



Ecological Genomics Symposium  
November 4 - 6, 2005 • Kansas City

Kansas NSF EPSCoR

## POSTER ABSTRACTS

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### **Gene x environment interactions in *Caenorhabditis elegans* using a genetical genomics approach**

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Gene x environment interactions (GEI) relate to non-additive effects of a genotype and its environment on the phenotypic expression of a particular trait. Such interactions play an important role in the evolution of life-histories under changing environmental conditions. Although previous molecular studies have unravelled specific genes affecting shade avoidance in plants and heat shock response in *Drosophila*, knowledge about the genetic pathways involved in GEI of life-histories is scant. We investigated the genetic architecture of gene x temperature interactions in a mapping population of the bacterivorous nematode *Caenorhabditis elegans*. We mapped several QTLs underlying body size changes at high and low temperatures. After extracting RNA we hybridized all RIL samples according to Fu & Jansen<sup>1</sup> (in prep) using whole genome oligo arrays (obtained from GSC, Washington University, St. Louis). QTL analysis was run on the expression profiles of the RILs. Using this genetical genomics approach we hope to identify gene networks underlying temperature body size changes.

<sup>1</sup>Fu, J., & R. Jansen How to double the power of half the arraying cost in genetical genomics. (in prep)

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### **Phosphotyrosine Residues of Kirre Mediate Muscle Fusion in *Drosophila***

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In *Drosophila*, somatic muscle is formed by the fusion of two cell types, founders and fusion competent cells (fccs). Kirre is expressed specifically on founder cells where it plays a role in the attraction, adhesion, and fusion. Kirre encodes a transmembrane protein with 5 extracellular immunoglobulin (Ig) domains and an intracellular autophosphorylation domain similar to receptor tyrosine kinases of vertebrates. Tyrosine phosphorylation plays a crucial role in activating this type of receptor. There are 11 tyrosine residues in the intracellular region and 2 are located in the autophosphorylation domain of Kirre. To investigate the function of these tyrosine residues in muscle fusion signaling we have produced transgenic flies in which the transgenic kirre gene has either all 11 or only 2 tyrosine residues into phenylalanine. When expressed in a Kirre deficient background, 11 Y-->F is unable to rescue muscle fusion. However, the transgene is sufficient for to attract and adhere the fccs to the founders. In rare instances some fusion does occur. These results indicate that the tyrosine's residues within Kirre are important for intracellular signaling. The transgene with mutations of only 2 Y-->F in the autophosphorylation region showed more fusion but the resulting muscle pattern does not match the wild type pattern. Taken together our results indicate that phosphorylation on tyrosine is required for kirre activity. Also it seems that tyrosines outside of the autophosphorylation domain are able to compensate for the 2 Y-->F mutations.

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### **Eavesdropping Plants: the molecular ecology of Arabidopsis responses to green leafy volatiles**

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Green leafy volatiles are released by plants when wounded mechanically or by herbivory. In corn seedlings, this complex mixture of six carbon aldehydes, alcohols, and esters has been shown to prime the release of indirect defenses (volatile sesquiterpenes) in neighboring seedlings. Plants with prior exposure to green leafy volatiles produce greater amounts of sesquiterpenes than plants without prior exposure in response to subsequent herbivore attack, simulated with mechanical damage and caterpillar regurgitant. The way in which plants detect and respond to green leafy volatiles is unknown, and we have begun a study of the effects of green leafy volatile production in *Arabidopsis thaliana*, a species with a sequenced genome and partially characterized defense signaling pathways. Here we report that *Arabidopsis thaliana* responds to green leafy volatile components with 1) increased production of direct defenses (aliphatic glucosinolates), 2) accelerated induction of direct defenses by caterpillar feeding, and 3) up-regulation of over a dozen genes including receptor like kinases, DNA-binding proteins, and ABC transporters. Significance of these findings to plant perception of green leafy volatiles and response signaling is discussed, as well as approaches to study their ecological significance.

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### **Identification of *Daphnia pulex* genes involved in resistance to the toxic cyanobacteria, *Microcystis aeruginosa***

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Cyanobacteria, important primary producers in the aquatic food chain, grow in estuarine, coastal marine and freshwater systems, including drinking water sources. Large visible blooms, often associated with eutrophic systems, deplete dissolved oxygen and release phosphates from the sediment, causing further eutrophication. A large number of these blooms are toxic, producing neurotoxins or hepatotoxins (microcystin, MC). MC is produced by three major genera of cyanobacteria: *Anabaena*, *Oscillatoria* and *Microcystis*, and has more than 50 structural variants. MC has a variety of adverse effects on animals, including *Daphnia*, and has been linked to primary liver cancer in humans. A natural isolate of *Daphnia pulex* (ODR) from the Old Durham Reservoir, NH, USA, seems to have evolved a unique resistance to toxic *Microcystis*. Our goal is to identify the genes involved in this resistance through a gene expression study comparing ODR to a *Microcystis* sensitive strain of *D. pulex*.

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### **High temperature effects on expression of genes involved in Russian wheat aphid-bread wheat interactions**

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The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) is a major pest of bread wheat, *Triticum aestivum* L. (em Thell), in most wheat-growing areas worldwide. Aphid-resistant cultivars are used to combat this pest but very little is known about the molecular basis of resistance and high temperature effects on aphid-wheat interactions. In this study, differential gene expression in *D. noxia* biotype 1-resistant wheat plants containing the Dnx gene and *D. noxia* biotype 1 feeding on Dnx plants was investigated using suppressive subtraction hybridization. The derived subtracted cDNA library includes sequences similar to Pto and Pti1 - genes involved in gene-for-gene recognition of and resistance to bacterial speck disease in tomato, *Lycopersicon esculentum* (L.). Pto and Pti1-like sequences contain an activation domain with conserved amino acid residues crucial for avr protein recognition and binding by Pto, and avr-Pto phosphorylation of Pti1. Wheat defense signaling is represented by sequences putatively involved in producing sterols, jasmonates, Ca<sup>2+</sup>, abscisic and gibberelic acids. Reductions in populations of *D. noxia* fed Dnx plants is likely related to the expression of sequences involved in defensive chemical production, cellular transport and exocytosis. Dnx plant tolerance of *D. noxia* feeding is proposed to be based on the expression of sequences putatively involved in self-defense against reactive oxygen species and toxins, proteolysis, DNA, RNA and protein synthesis, chloroplast and mitochondrial function, carbohydrate metabolism and maintenance of cell homeostasis. *D. noxia* unsuccessfully counter Dnx by differentially

expressing sequences putatively involved in detoxification, proteolysis, DNA, RNA, protein and lipid synthesis, carbohydrate metabolism and mitochondrial function.

A set of sequences described above was used to produce macroarrays containing resistance and defense response gene sequences expressed in resistant wheat plants being infested with *D. noxia* adults for 48 hr at 27°C and *D. noxia* sequences differentially expressed at 48 hr post-infestation. The macroarrays were used to identify changes in expression of these genes in resistant wheat plants and *D. noxia* at 48 hr post-infestation at 37°C. The results presented provide a first insight into temperature effects on resistant wheat and aphid gene expression.

## 6

### **The effects of body size, relatedness, and sperm storage on male reproductive success in an aggregate breeding amphibian (*Ambystoma maculatum*)**

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The outcome of sexual selection on males may depend on female mate choice and male-male competition, as well as post-copulatory processes such as sperm competition and cryptic female choice. We studied the outcome of sexual selection in the spotted salamander (*Ambystoma maculatum*), specifically examining the role of body size and relatedness to potential mates on male reproductive success. Using controlled mating experiments in the field, we gave females access to three males of different sizes. We then used seven microsatellite loci to determine paternity in the resulting larvae and to estimate relatedness,  $r$ , between females and their mates. We also calculated the average  $md^2$ , a measure of genomic divergence, across all larvae and compared it to values predicted by simulations under the assumptions of random mating and random mating with reproductive skew. Male body size did not affect reproductive success, suggesting a limited role for male-male competition in sexual selection in this system. Relatedness also did not significantly affect male reproductive success, and the relatedness of the males available to a female did not significantly influence the proportion of larvae sired by stored sperm. However, the average genomic divergence of the offspring was lower than predicted by chance, but not when the expected value from simulations incorporating reproductive skew were compared with the observed values of larvae not sired by stored sperm. The genomic divergence of larvae sired by stored sperm was significantly lower than that of larvae sired by the males in our experimental chambers. These results suggest that relatedness to mates may still influence a male's reproductive success by determining whether his sperm is stored for later breeding seasons. Sexual selection is likely to involve a complex interaction among many factors in this species.

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### **Environmental Regulation and Modeling of Flowering Time in *Arabidopsis***

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*Arabidopsis* flowering time is regulated by internal and environmental factors, such as light and ambient temperature. Four major pathways process environmental and internal growth status information, ultimately stimulating the production of flower meristem identity genes. We are using microarray analysis and real time RT-PCR to identify genes that are differentially expressed at different ambient temperatures, before the floral transition. Such genes may play a role in the temperature-mediated regulation of flowering time. In addition, we are developing a mathematical model that predicts flowering time based on the dynamics of gene expression over time, as influenced by the environment. In particular, a three-gene regulatory loop acts as a bistable switch to convert the apex to the reproductive stage and promote the formation of flowers. This model provides a framework for linking two common measures of flowering time, days to flowering and total leaf number. It also accounts for a rare but exquisitely precise developmental abnormality.

### **Changes in nematode gene expression in response to soil bacteria**

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We are using resident nematode populations sampled from the Konza Tallgrass Prairie Biological Station near Manhattan, Kansas, to link the responses of organisms to environmental change at the genetic level. Previous studies of nematode communities at Konza have shown trophic level responses to disturbances including nitrogen addition, altered soil moisture, and changes in 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. In addition, studies of bactivorous nematodes are 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. Almost all that is known about gene function in *C. elegans* has been determined in this environment. Yet, many of the genes in *C. elegans* genome have not been assigned a function. 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. We subsequently used it as a food source to develop a soil culture system for *C. elegans*. To begin analyzing genes involved in responses to altered soil environments, we used cDNA microarrays to identify genes that were differentially expressed in response to food resources. We initially compared plate-grown *C. elegans* fed either *E. coli* or *M. luteus* using an experimental loop design. 142 genes were differentially expressed in response to growth on *M. luteus*. Up-regulation of several of these genes was verified by real-time PCR. Surprisingly, no genes were significantly up- or down-regulated by growth in soil culture relative to growth on agar plates. These results indicate that differences in bacterial environment, or food sources, are of prime importance while substrate, agar or soil, is less important.

Viable loss of function mutants were available for five genes that were up-regulated by growth on *M. luteus*. This allowed us to easily test whether any of these genes “matter” for *C. elegans* responses to soil bacteria. In order to examine the contribution of these genes to nematode fitness in different bacterial environments, we have examined brood sizes. Mutation of four of these genes caused statistically significant reductions in brood size when grown on *M. luteus*. Furthermore, mutant phenotypes have not been reported for two of these genes. Our results show that expression of specific genes induced in response to a change in environment, can contribute to fitness traits. Our results demonstrate that assessment of *C. elegans* gene functions in a more natural context can identify functions for genes of unknown function.

### **Semi-conserved transcription factor binding sites in the Yeast genome.**

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Comparative genomics is now widely used to identify non-coding DNA sequences that are functionally constrained between genomes. The underlying assumption of these methods is that functional sequences will be conserved between genomes. While sequence conservation at appropriate evolutionary distances is a strong indicator of function, functional sequences which are either species specific or specific to a subset of related species also exist.

We have developed a computational method to identify semi-conserved transcription factor binding sites among multiple *Saccharomyces* genomes. Semi-conserved binding sites demonstrate clear evidence of functional constraint across 3 of 4 species examined, and lineage specific loss of constraint in one species. We predict these semi-conserved binding sites are indicative of cis-regulatory changes that have occurred in a lineage specific manner during the evolution of the *Saccharomyces* genomes.

A genome wide analysis of 90 transcription factors, whose binding sites are defined, reveals that between 3 and 6% (669 to 1443) of the functionally constrained transcription factor binding sites fit a semi-conserved pattern, with the loss of constraint occurring in *S. cerevisiae* or one of its two closest relatives. For some transcription factors as many as 25% of the functionally constrained binding sites are semi-conserved. 10 promoters containing semi-conserved binding sites for the transcription factor MSN2/4 have been cloned and the function of these binding sites in the related Yeast species is being examined.

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### **Genetic architecture of fitness in the nematode *Caenorhabditis elegans***

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Although the concept of fitness is the basis of evolutionary theory, the genetic architecture of fitness remains one of the least explored areas. Recent studies indicated high additive genetic variance for fitness and fitness-related traits. This situation is explained as the result of high number of loci underlying fitness traits – large target for mutations. However, the knowledge about approximate number of loci influencing fitness in a particular environment and their ‘per-locus’ additive contribution to fitness are still highly speculative.

We estimated relative fitness in optimal environmental conditions for a set of approximately 60 recombinant inbred lines (RILs) of the nematode *Caenorhabditis elegans*, derived from the crosses between two well-studied strains: N2 (Bristol) and CB4856 (Hawaii), in a competition experiment against the modified PD4792 line. We expect the analysis to reveal the distribution and relative importance of loci underlying fitness. The genomic regions of interest will be analysed by implementing candidate gene approach, introgression lines analysis and mutant analysis.

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### **Doing it until it hurts: effects of age, behavior, and tissue heterothermia on heat-shock protein activity in honey bees**

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In this study we investigated the relationship between honey bee adult behavioral development and heat shock proteins (Hsps), which are highly-conserved molecular chaperones that interact with other proteins that are in non-native conformations, whether due to protein-denaturing stress or because the peptides they comprise have not yet been fully synthesized, folded, assembled, or intracellularly localized. Adult honey bees spend their first 2-3 weeks working inside the constant environment of the hive (33-35°C and 70-80% RH) which is maintained by the endothermic shivering of the flight muscle, wing-fanning, evaporation via regurgitation and clustering of individuals. At about 3 weeks of age workers leave the hive and are exposed to a more variable environment as foragers who gather pollen and nectar. Foragers warm their thoraces (which house the flight muscles) to about 37°C before flight, during which the thoracic temperatures remain higher than brain or abdominal temperatures at all air temperatures. Specifically, we measured the activity of Hsp70 family genes and proteins in honey bee brains and thoraces across a variety of laboratory temperatures and in individuals of different ages and behavioral groups. Constitutive expression of Hsp70 family proteins was higher in the thoraces of foragers than in those of hive bees. Significantly higher levels of hsp70 but not hsc70 mRNA were present in the thoraces of foragers in comparison to hive bees. There was no significant difference in brain Hsp70 protein levels among behavioral groups, although hsp70 and hsc70 mRNA levels in the brain vary with behavioral group. Maximum induction in both tissues occurs at air temperatures of 48°C in the laboratory. The difference in thorax protein and mRNA expression in foragers vs. hive bees likely reflects prolonged thoracic hyperthermy and flight muscle damage in foragers.

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**Habitat Avoidance as an Integral Component of Ecological Speciation**

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What forces drive ecological speciation in sympatry? Current genetic models of adaptive divergence emphasize the importance of preferences for distinct habitats based on sensory cues. In this scenario, populations are kept from close contact by divergent preferences that allow them to discriminate between different niches. The consequence is a decrease in gene flow and effective premating isolation. However, recent studies show that avoidance of non-ideal habitats may be equally important in habitat choice, a finding that calls for a re-working of theoretical models of ecological speciation. Here we present three basic host discrimination models that evaluate the implications of habitat avoidance genes for a sympatrically speciating system

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**QTL Analyses of Host Odor Discrimination in the Phytophagous Insect *Rhagoletis pomonella***

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Host odor discrimination may play a key role in the ecological divergence between haw and apple tree populations of the phytophagous fly, *Rhagoletis pomonella*. Both haw and apple populations of *Rhagoletis* demonstrate strong fidelity to their natal hosts. Host recognition depends in part on olfactory cues: recent studies have suggested that both attraction to natal host odors and avoidance of non-natal host odors are important for host fidelity. Olfaction in flies may be governed by 60 or more loci, identified by recent bioinformatic analyses of the *Drosophila* genome. However, each locus may have a particular olfactory role, and odor attraction and avoidance can be modeled by the interaction of only two or three loci (see complimentary poster). Thus, we seek to identify specific regions of the *Rhagoletis* genome that are associated with haw and apple odor discrimination through the application of linkage maps and QTL analyses. Here, we present our ongoing efforts to identify the genomic regions and loci responsible for host odor recognition in *Rhagoletis pomonella*.

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**Applying microarrays in ecological field experiments**

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As microarrays for the study of gene expression become more readily available, their application in ecological field experiments will increase. Ecological field experiments often have more complicated designs than the designs of early microarray experiments, including split-plot and strip-plot designs. When microarrays are applied in this context, several issues arise. First, microarrays for ecologically important species may not be available. If microarrays for related species are employed, hybridization rates may be lower and the variability in intensity may be higher. The frequency distribution of the observed intensity for genes of interest should be studied to determine which samples can be included in analyses. Second, if spotted arrays are used, two dyes may be used on the same array, so that samples are paired. This pairing structure then needs to be superimposed on the experimental design in the field. Changing the structure for superimposing the pairing will generally change which experimental treatments can be analyzed with greater statistical power. Third, though ratios of intensities have commonly been used in microarray analyses, use of ratios will tend to be less desirable in the context of more complicated experimental designs.

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**Alpha-globin evolution at a high elevation contact zone**

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Recent discovery of a high altitude contact zone between two clades of deer mice provides a unique opportunity to observe patterns of adaptive variation in hypoxia tolerance. To understand the processes that have shaped this

variation, we are reconstructing the evolutionary history of deer mice on the western flank of the Sierra-Nevada using multi-locus genetic data. Using DNA obtained at two high elevation sites and a low elevation contrast, we are generating nucleotide sequences for four loci: two paralogs encoding the alpha chains of hemoglobin, an intron within the B-fibrinogen locus, and the mitochondrial cytochrome b gene. By contrasting patterns of nucleotide variation across these loci, we are building on electrophoretic studies of *P. maniculatus* that suggest an adaptive role for alpha-globin proteins in the face of selective forces governed by native elevation. The ultimate goal of this study is to understand the evolutionary trajectory of a trait's architecture and its consequences for population structure in a secondary contact zone.

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### **Analysis of *fed-2/rsd-2***

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The introduction of a specific dsRNA sequence by injection, feeding, soaking, or by in vivo transcription from transgenes can lead to systemic silencing in distal tissues and also in the progeny of the treated worm. I work on an RNAi defective mutant *ne319*. *ne319* failed to complement *rsd-2* and there is a premature stop codon in *rsd-2* sequence of *ne319*. We conclude that *ne319* is an allele of *rsd-2*. We report our genetic and phenotypic analysis of *rsd-2*.

## 17

### **Ecological Genomics: Evolution of Plant Defense in Competitive Environments**

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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, currently the best framework we have to understand the simultaneous evolution of defense and competitiveness, predicts a tradeoff between these factors. Plants that effectively grow and compete well against neighbors are expected to have lower defense levels either because growth diverts limited resources away from defense production, or because less valuable tissue that is consumed is more readily replaced. In contrast to these predictions, some recent studies have found evidence that some plant species may be able to compete and defend. One hypothesis for this result is that some defensive traits, such as toxin concentration, have dual functions in defense and competition. In two growth room experiments in which we examined transcript profiles of a close wild relative of *Arabidopsis thaliana*, we tested (1) whether neighboring plants elicit defense responses, and (2) whether there was overlap in gene expression patterns between herbivory and competition treatments that were either complementary or antagonistic. In one experiment involving three treatments (herbivory, competition, control) there were over 900 significantly differentially expressed genes, and evidence that competition elicits genes with known function in defensive pathways. Evidence will also be presented from a factorial experiment involving competition and herbivory.

## 18

### **Mating system and the evolution of quantitative traits: An experimental study of *Mimulus guttatus* (yellow monkeyflower)**

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The mating system of a population profoundly influences its evolution. Inbreeding alters the balance of evolutionary forces that determine the amount of genetic variation within a population. It redistributes that variation among individuals, altering heritabilities and genetic correlations among traits. Inbreeding even changes the basic relationships between these genetic statistics and response to selection. Here, we report the results of an experiment that addresses the following question: If populations differing only in mating system are exposed to the same selection pressures, will they respond in qualitatively different ways? Selection was imposed on an index of two negatively correlated traits (flower size and development rate) on populations that reproduced (a) entirely by outcrossing, (b) entirely by self-fertilizing, or (c) by a mixture of outcrossing and selfing. Selfing populations responded mainly by evolving larger flowers while outcrossing populations also evolved more rapid

development. Divergence occurred despite the fact that the selection regime was the same and mating system had no direct effect on fitness.

## 19

### **Bioinformatics Grid Service Recommendation in Gene Expression Profiling**

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This paper describes preliminary work on using graphical models to represent relational data in bioinformatics portals such as myGrid. The objective is to generate automated recommendations of software components in the domain of gene expression modeling especially using microarray data. Users in this domain work with online data, metadata, source code, desktop applications, web services and computational grid services. In this study, we develop an ontology and data model for gene expression profiling using data normalization and clustering algorithms for CDNA microarrays.

We present an implementation of this ontology for TIGR's TM4 suite of applications (especially the Multi-Experiment Viewer, which features many clustering algorithms. MEV provides a set of data transformations used to prepare for data mining and generates clustering output than can be visualized using other MEV components.

In addition, a number of graphical model toolkits that support gene regulatory network and pathway modeling.

We then discuss the development of a content-based and collaborative recommender system for MEV components and how it is extensible using description languages such as SCUFL from the European Molecular Biology Lab (EMBL) and European Bioinformatics Institute (EBI), and the TAVERNA editor (Oinn, 2003). We compare this recommender system to others that have been used in bioinformatics domains and examine the potential transfer to an integrative domain such as ecological genomics.

Recent systems such as ResearchIndex / CiteSeer provide collaborative recommendation through citation indexing, and systems such as SourceForge and the Open Bioinformatics project provide similar tools such as content-based indexing of software. Our current research aims at learning probabilistic relational models (PRMs) from data in order to support intelligent retrieval of data, source code, and experimental records. We present a system design and a précis of a test bed under development that applies PRM structure learning and inference to CF in repositories of bioinformatics data and software.

## 20

### **Changes in Nematode Species Composition in Response to Environmental Cues**

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While much is known about the roles genes play in development and physiology, little is known about how the environment affects organisms at the level of expression of individual genes. Using resident nematode populations sampled from the Konza Tallgrass Prairie Biological Station near Manhattan, Kansas, we are attempting to link the responses of organisms to environmental change at the genetic level. We hypothesize that different organisms may have varying genetic capacities to respond to changes in the environment; either by differences in the genes they possess or in how those genes are regulated. We are currently testing these possibilities by looking at the responses of bacterial feeding nematodes to environmental changes. Research on Konza has shown that nitrogen addition and reduced frequency of burning greatly impacts the tallgrass prairie ecosystem. In addition to changes to plant communities, these perturbations have also been shown to alter nematode communities at the family level. However it is not known whether there are differential responses to treatment at genus level or below, which is necessary to determine the genetic causations for environmental response. Therefore, the objective of this study was to identify taxa that are sensitive to changes in nitrogen addition and burning régime using sequencing and realtime PCR probes for the 18S ribosomal RNA gene and the ITS1 region. We have currently identified 19 nematode taxa across four families. The results show that some nematode taxa within a family respond similarly to nitrogen and burning, while others within the same family respond quite differently. Additionally, although nematodes from different taxonomic groups on average respond differently, similar responses were seen in nematode taxa that span three taxonomic families. Current and future work will strive to explain these nematode responses to treatment at the genomic level. Microarray studies using

*Caenorhabditis elegans* grown on alternate bacterial food resources, as well as in altered environmental conditions (e.g., gradients of nitrogen addition, temperature, and osmolarity) will indicate candidate genes to assess in native nematode taxa. By utilizing a combination of model system genomics and field ecology, we are now on the way to connecting ecosystem responses to the genes that cause those responses.

## 21

### **Ecogenomics consortium in The Netherlands**

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The Ecogenomics consortium in The Netherlands concerns an innovative cluster, headed under "safe and sustainable agro-production systems" of the Netherlands Genomics Initiative. The general aims and strategy of the consortium are focussed on enhancing our understanding of the functioning of ecosystems, with the aim to unlock the full genetic potential for sustainable use of ecosystems for agricultural and other anthropogenic purposes. Because this project provides a unique chance to bring together an internationally competitive consortium of groups that so far have been operating in relative isolation, we aim that this proposal is the starting point rather than the end of generating a lasting centre of outstanding, internationally oriented ecogenomics research. The key aims of the current knowledge project are:

- to enhance our ability to apply the full genetic potential for bioremediation purposes
- to improve our understanding of the contribution of species within an ecosystem to ecosystem functions and biodiversity
- to enhance disease suppressiveness of soils
- to improve our ability to define and measure environmental quality and health of ecosystems by developing novel genomics-based bio-analytical tools.

## 22

### **Foraging (for) gene in the study of dispersal behavior of a predatory mite, *Phytoseiulus persimilis***

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*Phytoseiulus persimilis* (Acari: Phytoseiidae) is a specialist predator on tetranychid mites, especially on the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). The foraging environment of the predatory mites consists of prey colonies distributed in patches within and among plants. Previous studies have found that several behavior related to foraging are governed by genetic factor(s), therefore we are using quantitative and molecular techniques to investigate the genes related to variation in these behaviors. We are particularly interested in the resource (i.e., prey)-dependent dispersal behavior of the predator. In the course of developing molecular markers to study the foraging behaviors, we isolated a partial sequence that is presumed to be the orthologue of the foraging (for) gene, which we have named Ppfor (*Phytoseiulus persimilis* foraging gene). The for gene is associated with the rover-sitter phenotypes in fruit flies and is known for its role in foraging behaviors in other arthropods like honeybee and ant. We studied the use of the Ppfor gene to develop molecular markers for and understand behavioral phenotypes.

## 23

### **Expression of the foraging gene in migratory and non-migratory monarch butterflies (*Danaus plexippus*)**

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The North American monarch butterfly (*Danaus plexippus*) offers an excellent system for studying single gene effects on large, discrete behavioral differences within a species because of the largely different migratory and non-migratory modes of behavior. The annual fall migration has been well studied, however, the underlying molecular regulation of the migratory mode, which consists of behavioral, physiological, and life history changes is not well understood. To investigate the genetic differences between the migratory and non-migratory modes, we examine the mRNA expression of a candidate gene, Dpfor, by using real time quantitative RT-PCR (qRT-PCR). This gene is the *Danaus plexippus* ortholog of the foraging (for) gene, which codes for cyclic GMP-dependent protein kinase G (cGMP-PKG). Differential expression of for has been implicated in differing

foraging strategies in *Drosophila melanogaster* and task changes in *Apis mellifera*. Results from the qRT-PCR indicate that the mean expression of *Dpfor* was greater in non-migratory monarchs than migratory monarchs but the difference was not statistically significant because of large variance in *Dpfor* copy number among non-migrants.

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#### **Assessing Genetic Structure in Balinese Macaques and Its Implications for Disease Transmission**

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Recently several anthrozoonotic diseases have emerged on the Asian landscape. Primates are implicated as both pathogen hosts and reservoirs. Simultaneously, human alteration of habitats has increased. However, few studies have examined the relationship between pathogens, changes in existing landscapes, and primate populations. We are interested in how human manipulation of landscapes impacts macaque social and genetic structure and how these changes might influence pathogen ecologies among primates on Bali, Indonesia. Using a multi-locus approach, we are investigating distinct populations of long-tailed macaques (*Macaca fascicularis*) across Bali.

Our goal in this preliminary study is to dissect how anthropogenic ecosystem level changes are effecting macaque populations on a genetic level. We utilize PCR sequencing techniques of markers for the sex-determining region - Y chromosome (SRY) and the displacement loop (Dloop) region of the mitochondrial genome with DNA extracted from blood. Preliminary results from 43 individuals across six populations provide contrasting views for parental markers, reflecting macaque social structure. Maternal markers indicate populations are separated geographically along matriline and have maintained stability for long time periods. In contrast, Y-specific markers show that males move frequently between different populations indicating that males may be an important component for pathogen movement across Balinese landscapes. More accurate estimates of gene flow will be made using autosomal markers that average the migration effects of males and females. Further, genetic patterns of variation of the macaque populations will be overlain on the patterns of pathogens collected from the same populations to determine how macaque population structure can influence the evolution of disease itself.

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#### **A Molecular Approach for Using Crayfish to Determine Water Quality in Small Streams**

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The purpose of this study was to develop a biologically-based assay (bioassay) that could be used to test sources of water in and around St. Joseph, MO for compounds that could adversely alter some measurable biological activity. Initial studies attempted to bring water samples into the lab and investigate gross morphological changes during embryonic development using zebrafish embryos. These early studies revealed little changes in zebrafish development when exposed to water from each of the nine ponds on the Missouri Western State University campus. Since the interactions occurring between biotic and abiotic factors in the environment can often be very complex and difficult to mimic in the laboratory, we have since developed a protocol that places living organisms, crayfish, in various bodies of water under restricted conditions, and allows for changes to be assessed after a designated period of time. In this presentation, we report the results of one such study addressing the biological activity of water at different sites along Roy's Branch near its outflow into the Missouri River in St. Joseph, MO. Our results indicate that adverse changes in gene expression in crayfish tissues do occur in a site specific manner; however, the causative mechanism is yet to be determined.

### **The Genetic Basis of Standing Variation within *Mimulus guttatus* (Common Monkeyflowers)**

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A fundamental goal of evolutionary genetics is to describe how standing genetic variation is maintained and to assess its role in the divergence of traits observed between populations and species. Current models on how variation is maintained in populations fall into two broad classes: mutation-selection hypotheses and balancing selection hypotheses. We intend to experimentally distinguish competing hypotheses at the scale of individual loci affecting quantitative traits (QTL). Substantial standing genetic variation has been demonstrated in an Iron Mountain, OR population of *Mimulus guttatus* for flower size (an ecologically significant trait in terms of such factors as water use efficiency and pollinator visitation). To map genetic variation in a heterogeneous outbred population, we have chosen a replicated F2 QTL mapping scheme using three separate crosses between outbred parents with extreme floral phenotypes. Phenotypic measurements of F2 progeny exhibit similar distributions across all three crosses of floral traits but different distributions of fitness traits. The phenotypic correlations between floral and fitness traits also differed substantially among crosses, suggesting that QTLs are heterogeneous in their trait-fitness relationships. Preliminary marker data identify numerous genomic regions that are significantly associated with floral and/or fitness traits. Some QTLs exhibit no pleiotropy, some exhibit limited pleiotropy (affecting multiple floral traits but not fitness measures or vice versa), while some QTL affect both floral and fitness traits. Heterogeneity in pleiotropic effects among QTL is consistent with previous studies demonstrating that the deleterious mutation model (DMM) is not sufficient to explain observed levels of variation in flower size in the Iron Mountain population. Balancing selection, of some form, is thus indicated. Pleiotropy among morphological traits has been previously observed in crosses between perennial and annual *Mimulus guttatus* and between *Mimulus nasutus* (a small flowered selfer) and *Mimulus guttatus*, suggesting that pleiotropy has been important in the diversification of floral form within the species complex.

### **Role of house flies in the ecology of enterococci and associated antibiotic resistance and virulence genes**

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Antibiotic resistance genes in the gastro-intestinal microbial community of domestic animals and their feces/manure have become common in the United States due to heavy use of antimicrobial agents in animal productions for growth promotion and prophylaxis. Multi-drug resistance of clinical isolates has become a serious problem due to a progressive decline in the number of antibiotics effective for treatment of human infections. The connection between the antibiotic resistance of food animal origin and that of clinical isolates has been suggested in several studies; however, the ecology of antibiotic resistance and virulence genes in the environment is poorly understood.

Manure-borne insects, primarily house flies, potentially transmit antibiotic resistant bacteria from animal manure to residential settings and therefore represent a link between agricultural and urban environments. The ubiquity of enterococci in animal and human digestive tracts, their medical importance, frequent multiple antibiotic resistance, and seemingly limitless capacity for horizontal gene transfer makes the enterococci an ideal bacterial group for investigating the ecology of antibiotic resistance genes. In this project, we have isolated and characterized enterococci from the digestive tract of 260 house flies (*Musca domestica*) collected from five fast-food restaurants in Kansas. House flies frequently (97% positive flies) carried enterococci ( $2.1 \times 10^3$  to  $4.6 \times 10^3$  CFU per fly). We randomly selected 355 enterococcal isolates for further identification and characterization. Using multiplex-PCR, we identified 205 (57.7%) of the isolates. These represented *Enterococcus faecalis* (88.2%), *E. faecium* (6.8%), and *E. casseliflavus* (4.9%). Identified isolates showed phenotypic resistance to neomycin (77.1%), streptomycin (74.7%), tetracycline (46.8%), erythromycin (22.0%), and ciproflaxacin (10.8%). Tetracycline resistance was coded by several genes, including *tetM* (86.5%), *tetK* (64.5%), *tetC* (5.2%), *tetO* (5.2%), and *tetA* (3.1%) representing both resistance mechanisms - ribosomal protection and active efflux. The *ermA,B,C* determinants were also investigated; 89% of erythromycin resistant isolates carried *ermB* gene and 11% had an unknown gene. Transposon family Tn916/1545, likely carrying *tetM* (two different genotypes as assessed by RFLP and sequencing), was detected in 71 (35.0%) isolates. Multiplex PCR was used for screening of *E. faecalis* for virulence genes; *gelE* (gelatinase) (70.1%), *asa1* (aggregation substance) (33.1%), *esp* (surface

protein) (8.8%) and *cylA* (cytolysin) (8.8%) were detected. Phenotypical testing revealed that 90.0% of gel E genes were expressed, and 8.8% of isolates carried unknown gelatinase gene(s). Fifty-seven percent of *asaI* genes were expressed. The phenotypic tests using human blood showed that all *cylA* genes were expressed while 2.2% of the total isolates carried unknown cytolysis determinant(s). This study shows that house flies play a role in the ecology of antibiotic resistance and virulence genes and likely represent an ecological link between agricultural and urban environments.

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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.

## 29

### **Distribution of a chromosomal polymorphism in relation to a climatic gradient**

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Geographically structured genetic variation, as represented by clines, offers unique opportunities to study adaptation in natural populations. A previous study (McAllister 2002, *Genome* 45:13-21) of a latitudinal transect in *Drosophila americana* revealed the presence of a latitudinally distributed polymorphism involving a centric fusion between the X and 4th chromosomes. However, due to limited sampling on only five populations restricted to mid-latitudes of the Mississippi River Valley, the extent of the cline for the X-4 fusion was not fully characterized. This study presents a more extensive analysis of chromosomal polymorphism in populations of *D. americana* collected throughout the Mississippi River Valley. A total of 15 samples were obtained from the region bounded in longitude by the -88.5° and -92° meridians and in latitude by the 30.5° and 44.5° parallels. Populations at the latitudinal extremes are at, or near, fixation for the alternative centromeric arrangements.

Frequencies of the derived X-4 fusion arrangement are highly correlated with latitude among polymorphic populations. Records from NOAA weather stations nearest to the collection localities were obtained to determine climatic means for the previous 30 years. Climatic variables exhibiting the strongest correlations with the frequency of the X-4 fusion represent measures of winter severity. Genetic variation for cold tolerance or overwintering ability is likely associated with the alternative chromosomal arrangements, and thus potentially responsible for the maintenance of this polymorphism.

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### **Genomic incompatibility in an interpopulation cross results from epistatic interactions at four major loci in the moss *Ceratodon purpureus*.**

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The evolution of post-zygotic reproductive isolation is a critical step in the generation of new species. The genetic basis of reproductive isolation, the causative evolutionary forces, and their biogeographic context remain largely unexplored, however, outside of a handful of model systems. Here we examine the genetic basis of outbreeding depression between geographically distant populations of the moss *Ceratodon purpureus*. We crossed a male from Ithaca, New York, USA, to a female from Otavalo, Ecuador, and cultivated 288 recombinant progeny. Molecular population genetic evidence indicates these populations exchange neutral genes. Nevertheless, approximately half of the hybrid progeny exhibited severely reduced growth rate at the juvenile

(protonemal) stage; this low-fitness phenotype is almost completely absent in natural populations. To dissect the genetic basis of the dwarf phenotype, we constructed a linkage map using 121 polymorphic AFLP loci and restriction site polymorphisms in three nuclear loci. Using composite interval mapping we identified four autosomal QTLs associated with outbreeding depression. To understand the genetic interactions underlying this phenotype, we classified the hybrids as normal or low-fitness, and constructed a full-factorial ANOVA with the marker genotype at the marker most tightly linked to the four autosomal QTL peaks specified in the model. We found significant pairwise interactions between one QTL and the other three, and a nearly significant four-way interaction effect ( $p = 0.09$ ). We found asymmetry in effects of introgression at all four loci. In addition to the loci implicated in hybrid breakdown, six regions of the genome exhibited significant transmission ratio distortion. Five of these were distorted toward the Ecuador (female) parent, while only the sex-determining region was distorted toward the New York (male) parent. Current work is aimed at 1) testing for correlations between the strength of outbreeding depression and geographic distance between populations; 2) testing whether QTLs that exhibit negative epistasis in a hybrid genetic background underlie locally adapted phenotypes in the New York or Ecuador populations; and 3) determining whether segregation distortion results from population divergence or genomic conflict occurring within populations.

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#### **Plant Virus Biodiversity and Ecology at Oklahoma's Tallgrass Prairie**

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The International Committee for the Taxonomy of Viruses recognizes a global total of close to 2,000 species of viruses. DNA sequencing of marine environmental samples suggests that 2,000 is a gross underestimate of the number of viral species on earth. Estimation of the actual number can be approached using quantitative biodiversity theory. While these estimates have been made for viruses infecting single-celled organisms in an aqueous environment, studies assessing viruses infecting multicellular organisms lag far behind. We have initiated a survey of the biodiversity of viruses infecting vascular plants in the Tallgrass Prairie Preserve of Oklahoma, home to over 700 species of vascular plants. In our first approach, we are screening the majority of preserve plant species for viruses. Detection and identification is by double-stranded RNA analysis, cloning, sequencing, and microarray analysis. The fraction of previously unknown viral species we identify in the survey will provide an indication of the extent of global underestimation of known viruses. The percentage of the plant species examined that contain each virus will distinguish generalist from specialist viruses. Subsequent surveying will collect data for individual plants to allow calculation of the percentage of all plants examined from one species that contain a particular virus. Another approach is the "lawnmower approach" in which total plant material from a defined area constitutes an environmental sample that is used for sequence analysis. Data will be mined to address questions such as: does the ratio of number of viral species to the number of plant species in an area depend on plant species richness? Does the mix of viral species decay with distance along a transect? Does plant diversity lower the load of virus species? Our guiding hypotheses are that the majority of plants harbor some viruses and that most virus infections in plants do not cause disease. Since we are not limiting our search to economically important plants, already well studied, we expect to find many new viruses. These new viruses will feed into basic research on the mechanisms of virus replication, translation, particle assembly and movement, as well as providing tools for biotechnology.

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#### **Effects of Chromosomal Arrangements on Patterns of Sequence Variation**

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Geographically distributed genetic polymorphisms suggest the presence of natural selection in response to variable climatic conditions. Chromosomal inversions are a common type of polymorphism that is often associated with climatic and/or seasonal gradients. The hypothesized role for chromosomal inversions is to

modulate patterns of recombination and to protect co-adapted gene complexes, thus potentially organizing genetic variation in response to environmental gradients. The fly species *Drosophila americana* presents an excellent model for examining the role of genome organization in facilitating a correlated response to environmental conditions. A derived chromosomal rearrangement consisting of a centromeric fusion between the autosomal 4th and the X chromosomes is present in *D. americana*. Frequency of the X-4 fusion is positively correlated with latitude among populations sampled in the central United States. Analyses of neutral loci throughout the genome, however, indicate the absence of detectable population structure, suggesting substantial gene flow among populations. The maintenance of this cline for the X-4 fusion apparently reflects a balance between gene flow and natural selection acting on the karyotypes themselves or on genes associated with them. Several polymorphic inversions are also present in *D. americana*. Older literature reports an association between inversion Xc and the X-4 fusion, and therefore, the inversion should show a similar clinal distribution. The geographic distribution of In(X)c and its association with the centromeric arrangement was determined by examining samples of *D. americana* representing the latitudinal extent of the species' range. Centromeric arrangement of the X and 4th chromosomes and presence or absence of In(X)c was determined for over 100 wild caught males. The results indicate that In(X)c is strongly associated with the arrangement of the centromere, being found almost exclusively on chromosomes with the X-4 fusion. Analyses of DNA sequence variation were used to examine the history and the effects of these chromosomal rearrangements. Sequences were obtained for 5 gene regions distributed along the X chromosome for 25 inbred lines with known chromosomal arrangements. Within and around In(X)c, sequence differentiation is high between fused and unfused chromosomes, and the derived X-4 fusion exhibits less nucleotide variation than the unfused X. Nearest to the centromere, however, the level of nucleotide variation on the ancestral unfused X is extremely low and no variation is observed on the derived X-4 arrangement. Furthermore, no sequence divergence is observed in this region between the alternative chromosomal arrangements. Observed patterns of sequence variation along the X chromosome are consistent with the fusion occurring recently on an X chromosome already containing the In(X)c arrangement followed by a rapid increase in its frequency.

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#### **Evolutionary Genomics of Behavioral Isolation in *Drosophila***

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Courtship plays a major role in the sexual isolation of species, yet the genetics underlying courtship behavior are poorly understood. Cuticular hydrocarbons acting as pheromones influence sexual isolation of many *Drosophila* species. Sexual isolation of *D. simulans* and *D. sechellia* is affected by a pheromonal difference expressed through cuticular hydrocarbons found on the surface of the fly. *D. simulans* is a monomorphic species in that male and female *D. simulans* both have *cis* 7-tricosene (7-T) as their major cuticular hydrocarbon. In contrast, its sister species, *D. sechellia*, is sexually dimorphic: males have 7-T but females have *cis*, *cis* 7,11-heptacosadiene (7,11-HD). This difference results in asymmetric sexual isolation between these species, such that *D. sechellia* males court *D. simulans* females whereas *D. simulans* males do not court *D. sechellia* females. A genetic analysis using quantitative trait loci (QTL) mapping has identified three genomic regions (on the X chromosome, and the left and right arms of the 3<sup>rd</sup> chromosome) that contribute to the differential production of the cuticular hydrocarbons. We are performing more refined genetic mapping to identify the location and number of genes contributing to cuticular hydrocarbon production by creating nearly isogenic lines containing intervals around each QTL introgressed from *D. sechellia* into *D. simulans*. The third chromosome, left arm QTL has been introgressed from *D. sechellia* into *D. simulans* for three of the seven molecular marker intervals. These intervals were completed using selective crossing and genetic analysis. We are currently completing the remaining intervals of the right arm and have begun work on the left arm of the third chromosome, as well as beginning testing of the cuticular hydrocarbon composition. Because of chromosomal incompatibility, the X chromosome has not been successfully introgressed. We are also establishing a more detailed QTL analysis through the use of Recombinant Inbred (RI) lines selected to create a more comprehensive introgression of the *D. simulans* and *D. sechellia* genomes. *D. simulans* females were crossed with *D. sechellia* males. The female progeny were backcrossed to *D. simulans* males and the resulting progeny were inbred for 20 generations to establish nearly isogenic lines. In this crossing scheme, the majority of the, *D. simulans* loci tend to fix within the RI lines. Each generation we have selected against *D. simulans* markers in order to create lines with a greater percentage of *D. sechellia* loci fixed. Twenty-nine RI lines have been established and genotyped. Over 200 other RI lines are currently being established. We are also analyzing the cuticular hydrocarbon composition for the established RI lines, which will then be used in the QTL analysis. The established RI lines will also be used for QTL mapping

of male courtship song, sperm length, and male and female choice. Mapping of these traits will provide a comprehensive genetic overview of sexually selected traits in *Drosophila*.

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### **The genetic architecture of thermotolerance *Drosophila***

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For insects, temperature is a major environmental variable that can influence an individual's behavioral activities and fitness. *Drosophila melanogaster* is a cosmopolitan species that has had great success in adapting to and colonizing diverse thermal niches. This adaptation and colonization has resulted in complex patterns of genetic variation in thermotolerance phenotypes in nature. Although extensive work has been conducted documenting patterns of genetic variation substantially less is known about the genomic regions or genes that underlie this ecologically and evolutionarily important genetic variation. We have employed a complementary approach of quantitative trait locus (QTL) mapping, P-element mutagenesis, artificial selection, and surveys of natural genetic variation to dissect the genetic architecture of survival after heat stress and recovery from chill coma. We mapped QTL affecting variation in heat- and cold-stress resistance in a recombinant-inbred mapping population derived from a cross between Oregon and 2b3. We identified four QTL for heat stress and three QTL for chill-coma recovery, most of the QTL were trait- or sex-specific, suggesting overlapping but generally unique genetic architectures underlie resistance to low and high temperature extremes. For our P-element screen we have identified a remarkably large number of candidate genes for both heat and cold tolerance that influence a diverse array of cellular and molecular functions. We have used artificial selection to create lines that are divergent for chill-coma recovery time. This experiment has yielded highly significant levels of mean divergence between the resistant and susceptible lines. We have also detected many correlated responses (CR) to selection, including a positive CR in starvation resistance and a negative CR in heat resistance. Furthermore, selection has resulted in strong genetic effects on the plasticity of cold tolerance phenotypes. That is when flies are grown at low temperatures the cold susceptible lines become much more resistant to cold, however the cold resistant lines do not change. This implies that selection has acted upon environmentally inducible cold tolerance pathways. We are currently directly addressing this hypothesis through the use of whole-genome expression analysis of each selection line reared at low and normal temperatures. These complementary experiments have identified numerous loci and patterns of natural genetic variation that suggest a complex genetic architecture underlies thermotolerance phenotypes. These loci and patterns of natural genetic variation will be further dissected using experiments involving fine mapping, molecular characterization of candidate mutants, and genome-wide transcription profiling.

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### **Microarrays and Global Climate Change: Exploring a Pine Forest under Elevated CO<sub>2</sub>**

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Forest ecosystems play an important role in the global carbon cycle and can be directly affected by changes in atmospheric CO<sub>2</sub> concentration. Acclimation of forest trees to a future scenario of elevated atmospheric CO<sub>2</sub> is likely to involve changes in the expression of many genes controlling key physiological and structural responses. Genomic approaches such as microarray technology are improving our capacity to evaluate the behavior of hundreds or thousands of genes simultaneously, and contributing therefore to a better understanding of molecular mechanisms underlying important ecological processes. Using cDNA microarrays, we compared gene expression patterns of loblolly pine trees growing in the field under ambient and elevated (ambient + 200  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> concentrations. Each microarray contained 1790 replicated pine ESTs assigned to a broad range of functional categories. There were strong seasonal effects on the total number of genes differentially expressed between ambient and elevated CO<sub>2</sub>, with a tendency of more genes being upregulated under elevated CO<sub>2</sub> than downregulated throughout the season. Genes coding for enzymes involved in carbon metabolism, such as citrate synthase and glucose-6-phosphate isomerase, were upregulated in elevated CO<sub>2</sub> during most of the growing season. The same pattern was observed for an alanine-aminotransferase gene involved in both carbon and aminoacids metabolism. Genes coding for the enzyme rubisco showed no differential expression between ambient

and elevated CO<sub>2</sub>. This application of genomic-scale analysis to field experiments is an important step towards a more mechanistic understanding of the response of forests to global climate change.

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### **Genomic Evidence for Divergent Selection in Eastern Oyster Populations across an Ecotone**

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The eastern oyster, *Crassostrea virginica*, is broadly distributed along the western North Atlantic coastline. Inside this range, a steep latitudinal temperature gradient and a transition in species composition from temperate to subtropical communities generate an ecotone along eastern Florida. Despite evidence for homogenizing gene flow within large continental regions, presumably mediated by a three-week planktonic larval stage, oyster populations display a sharp genetic cline in eastern Florida. We are interested in discovering the abiotic and biotic mechanisms by which a high gene flow species maintains genetic diversity along the ecotone. Population genomics approaches offer effective ways to statistically test for genetic evidence of divergent selection in this non-model species. We report genomic evidence for the role of selection across the ecotone using 215 amplified fragment length polymorphism loci sampled from two populations experiencing the environmental extremes. Simulations of neutral population divergence provided a statistical framework for testing whether differentiation at some loci is greater than expected by genetic drift. For a small portion (2-7%) of loci, allele frequency differentiation was high enough across the ecotone to reject the neutral drift model ( $p < 0.01$ ). We infer that divergent selection along the ecotone plays a role in maintenance of genetic differences. These results provide focal loci for more intensive study of local adaptation and gene flow in oyster populations along the eastern Florida ecotone.

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### **Molecular Ecology of Reproductive Allocation: an ecogenomic approach**

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The ability to allocate resources to sexual and asexual reproduction is widely distributed in the plant kingdom. The percentage allocation to either of these functions has a heritable basis, and has profound fitness consequences. As such the trait will be under the influence of selection, driven by environmental cues like light conditions etc. Here we present the first results of an ecogenomic research programme in the *Trifolium* genus, a genus where several species have the capability of asexual (clonal) reproduction. LEAFY (LFY) is the central gene in the regulatory pathway, its onset causes flowering. LFY, reported to be a single-copy gene, is quite variable in sequence between 24 investigated *Trifolium* species. Several SNPs were found in the LFY exon 1 among 36 genotypes of the clonal *trifolium repens*, characterised by their strongly diverging allocation pattern. Evidence was found that LFY has been duplicated in an early phase of the *Trifolium* evolution; whether various copies are fully functional is currently being investigated. In aqcn cDNA-AFLP experiment, involving 129 primersets, 240 genes were identified that are differentially expressed between non-flowering and very early-flowering meristems. These genes are currently used in QPCR trials to screen for expression under various conditions, at various life stages and in various natural populations. The ultimate goal of the programme is to get insight in the underlying genomic changes when selection changes reproductive allocation, either as a consequence of natural occurring environmental selection pressures or when artificial selection regimes are imposed.

**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 2) characterize genes in big bluestem roots which are differentially regulated in response to nitrogen and water stress. Ultimately, we plan to extend these growth chamber studies to field plots at Konza Prairie. We have constructed subtractive cDNA libraries from the roots of two month-old bluestem 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 not only in amino acid metabolism but also those involved in stress/defense signaling, transcription/translation, protein processing, cell wall metabolism, secondary metabolism, respiration/redox reactions. We are in the process of validating the expression of some of these genes.

**39****Finding biological processes underlying complex multiple-drug resistance in the malaria parasite**

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The malaria parasite, *Plasmodium falciparum* has thwarted all efforts to eliminate it with drugs. In fact, widespread, increasing levels of resistances to diverse pharmacologically active compounds is yielding a generalized multiple-drug resistant (MDR) phenotype in populations of *P. falciparum* from several regions of the world. The observed steady march towards MDR is clearly not the result of a simple, single-gene mechanism. Past studies have emphasized the role of single-gene determinants of resistance; however, this focus necessarily has ignored the genomic context in which these genes function. Consequently, rather than searching for resistance genes, we are exploring the complex, multi-genic physiological processes that underpin decreasing sensitivity to various drugs in the progeny of a genetic cross. Alignment of QTL scans of response phenotypes to different drugs reveals shared loci that may account for observed correlations (cross-resistance patterns) between drug responses in the progeny and in natural populations. Using an integrative genomic approach that combines QTL's with gene expression microarrays, comparative genome hybridization and population genetics, we have identified candidate genes in these loci that could highlight the biological processes that can contribute gene variants that influence resistance evolution.

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### **Transposable element proliferation in bacterial symbionts of grain weevils**

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Rice weevils and maize weevils harbor obligate endosymbiotic bacteria that provision nutrients to their hosts (*Sitophilus oryzae* primary endosymbiont, or SOPE, and *Sitophilus zeamais* primary endosymbiont, or SZPE). Although they are clearly mutualistic symbionts, SOPE and SZPE lack most of the genomic hallmarks of very ancient bacterial endosymbionts, and instead apparently represent transitional taxa along the evolutionary trajectory from free-living bacteria to intimate intracellular mutualists. Insertion sequence (IS) elements often proliferate in evolutionarily young intracellular symbionts and are then lost in ancient symbionts. Therefore, our goal was to quantify the IS elements within SOPE and SZPE in order to assess their potential impact on the ecology and evolution of these symbioses. We found that SOPE and SZPE harbor ~110 and ~50 IS903 elements, and ~2300 and ~5900 IS256 elements, respectively. These prolific IS elements could profoundly impact the genomic evolution of SOPE and SZPE, which in turn could affect their long-term efficacy as mutualists.

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### **Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis***

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HSP30 is a so-called "small" heat shock protein that has been shown by Weber and colleagues to occur in trout and salmon muscle and to respond strongly to heat shock. We have identified an *hsp30* gene in the zebrafish genome and verified, by RT-PCR, that transcript levels for this gene increase when zebrafish is heat-shocked. We have cloned a stoneroller HSP30 cDNA and found very high sequence similarity between zebrafish and stoneroller proteins. We are working to extend our analyses studies to the protein level. Unfortunately the antibodies against the trout protein do not cross-react with the stoneroller or zebrafish proteins. Therefore we have expressed zebrafish HSP30 in *E. coli* and are now waiting for rabbit antiserum against this protein.

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### **Abundance of *nifH* Genes in Urban, Agricultural, and Pristine Prairie Streams Exposed to Different Levels of Nitrogen Loading**

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The biosphere has been highly enriched with nutrients, especially nitrogen, by human activities. In most ecosystems, nitrogen availability should be limited, but soils and aquatic ecosystems have been heavily impacted by human activities such as agriculture, recreation, and urbanization. Nutrient dynamics are important to our understanding of the processes of eutrophication and oligotrophication. Ecosystem processes drive biogeochemical cycles that influence input and losses of nutrients in the environment. In streams, the availability of nutrients, geochemical characteristics, hydrodynamics, and human activities influence the metabolic activities and structure of microbial communities. A combination of process-level and molecular microbial ecology techniques are being applied to study nitrogen fixation in small prairie streams with different nitrogen impact histories, from pristine to heavily polluted. Nitrogen fixation was measured with acetylene reduction assays.

Impacted urban and agricultural streams exhibited low nitrogen fixation activity. On the other hand, nitrogen fixation was relatively high in a pristine stream at Konza prairie, particularly in leaf litter samples. Simultaneous sampling of sediments and leaf litter was performed for later molecular analyses of the nitrogen-fixing microbial guild. Direct DNA extracts were examined using real-time PCR of a nitrogenase complex gene (*nifH*) to determine the abundance of nitrogen-fixing organisms. The *nifH* gene has one of the largest non-ribosomal sequence databases from diverse uncultivated microorganisms. The construction and sequencing of PCR-based clone libraries will assess diversity and community distribution in these different streams. This study will provide a link between the abundance of *nifH* genes and nitrogen fixation activity. An understanding of the effect of nitrogen pollution on nitrogen cycling communities in small streams will increase our ability to overcome the challenges of nutrient pollution. This work is supported by Kansas NSF EPSCoR.

### **Rac GTPases and UNC-34 Enabled molecules show functional redundancy in *C. elegans* axon pathfinding and neuronal migration**

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It has been shown earlier that many genes affect guidance and axonal growth as well as cellular migration. The mechanisms by which molecules interact with each other are still under study in several labs and address the process of functional redundancy among molecules. Previously we have presented results from a *ced-10(n1993)* synthetic lethal screen suggesting, three *C. elegans* RAC proteins (CED-10, MIG-2 and RAC-2) are functionally redundant in cell migration and axon pathfinding (e.g. single mutants show few defects whereas double mutants display strong axon pathfinding and cell migration defects). Further, we found that new *mig-2(lq13)*, *ced-10(n1993lq20)*, and the existing *ced-10(n3246)* (Reddien and Horvitz, 2000), are gain-of-function alleles. All three gain-of-function mutations affected residues near the switch 1 and switch 2 regions of the GTPases, which mediate interaction of the GTPases with Dblhomology GTP exchange factors (DH-GEFs). Possibly, *lq13*, *lq20* and *n3246* are dominant-negative mutations that perturb interaction of the GTPases with their GEFs.

We found that *lq17* was a hypomorphic mutation in *unc-34*, which encodes an Enabled-like protein involved in cytoskeletal signaling, which has previously been shown to affect axon pathfinding on its own (Withee et al., 2004). However, *unc-34(lq17)* mutants had few defects in axon pathfinding and cell migration whereas double mutants with *ced-10*, *mig-2* and *rac-2* had strong defects. These data indicate that UNC-34 and Rac signaling have overlapping roles in axon pathfinding and cell migration and are consistent with previous results suggesting that CED-10 and UNC-34 act in parallel pathways downstream of the UNC-40 guidance receptor (Gitai et al., 2003).

Further, UNC-115 aBLIM, an actin-binding protein, was previously shown to act downstream of RAC-2 and possibly CED-10 in axon pathfinding. Indeed, we found that *unc-115*; *unc-34* double mutants displayed synthetic defects in axon pathfinding suggesting functional redundancy among UNC-115 and Rac molecules. Possibly, UNC-115 modulates the growth cone actin cytoskeleton in response to Rac signaling and in parallel to UNC-34. In addition, mutations in MIG-15 NIK kinase affect axon pathfinding and show interaction with Rac molecules (Poinat et al. 2002). Our results show enhancement in synthetic defects in axon pathfinding in *unc-34(lq17)*; *mig-15(rh148)* double in comparison to *mig-15*; *rac* doubles. These results suggest that MIG-15 acts in Rac pathway and parallel to UNC-34 Enabled in axon pathfinding.

### **Mechanisms controlling plant developmental timing at elevated CO<sub>2</sub>**

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We compared the biochemistry, growth and developmental timing of four closely related genotypes of *Arabidopsis thaliana* grown at current (380 ppm) and elevated CO<sub>2</sub> (700 ppm). The four genotypes were as follows: a genotype previously selected for high seed production at elevated CO<sub>2</sub> (hereafter SG); a control genotype randomly selected at elevated CO<sub>2</sub> (hereafter CG); and two parental genotypes initially crossed to produce SG and CG (hereafter P1, P2). We found that SG flowered 12 d later when grown at 700 ppm CO<sub>2</sub> relative to 380 ppm CO<sub>2</sub>, whereas P1, P2, and CG did not exhibit differences in the timing of floral initiation between 380 and 700 ppm CO<sub>2</sub>. Size at flowering was disrupted in SG, such that elevated CO<sub>2</sub> increased the biomass of SG by 102% at floral initiation versus only by 80% for P1, P2 and CG. Interestingly, the phenotypic responses of SG to growth at elevated CO<sub>2</sub> mimic the observations made by others in *A. thaliana* grown on medium containing increased exogenous sucrose levels at current CO<sub>2</sub>. Therefore, in a subsequent experiment we investigated the relationship between foliar sugar concentration and time to floral initiation. To alter foliar sugar concentration, we grew each of the four genotypes previously mentioned at 270, 380, 700, and 1000 ppm CO<sub>2</sub>. We found that as foliar sugar concentrations increased the time to floral initiation also increased. However, a threshold was also observed in this response where further increases in sugar concentration did not further delay the timing of floral initiation. This suggests that signaling by excess foliar sugars may play a role in delayed floral initiation at elevated CO<sub>2</sub>, possibly by influencing the expression of genes involved in floral initiation.

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### **Genetic basis of adaptation to high-altitude in deer mice**

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One of the main goals of evolutionary biology is to elucidate the adaptive significance of genetic variation and the evolutionary forces responsible for maintaining that variation. One particularly powerful approach is to analyze DNA sequence variation at genes that underlie ecologically important traits in natural populations. The deer mouse (*Peromyscus maniculatus*) is particularly well-suited to such an approach because it exhibits high levels of geographic variation in fitness-related traits that are amenable to genetic analysis. For example, experimental evidence indicates that adaptive variation in aerobic capacity and thermogenic capacity among deer mice from different elevations is associated with a complex hemoglobin polymorphism that involves multiple genes from the alpha- and beta-like globin gene families. Specifically, physiological experiments have revealed that the maximum rate of oxygen consumption (VO<sub>2</sub>max) elicited by exercise and cold exposure is strongly associated with allelic variation at two closely linked alpha-globin genes. This is a unique situation where fitness-related variation in whole-organism physiology can be related to a simple biochemical phenotype (O<sub>2</sub>-binding affinity of the globin polypeptides) that has a well-characterized genetic basis. In this talk I will present the results of a genetic analysis of hemoglobin polymorphism in populations of *P. maniculatus* that are distributed across steep elevational gradients in montane regions of western North America. By analyzing patterns of DNA sequence variation in the genes that underlie adaptation to high-altitude hypoxia, this study provides insights into the evolutionary forces that have shaped fitness-related variation in physiological performance.

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### **haf-6(ne335) is RNAi defective**

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RNAi is a conserved mechanism where a dsRNA molecule triggers the cleavage of a homologous mRNA. ne335 is an RNAi defective mutant with a deletion in the haf-6 gene. haf-6(ne335) is a member of the highly conserved ATP Binding Cassette (ABC) family of proteins in *C. elegans*. ABC proteins are usually membrane proteins that collectively transport a variety of substances. Defects in ABC proteins in humans have been linked to a number of genetic disorders like cystic fibrosis, Stargardt disease, X-linked sideroblastic anemia and to multi-drug resistance in cancer. Further studies on haf-6(ne335) are being carried out to elucidate its role in the mechanism of RNAi.

(See # 13)

### **QTL Analyses of Host Odor Discrimination in the Phytophagous Insect *Rhagoletis pomonella***

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### **EXC-9 and EXC-5 Regulate the Structure of the Nematode Osmoregulatory Organ**

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The small soil-dwelling nematode *C. elegans* contains a large H-shaped cell, called the excretory canal cell, which serves as the *C. elegans* osmoregulatory organ. This cell is necessary for the animal to maintain an osmotic balance with its environment and to allow the animal to adapt to changes in soil conditions. Many genes function together in order to regulate the formation and structure and integrity of this cell. These genes likely play a role in the animal's ability to adapt to different environmental conditions. Mutations in several genes cause the canal cell to develop large fluid-filled cysts. We have examined the function of two of these genes, *exc-9* and *exc-5*.

We have cloned *exc-9*; it encodes a small protein containing a single protein-binding LIM domain and a short C-terminal region. EXC-9 shows close homology to CRIP (Cysteine-Rich Intestinal Protein), first identified as a protein highly expressed in the mammalian intestine. It has also been found in invertebrate neurons, and overexpression of CRIP alters mouse cytokine expression. We have examined the expression pattern of EXC-9

and a homologue, B0496.7, in *C. elegans*. The genes are expressed in different cell types, and are found together only in the uterus. One or the other gene is expressed in multiple epithelial cell types and some neurons.

Mutations in *exc-5* cause cystic defects similar to those of *exc-9* mutations. *Exc-5* encodes a putative guanine nucleotide exchange factor (GEF). GEFs activate small GTPases such as Rho, Rac, and Cdc42. We performed an EMS non-complementation screen to isolate new alleles of *exc-5*. A total of 41 new alleles were obtained. Three of these alleles appear to be novel hypomorphs, and are currently being sequenced. One temperature-sensitive strain was collected from the non-complementation screen.

Overexpression of either *exc-5* or *exc-9* prevents cyst formation, but causes defects in canal migration and growth. We are using genetic and immunological techniques to find other proteins that interact with these proteins to act in pathways to determine cell shape.

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### **The influence of global climate change on gene expression in a tallgrass prairie plant**

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Most models of global climate change predict a reduction in the frequency of rain events in North America that will decrease mean and increase the variance in soil moisture availability. We measured changes in gene expression of big bluestem plants (*Andropogon gerardii*) in response to reduced frequency of rain events in the field. Tissue was sampled from plants growing on the Konza prairie reserve under rainout shelters designed to control water application to replicate plots. By using microarrays developed for maize we were able to compare the expression levels of over 9,000 genes for plants in delayed and ambient watering treatments. Our results indicate that a variety of stress signaling and response proteins were upregulated in response to greater variation in precipitation, while those of the hypersensitive response were consistently downregulated. Thus, bluestem may be experiencing an ecological tradeoff between different genetic mechanisms for responding to stress.

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### **Evolution of *Daphnia* gene expression following the eutrophication of York Pond, USA**

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This project aims to understand how gene expression in *Daphnia* has evolved in response to major changes in a local ecosystem over the course of many decades. The Berlin Fish Hatchery, owned and operated by the New Hampshire Fish and Game Department, opened in the early 1930s and was substantially upgraded in 1952. Waste and runoff from the hatchery transformed nearby York Pond into one of the most eutrophic lakes in NH. High levels of toxic cyanobacteria, an ecological consequence of biological pollution and eutrophication, have been found in York Pond, as have large populations of *Daphnia*, which are presumably resistant to the high toxicity levels. Diapausing eggs (ephippia) of past generations of *Daphnia* have been found in the lake sediment dating back to before and during the introduction of the fish hatchery. Using DNA extracted from ephippia, their hatchlings and adult *Daphnia* from the water column, it is possible to identify *Daphnia* species present, assess ancestor/descendent relationships as well as relative abundance of species over time in York Pond. Furthermore, successful hatching of ephippia from past populations will provide the opportunity to conduct laboratory-based analysis such as QTL mapping and comparative gene expression studies of a resurrected *Daphnia* population. While historical populations of *Daphnia* have been successfully studied for signs of evolutionary change, there has not been a gene expression analysis on an 'ancient' population. Currently, a hatching experiment is underway, with the intention of measuring gene expression differences between a current York Pond *Daphnia* population and an ancestral, pre-hatchery population. As a first step to this expression study, a cDNA library of a modern *Daphnia* population was made directly from natural isolates of *Daphnia catawba* from York Pond. Genes involved

in toxic cyanobacteria resistance are suspected to play a role in *Daphnia* adaptation to York Pond's eutrophication. Results of this project may corroborate other studies aiming to identify genes responsible for resistance to toxic cyanobacteria and other natural pollutants.

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**Retrotransposon proliferation and large-scale genome expansion in diploid hybrid sunflower species**

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Long terminal repeat (LTR) retrotransposons are mobile genetic elements that are ubiquitous in plants and represent a significant genomic fraction in plant species with large genomes. The factors governing the activation and proliferation of these elements are poorly known, although mounting evidence suggests roles for natural agents such as interspecific hybridization and abiotic stress. Wild sunflowers in the genus *Helianthus* provide an excellent system for studying the evolutionary dynamics of LTR retrotransposons. Three species (*H. anomalus*, *H. deserticola*, and *H. paradoxus*) have arisen, independently, via hybridization between the same two parental species (*H. annuus* and *H. petiolaris*). All three hybrid species are adapted to abiotically extreme habitats. Previous work has demonstrated large-scale differences in nuclear genome size between the hybrid and parental sunflower species, with hybrid species possessing nuclear genomes from 50 to 75% larger than either parent species. These observations are in spite of the fact that all parent and hybrid species have the same base chromosome number and are diploids. Using Southern hybridization and quantitative PCR approaches, we demonstrate that nuclear genome size increases in sunflower hybrid species are attributable, in large measure, to recent, independent, and massive proliferation of Ty3/gypsy-like LTR retrotransposons. Cloning and sequencing of several hundred degenerate PCR amplification products (Ty3/gypsy reverse transcriptase domain) demonstrates extreme sequence heterogeneity of these elements in *Helianthus* and identified candidate lineages that may have proliferated in the hybrid species.

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**Comprehensive mutation identification in an experimentally evolved bacterium**

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Identification of adaptive mutations in organisms with known evolutionary histories has been constrained by traditional genetic and sequencing techniques. Using sepharose bead-based technology, we have comprehensively identified accumulated mutations in an experimentally evolved genotype of the social bacterium *Myxococcus xanthus* that exhibits greatly altered behavior and fitness relative to its ancestor. *M. xanthus* exhibits several distinctive social behaviors, including cooperative predation, social motility and multicellular development. The entire 9.14 Mb genome of strain 'SI' was sequenced to an average depth of 19 with bead technology and 45% of the same genome was sequenced independently by the traditional shotgun cloning approach. The resulting sequence revealed 15 single nucleotide mutations relative to the laboratory ancestor of SI (GJV1), but no evidence of duplications, transpositions or multiple-base deletions. Fourteen mutations appeared during 1000 generations of evolution in liquid culture. During this phase, GJV1 evolved into a genotype ('OC') that exhibits an obligate social cheating phenotype during *M. xanthus* multi-cellular development. The final mutation appeared during an ~60 generation competition experiment in which the obligate social cheater (OC) evolved into a socially independent and competitively superior cooperator. This high-fidelity, whole-genome re-sequencing analysis of an experimentally evolved organism demonstrates the feasibility of comprehensive and precise characterization of molecular evolution in experimentally evolved bacteria.

### **Identification of Ecdysone-Regulated Genes during leg development in *Drosophila***

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The elongation and eversion of the leg imaginal discs in *Drosophila* is an ideal system for studying hormone-regulated morphogenesis. Broad (br) is a key ecdysone-inducible early gene for leg morphogenesis. Previous studies suggest that the Rho1 small GTPase is necessary to direct the cell shape changes that drive morphogenesis of the adult legs. To identify genes whose expression are ecdysone-inducible and br dependent during imaginal disc morphogenesis, we probed Affymetrix whole *Drosophila* genome microarray chips with RNA isolated from leg discs of staged larvae and prepupae from amorphic br5/Y mutant and w1118/Y control animals. We have identified several interesting candidate genes that we are currently validating by Northern blot analysis. We plan to use RNA interference to conduct functional analysis of many of these genes. In a related project, we are cloning a br interacting gene that we recovered from a previous screen E(br)165 shows strong interaction with br and with Rho1. We have mapped the mutation to 26B-E on the left arm of the second chromosome and are continuing to refine the mapping with the goal of identifying the affected gene. We will present the preliminary phenotypic analysis of this mutant.

### **Ecological Genomics at Oak Ridge National Laboratory**

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Genomics provides an opportunity to explore in ever-increasing detail mechanisms that enable organisms to develop, survive, carry out normal function, and reproduce across a range of environmental conditions. Technologies developed in this field have the potential to not only enhance our understanding of basic biology, but they also have far-reaching implications for research in ecology and the environmental sciences. Although the future looks bright, it remains a significant challenge to move the emerging field of ecological genomics from the laboratory to natural environments where genomics, proteomics, and metabolomics can be used to address important questions.

Scientists at Oak Ridge National Laboratory (ORNL) are engaged in several studies aimed at integrating genomics into the ecological and environmental sciences. First, and foremost, has been the sequencing of the first tree genome – *Populus*. In this activity, the Department of Energy, Office of Science, in collaboration with ORNL, the Joint Genome Institute, and other international partners, has sequenced the *Populus trichocarpa* (i.e., black cottonwood) genome to an 8.2x depth, assembled scaffolds into 19 chromosome-level linkage groups, and annotated the genome based on four gene-calling algorithms. This project provides an unprecedented resource for the most widespread native tree species in North America. The genomic tools derived from this effort will provide novel platforms for the investigation of ecosystem processes. An example of this is seen in a newly-funded project that seeks to identify genomic regions associated with adaptive variation in natural *Populus* populations using single nucleotide polymorphisms (i.e., SNPs) and quantitative trait loci (i.e., QTLs). By utilizing natural populations and surveying the genetic information contained within the genomes of such organisms, it should be possible to determine the range of adaptive variation that may be harbored within and among populations, and thus determine the stability and adaptability of a species over a range of climatic conditions. These estimates will greatly aid in the design of more intensive studies of molecular adaptation in natural populations, and could lead to strategies to mitigate the ecological effects of climatic change through land management practices.

In addition to studies involving natural populations, studies designed to examine the conceptual framework for ecological genomics are also underway. The HERMES project, Hierarchical Experimental Responses to Macromolecular at Ecosystem Scales, attempts to scale the effects of a single gene modification in nitrogen assimilation from cell to ecosystem under variable CO<sub>2</sub> and nitrogen concentrations. Global gene expression profiling and metabolomics are linked to microbial and plant community dynamics to determine mesocosm-wide regulation of carbon and nitrogen pools. This project provides an initial attempt to understand the genetic constraints on nitrogen assimilation as a possible limitation to CO<sub>2</sub> fertilization across multiple levels of biological organization. It is hoped that the outcome of the above experiment will illustrate the plausibility and importance of relating underlying genetic mechanisms to ecosystem-scale processes.

### Ecological Genomics at the Continental Scale

*Frontiers of Integrated Biological Research (FIBR)*

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Plants have the capacity to integrate multiple cues from the environment in order to generate adaptive plastic responses, but despite the key role they play in adaptation the mechanisms of integration and response remain largely unknown. We are taking a multi-level approach to understanding environmental response in *Arabidopsis thaliana* as part of an NSF/FIBR project entitled “Molecular evolutionary ecology of developmental signaling pathways in complex environments”. This joint effort of Brown University, Kansas State University, North Carolina State University, the University of Wisconsin, and six European molecular genetics laboratories focuses on the key life history trait of flowering time, which is likely to experience geographic variation in selection pressure across the broad and climatically diverse native range of *A. thaliana*. Our approach includes analysis of flowering time response to a wide range of natural and manipulated environments at scales ranging from DNA sequence variation to continental ecotype distributions. Part of this study entails establishing five common gardens from Valencia, Spain (39°29’N, 00°24’W) to Oulu, Finland (65°00’N, 25°26’E). At these sites many *A. thaliana* accessions will be grown in multiple plantings synchronized with local populations in order to assess geographic and seasonal variation in selection on flowering time. Measurements of bolting time and fitness will be combined with mathematical gene network models; genotype information; and on-site, high-resolution weather data to infer the functional and evolutionary significance of natural genetic variation in the converging signaling pathways of flowering time control. We present here a description of the field sites, the experimental design, and illustrative preliminary data from the experiments in progress.

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### Ecotoxicogenomics of a Small Mammal Community Exposed to Heavy Metals

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One hundred years of lead, zinc, and cadmium mining in the Tri-State area of Kansas, Missouri, and Oklahoma has resulted in 2500 sq. mi. of heavy metal contamination to soil and groundwater. Although the area has been remediated for human health, the effects of heavy metal exposure on ecological processes are poorly understood. Disturbance regimes have a cascading effect on biological systems; we examined the effect of heavy metals on a hierarchy of biological functions from gene to ecosystem. We hypothesized that heavy metal contamination altered gene expression in biochemical pathways leading to disruptions at physiological, organismal, population and community levels. Using the deer mouse (*Peromyscus maniculatus*) as our focal species, we compared gene expression and tissue pathology in liver and kidney, body condition (body mass, hind foot length, and categorical score), population genetic diversity, and small mammal community diversity at a contaminated site in Galena, KS with a reference site on Neosho State Wildlife Refuge near St. Paul, KS. We maintained 25 trap stations at each site for five nights. Traps were checked before dawn, trapped mice were anesthetized with halothane, euthanized by cervical dislocation, and necropsied. Mice were identified to species, weighed, sexed, measured and body condition was scored. For a maximum of 5 animals per site, the caudal half of the left kidney and the proximal half of the left lobe of the liver were collected and preserved for total RNA extraction. cDNA will be hybridized to Affymetrix mouse chips to determine differential gene expression between sites. The remaining liver and kidney and the brain were preserved in 10% formalin for histopathologic evaluation. All other small mammals trapped on site were euthanized and frozen to estimate total body heavy metal content, although a small amount of epithelial tissue was placed in 95% ethanol for DNA analysis. 10 microsatellites will be used to determine overall genetic diversity within sites and to estimate levels of gene flow among sites. Gene expression analyses are pending, however we found significant pathology and apoptosis in tissues from the contaminated site; no pathology was found on the reference site. *P. maniculatus* at the contaminated site (n=10) vs. the non-contaminated site (n=2) had a lower average body weight ( $14.4 \pm 1.0$  g (mean  $\pm$  s.e.) vs.  $19.0 \pm 2.0$  g,  $t = -1.82$ ,  $P = .048$ ), poorer body condition ( $3.5 \pm 0.2$  vs.  $4.0 \pm 0.0$ ,  $P = .003$ ), and shorter hind foot length ( $18.5 \pm 0.2$  mm vs.  $19 \pm 0.0$  mm,  $t = 1.81$ ,  $P = 0.048$ ). Microsatellite markers are currently being optimized. A total of 28 small mammals of 4 species were captured over all sites. Small mammal community diversity was greater on the

reference site than on the contaminated site (Shannon diversity index = 1.43 on reference site and 0.391 on contaminated site). We infer from the histopathological findings that heavy metals had a disruptive effect on physiological processes which resulted in low body mass, small body size and poor body condition for *P. maniculatus*. This organism level effect likely lowered individual fitness on the contaminated site. Genetic analyses will allow us to determine if organism level effects have reduced the effective population size at the contaminated site. At the community level, small mammal biodiversity was significantly lower at the contaminated site. Initial results are indicative of hierarchical ecological impact due to heavy metal exposure. Addition of genomic data will allow us to articulate the genomic interaction that precipitated this biological cascade.

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### **QTL Mapping of Ecologically Significant Traits in *Populus*: A First Step Toward Genotyping Extended Phenotypes**

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QTL mapping provides a potentially useful tool for studying the relationship between genetic variation in a species and ecological processes at multiple levels. Thirty years of research on a cottonwood hybrid zone (*Populus fremontii* x *P. angustifolia*) in northern Utah has shown that genes in a dominant riparian organism can have Extended Phenotypes. These extended phenotypic effects act at levels higher than the population, and can have community- and even ecosystem-level consequences. Despite this, little is known about the molecular basis of the traits involved. Here, we have created a genetic mapping population in order to determine whether extended phenotypes can be quantified and used to identify QTL with large-scale ecological effects. Given that traditional three-generation hybrid pedigrees trees do not exist for *P. fremontii* and *P. angustifolia* we used individuals from naturally occurring hybrid zone to create a segregating mapping population. Using DNA markers, we identified a pure F1-hybrid from the Weber River in northern Utah, and crossed it to a pure *P. angustifolia* from the same population. The resulting 246 backcross progeny were then used to create a dense linkage map consisting of 563 AFLP markers distributed across 23 linkage groups and covering 2422.2 cM. Patterns of segregation distortion, heterozygosity levels, and estimated and observed map lengths were all comparable to those reported for other poplar maps. Using this map, we have begun to identify QTL for traits with known ecological effects including chemical (tannin, salicortin, and HCH-salicortin content), phenological (timing of leaf flush), and architectural (bud density) traits, all of which appear to have extended phenotypic effects. Our results have two major implications for future ecological genetic and genomic studies. First, we have shown that natural hybrids provide tools for rapid genetic mapping in ecologically significant species for which traditional pedigreed crosses are not available. Second, the links between trait variation and ecological consequences identified in previous studies, coupled with QTL for the traits identified here, suggest we may be able to directly identify QTL that may have extended phenotypic effects once our experimental population is planted in the natural setting.

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### **Creating an Ecological Genome Consortium: Putting Genomics to Work in Ecology and the Environmental Sciences**

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Recent success in sequencing the genomes of *Populus* (tree) and *Daphnia* (zooplankton) will prove to be watershed events in the ecological and environmental sciences, and will help define the emerging field of ecosystem genomics. These accomplishments will facilitate not only an enhanced understanding of basic

organismal biology, but they will also provide an unprecedented opportunity to interpret sequence data within an ecological and environmental context. For example, *Daphnia* is widely used for toxicity testing in aquatic ecosystems, whereas *Populus* grows throughout the Northern Hemisphere and has become a model perennial organism for understanding structure and function in terrestrial ecosystems.

While *Populus* and *Daphnia* are among a growing list of large (i.e., > 250 Mbp) genomes to be sequenced, efforts must be made to build upon these accomplishments by developing a framework whereby other organisms can be selected for sequencing based on ecologically-relevant traits. Adding ecological relevance to the list of characteristics used to select organisms for sequencing would add a new dimension to the use of post-sequencing genomic information. Moreover, sequencing organisms across multiple trophic levels or communities isolated from the plant rhizosphere would provide much-needed resources for linking structure and function in ecological and environmental systems. The scientific community, however, must be proactive in developing the rationale for additional sequencing projects and in identifying how such efforts will lead to an improved understanding of terrestrial, freshwater aquatic and marine ecosystems.

An Ecological Genome Consortium has been organized to promote sequencing of organisms of interest to the ecological and environmental sciences. The near-term goals of the consortium are to provide a forum that will (1) bring together representatives from state and federal agencies, national laboratories, and academia to discuss how genomic technologies can most effectively be used in the ecological and environmental sciences, (2) allow scientists to develop the rationale for how sequencing organisms selected from terrestrial, freshwater and marine environments can expand our understanding of the world around us, and (3) provide a mechanism to identify a well-justified and compelling list of organisms for future sequencing. This list would include organisms of broad interest to those working in the ecological and environmental sciences. The long-term goal of the consortium is to create a community-wide resource that will encourage and facilitate the continued development of ecosystem genomics. The consortium web site (<http://www.ornl.gov/sci/egc/index.html>) provides an on-line mechanism whereby scientists from the national and international community can join the consortium, identify areas of research interest, specify types of sequencing required, and suggest organisms and/or collections of organisms for future sequencing. Membership in the consortium is open to researchers from all scientific disciplines. Information obtained through the consortium will be made available to all members through an on-line newsletter.

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### **An investigation of Rho signaling in ecdysone-triggered morphogenesis in *Drosophila***

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Precise spatial and temporal control over cell shape changes are essential for the development of all metazoans. Endocrine signaling provides a critical level of temporal control that additionally aids in the coordination of morphogenetic events. The evagination of *Drosophila* leg imaginal discs provides an ideal system for defining the molecular mechanisms of hormone-regulated tissue morphogenesis. A pulse of the steroid hormone ecdysone triggers the transformation of an epithelial sac into an immature adult leg through coordinated changes in cell shape. Our previous studies indicate that leg morphogenesis requires signaling through the Rho1 small GTPase. Although the direct effects that Rho1 signaling has on the actin cytoskeleton are well established, little is known about the mechanisms that control Rho1 activity in a developmental context. We have been using genetics to identify genes that regulate Rho1 during imaginal disc morphogenesis. One such gene, *E(br)65*, interacts strongly with mutations in *Rho1*, *RhoGEF2*, and nonmuscle myosin, and additionally displays defects in embryonic morphogenesis. We have mapped *E(br)65* to the second chromosome in a region of about 125 kb containing 12 genes. Additionally, we are attempting to monitor the activation of Rho1 during imaginal disc morphogenesis. To accomplish this, we have generated antibodies against the phosphorylated forms of the myosin regulatory light chain (MLC), since activating Rho leads to the phosphorylation of MLC first at Serine-21 (MLC-1P) and then at Threonine-20 (MLC-2P). Interestingly, MLC-1P is predominantly expressed in the adherens junction, whereas MLC-2P is expressed on the apical surface and in filopodia. Furthermore, whereas MLC-1P is found in all cells of the imaginal disc, MLC-2P appears to be expressed more strongly in cells that are undergoing cell shape changes, particularly cells that line deep folds in the epithelium.

### **Quantitative trait loci (QTL) mapping of key ecosystem and community traits in naturally occurring cottonwood hybrid zones.**

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The role that genes play in determining ecosystem and community level trait variation is poorly studied in most ecological systems. One system that provides unparalleled opportunities for defining the interactions between genes and ecology is the cottonwood system, which consists of extensive hybrid zones formed between two or more cottonwood species. In this system, we have identified strong correlations between host plant genetics and key ecosystem functions such as nitrogen mineralization and biomass production, and community-level traits including arthropod community composition and plant chemical defense. To better understand the genetic variation that underlies these correlations, we explore the utility of quantitative trait locus (QTL) mapping in natural hybrid zones between broad (*P. fremontii*) and narrowleaf (*P. angustifolia*) cottonwood species. Although QTL mapping is a widely accepted method for exploring quantitative trait variation in plant and animal populations, these methods have been restricted to experimental populations of known pedigree. Our study proposes to develop QTL mapping techniques for insitu exploration of quantitative trait variation in cottonwood hybrid zones. Here, we use amplified fragment length polymorphisms (AFLPs) to construct a linkage map for 282 cottonwood clones (*P. Fremonti* - 21, *P. Angustifolia* - 21, *P. Fremonti* x *P. Angustifolia* - 3 and [*P. Fremonti* x *P. Angustifolia*] x *P. Angustifolia*] - 237) from the Weber River in Ogden, UT. Data generated from this mapping population is being used to identify QTL associated with foliar chemistry (condensed tannins, salicortin and phenolic glycosides), leaf flush, and arthropod community composition. Once QTL for these traits are verified we will explore how genes controlling these traits ultimately affect the function of a dominant riparian species and its associated community in a natural environment.

### **Metagenomics on Soil-borne Microbial Communities**

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Soils are recognized as essential media for sustainability, because the living soil represents so-called life-support functions, i.e. functions that sustain life in the ecosystem and on this planet. There are several important links between the life-support functions of soils. The links all depend on the genetic pool of soil microorganisms and other communities. Tackling the complexity of soil microbial ecosystems with the aim of understanding and improving soil characteristics requires a novel approach. Traditional approaches have so far proven to be limited in their potential to unlock these functional capacities. For example, more than 95% of all microorganisms estimated to be present in soil are refractory to isolation and cultivation. These so-called “unculturables” may contain novel agents for use in both medicine and agriculture. By implementing a metagenomics-approach into soil and environmental sciences, we are at the beginning of understanding and unlocking the functional capacities of the living soil. With “metagenome”, we mean the collective genomes of all microorganisms present in a soil. The metagenomics-approach involves the extraction and cloning of large fragments of DNA. In addition we will apply and use DNA micro-array technology for the exploration of the so far hidden functional diversity of the soil microbiota. ‘1st generation’ phylogenetic DNA micro-arrays have been developed, containing specific probes for the detection of hundreds of different, environmentally relevant microbial lineages. These arrays allow a rapid screening for terrestrial microbial populations, and produce information about key terrestrial ecosystem functions. ‘1st generation’ phylogenetic microarrays will be continuously improved by elimination of poor and uninformative probes and inclusion of new probes (especially those targeting species specific to an environment of interest). When extended to mRNA it will allow us to monitor the actual functioning of the genomes of soil microorganisms. Therefore, metagenomics-based approaches will foster our understanding of soil functioning, and will bring a solution to the various questions about soil functioning with much more scientific depth than has been achieved so far.

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High salinity causes ion imbalance and hyperosmotic stress in plants. Organisms that thrive in hypersaline environments possess specific mechanisms for the adjustment of their internal osmotic status. One such mechanism is the ability to accumulate low-molecular-weight organic-compatible solutes such as sugars, some amino acids, and quaternary ammonium compounds. Glycine betaine (N,N,N-trimethylglycine, hereafter betaine) is a major osmolyte. Another mechanism for adaptation to high salinity is the exclusion of the Na<sup>+</sup> ion from sodium-sensitive sites. The accumulation of osmoprotectants is an important process for the adaptation to adverse environmental conditions. The Betaine is an important osmoprotectant, synthesized by many plants in response to abiotic stresses. Almost all known biosynthetic pathways of betaine are two-step oxidations of choline. Recently, a biosynthetic pathway of betaine from glycine, catalyzed by two N-methyltransferase enzymes, was found. Here, the potential role of N-methyltransferase genes for betaine synthesis was examined in a freshwater cyanobacterium, *Synechococcus* sp. PCC 7942, and in *Arabidopsis* plants. It was found that the coexpression of N-methyltransferase genes in *Synechococcus* caused accumulation of a significant amount of betaine and conferred salt tolerance to a freshwater cyanobacterium sufficient for it to become capable of growth in seawater. *Arabidopsis* plants expressing N-methyltransferase genes also accumulated betaine to a high level in roots, stems, leaves, and flowers and improved seed yield under stress conditions. Betaine levels were higher than those produced by choline-oxidizing enzymes. These results demonstrate the usefulness of glycine N-methyltransferase genes for the improvement of abiotic stress tolerance in crop plants.