in previous analyses of both inter- and intraspecific crosses in *Mimulus*. The presence of genomic regions containing QTL with effects on both traits suggests a potential pleiotropic relationship that contributes to differences in drought response strategies; however, polygenic control of individual traits may allow these traits to evolve independently from one another.

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Ecological genomics of intermediary metabolism and life history trade-offs in a wing-polymorphic cricket

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The physiological-metabolic causes of life history trade-offs is a central but poorly understood problem in life history evolution. We have been investigating alterations in lipid and amino acid metabolism underlying the trade-off between flight-capability (somatic allocation) and reproduction in a wing-polymorphic insect. The polymorphism, found in natural populations, consists of a flight-capable morph that delays reproduction and a flightless morph with substantially enhanced early-age reproduction. The flight-capable morph accumulates substantially greater lipid reserves (flight fuel) which causes it to decrease allocation of nutrients to egg production. The basis of increased lipid accumulation is mainly due to increased biosynthesis of fatty acids, increased diversion of fatty acid to triglyceride biosynthesis, and increased conversion of amino-acids to fatty acids. Global increases in activities of lipogenic enzymes underlie the increased flux through lipogenic pathways in the flight-capable morph. To identify the molecular and biochemical bases of increased activities of lipogenic enzymes we are purifying four representative enzymes and cloning the genes encoding these enzymes: NADP-isocitrate dehydrogenase (NADP-IDH), glucose-6-phosphate dehydrogenase (G-6-PDH), ATP-citrate lyase (ACL), and fatty acid synthase (FAS). A full-length NADP-IDH cDNA has been obtained and sequenced, the enzyme protein has been purified to homogeneity, and antibodies are being raised against this protein. DNA sequence, transcript abundance, enzyme protein concentration, and degree of postranslational modification will be measured on IDH, and subsequently, the other representative genes, in genetic stocks nearly-purebreeding for flight-capable or flightless morphs.