

Ecological Genomics Institute

**Abstracts of Research Projects
Funded in 2005**

Carolyn Ferguson

Flocks of *Phlox* flowers and genome mapping: towards unraveling the functional significance of reproductive character position

SUMMARY

A developing avenue of research in the PI's lab explores the adaptive significance of diverse floral morphologies in *Phlox*, a genus for which we have a comprehensive phylogenetic framework and strong morphological context. Pollinators, a primary part of the local biotic environment, can exert tremendous selection pressure on particular floral characters, potentially driving population diversification and speciation. The mapping of Quantitative Trait Loci (QTL) is a powerful approach to investigating the genetic basis of particular traits. The proposed seed grant study completes critical initial steps on the pathway to construction of a genetic linkage map for *Phlox* (n=7) that will be used to map QTLs with significance to pollination ecology.

Four specific objectives will be accomplished:

- 1) *Molecular markers*. A large number of highly variable, co-dominant micro satellite markers will be developed for building of a linkage map for *Phlox*;
- 2) *Floral phenotypes*. Floral characters for species in target clades will be assessed via quantitative phenotypic analyses of material from natural populations;
- 3) *Assessment of genome size*. Flow cytometry will be conducted to determine genome size for species in target clades (as a proxy for ploidy level);
- 4) *Genetic crosses*. Artificial interspecific crosses will be effected, followed by subsequent breeding of lines.

Following success of "proof of concept" for the marker development and the breeding of plants, the investigator will propose, as part of a full-fledged NSF proposal: 1) development of a genetic linkage map for *Phlox* from a cross between closely related *Phlox* species with contrasting floral morphologies, and 2) intensive QTL mapping focused on traits relevant to pollination ecology. In the long term, integration of genomic tools into the PI's research program on *Phlox* will enable the linking of the genetics of functionally significant floral traits to field-based pollination ecology, all within the context of phylogeny.

Loretta Johnson/Jyoti Shah

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

SUMMARY

Thirty-seven percent of the U.S. and 1/4 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 (N) 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*). The common interest in understanding the molecular response of roots to environmental stress in natural ecosystems stimulated Drs. Jyoti Shah (expertise in molecular basis of plant response to stress) and Loretta Johnson (expertise in prairie grass ecology and belowground productivity) to link environmental factors controlling root productivity in natural systems to temporal and spatial changes in gene expression in roots. As part of the Kansas NSF EPSCoR Ecological Genomics Initiative (www.ksu.edu/ecogen), Drs. Shah and Johnson have been collaborating to develop and introduce molecular tools to study long-term changes in belowground productivity at the Konza Prairie. We have taken a genomic approach, which involves identifying genes that are differentially expressed in proliferating roots in response to N and water availability. We have constructed subtractive cDNA libraries from the roots of three month-old big bluestem plants subjected to N and water stress under growth chamber conditions. cDNA libraries from these plants as well from N- plus water-supplemented plants were constructed to isolate and identify the genes differentially induced by N and water stress. So far, 1920 (out of over 4000) clones from these four libraries have been 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 request funds to continue sequencing and validating the expression of some of these genes, much needed preliminary data in order to make us competitive for a resubmission to USDA. Finally, we plan to extend these growth chamber studies to field plots at Konza Prairie. The specific objectives of the project are to: 1) Validate subtractive libraries of big bluestem roots subjected to N and water stress; 2) Correlate gene expression pattern with plant growth and productivity parameters and metabolite content in plants exposed to stress; 3) Conduct studies on the molecular response of roots of prairie grasses to abiotic stress under field conditions at Konza Prairie.

Ken Jones, Michael Herman, & Timothy Todd

**Changes in nematode community composition in response to environmental cues:
a genomic approach**

SUMMARY

We are using resident soil nematode populations sampled from the Konza Prairie Biological Station, an NSF-funded LTER site near Manhattan, Kansas, to link the responses of organisms to environmental change at the genetic level. Prior studies, including our own preliminary data, demonstrated that some soil nematodes respond differentially to nitrogen addition, increased soil moisture, and different experimental fire regimes. What could account for the differential success of some species, especially those in the same trophic level? We hypothesize that species may have different genetic capacities to respond to changes in the environment, either due to differences in the genes they possess or how those genes are regulated. From previous studies, we know that the abundances of some nematode taxa were increased with nitrogenous soil enrichment and annual burning, while others are not. As nitrogen addition is correlated to a decrease in pH and changes in ionic concentration, it is likely that the changes in the nematode community seen are at least in part controlled by changes in soil chemistry. However as pH and the ionic concentration of the water in the soil (i.e., osmolarity) cannot be easily decoupled, independent manipulation of pH and osmolarity in laboratory cultures will help determine which of the parameters illicit the greatest response in each taxa. We will focus on describing the differences in sensitivity across native Konza taxa to determine whether changes in the nematode community are driven by changes in soil chemistry. Once sensitivities to pH and osmolarity are determined, microarray analyses using *Caenorhabditis elegans* will assist in determining the genetic basis of these sensitivities. Since a recent study indicates that at least half of the *C. elegans* genes have homology in other nematode taxa, we will use array-based comparative genome hybridization to determine which genes *C. elegans*' shares with each native Konza taxa. This will allow a candidate gene approach to be used to assess the differential expression of those genes in native taxa.

Ari Jumpponen

Identification of the function of mutualistic root endophytic fungi using an *Arabidopsis* transcriptome model system

SUMMARY

Root-associated mutualistic fungi are important determinants of many ecosystem functions because they control community dynamics and net primary productivity of their host plants. Mycorrhizal fungi are an abundant and relatively well-understood group of such mutualists. However, our recent observations challenge the overwhelming abundance of the mycorrhizal root symbionts: non-mycorrhizal endophytes in Konza Prairie Long Term Ecological Research site equal or exceed the mycorrhizal fungi in abundance. During experiments aiming to determine the host ranges of these endophytes, we discovered that at least two of the endophyte species form functional mutualisms with *Arabidopsis thaliana* and increase the host's growth two- to four-fold. This finding is novel: *Arabidopsis* does not associate with mycorrhizal fungi and there is only one prior example of endophyte associations in *Arabidopsis*. We aim to take advantage of this unique and fortuitous mutualism to further understanding of the function of root associated symbioses. We argue that this system is broadly applicable to root symbioses and the wealth of information available for *Arabidopsis* will allow advances in understanding mycorrhizal and non-mycorrhizal root symbioses alike. In absence of preceding examples of studies similar to those proposed here, we draw from analogues from mycorrhizal and pathogen systems and hypothesize that, similar to those systems, root endophyte colonization will 1) induce a systemic host defense response improving the plant resistance against pathogens; 2) improve host nutrient metabolism; and, 3) improve host water use efficiency and drought tolerance. These hypotheses will be tested by analyzing differential distal gene expression in endophyte-colonized and endophyte free hosts using an Affymetrix A THI array for *Arabidopsis*. The proposed experiments will be used to direct future research effort and to provide preliminary data for future proposals.

Srini Kambhampati
Ecological Genomics of Eusociality in Termites

SUMMARY

Termites (Isoptera) are hemimetabolous eusocial insects that live in complex societies. Termites exhibit eusociality not unlike that found in ants and some bees and wasps: presence of castes (reproductives, soldiers, workers), polyphenisms (one genotype developing into several specialized phenotypes), multiple developmental pathways, complex communication, symbiosis with protozoans and bacteria, construction and maintenance of complex nests, and use of "agriculture." Whereas Kin Selection explains the maintenance of eusociality in haplodiploid Hymenoptera, a comparable theory for termites, in which both sexes are diploid, is not available. Since all extant termites are eusocial, comparisons between non-eusocial and eusocial taxa are not possible. Finally, termites differ in substantial ways from honey bees. Thus, genomic studies on termites will provide information on the genetic basis of cooperation and social living and lead to comparative studies with evolutionarily divergent honey bees, ants, and some wasps. Here, I propose to develop resources that will set the stage for **long term studies on the ecological genomics of eusociality in termites**. The specific aims are: 1. Construct 7 caste- and life stage-specific cDNA libraries for the termite *Reticulitermes flavipes*. 2. Construct a BAC library for *R. flavipes*. 3. Generate a 25,000 EST library and sequence 1-3 BAC clones to characterize the termite genome. 4. Submit a proposal for a large scale (250,000) EST project and sequencing of 20 BAC clones. 5. Submit grant proposals to utilize the EST library in microarray studies of caste-specific gene expression. 6. Submit a white paper for complete genome sequencing of *R. flavipes*. A consortium of termite researchers has been assembled to collaboratively and synergistically utilize the proposed resources. These resources are expected to significantly advance our knowledge of ecological genomics of termite eusociality. They will also help elucidate the interactions between genes, behavior, and environment that together result in a highly complex society encompassing many traits also observed in human societies.

Brett Sandercock & Samantha Wisely
Development of Microsatellite Markers for a Migratory Shorebird

SUMMARY

New molecular methods have facilitated interdisciplinary research between evolutionary ecology and molecular biology. Microsatellites are one of the most useful molecular markers for studies of population genetics, but can be difficult to isolate from avian genomes. The main objective of this project is to develop micro satellites for the Upland Sandpiper (*Bartramia longicauda*). The genetic mating system of this migratory shorebird is unknown, but five hypotheses make predictions for expected rates of extra-pair paternity (EPP). The *breeding density* hypothesis predicts high rates of EPP in habitats where sandpipers are breeding as semi-colonial nesters. The *breeding synchrony* hypothesis predicts higher rates of EPP at the seasonal peak of clutch initiation. The *adult mortality* and *parental care* hypotheses predict low EPP because sandpipers are long-lived and males provide sole parental care after hatching. The *genetic compatibility* hypothesis predicts higher EPP among pairs where social partners are genetically related.

In a 5-year field study, blood samples were obtained for 33 families, and DNA has been successfully extracted from all samples. In August 2005, we developed genomic DNA libraries enriched for microsatellites. We request funding from the Seed Grant Program of the Ecological Genomics Institute to complete four major tasks to demonstrate proof-of-concept for a grant to seek major funding for this field project. Our specific aims are to: sequence plasmids from positive clones, design new primers, screen the primers for visualization, and genotype a subset of individuals for baseline estimates of EPP. The *intellectual merit* of this proposal includes development of new molecular markers, and it will be one of the first molecular studies of a socially monogamous shorebird. The *broader impacts* include development of an interdisciplinary collaboration between Sandercock and Wisely, estimation of genetic diversity for a species of conservation concern, and research opportunities for undergraduates.

Samantha Wisely & Sue J. Brown

Ecological, physiological and genetic consequences of environmental toxins

SUMMARY

Heavy metals toxicants are highly disruptive to normal physiological function. Mammals are particularly susceptible to these toxicants, because heavy metals accumulate and concentrate as they are shuttled up the food chain. More than 100 years of ore-mining in the tri-state area of Oklahoma, Kansas and Missouri have left approximately 5000 square miles of agricultural, suburban, and urban sites with toxic levels of lead, zinc and cadmium contamination. Although remediation of the environment to protect human health is nearly completed, virtually nothing is known about how such contamination disrupts ecological and evolutionary processes in this ecosystem. We propose to focus *in situ* physiological and molecular studies on a ubiquitous small mammal, the deer mouse (*Peromyscus manicu/atus*), located in the Tri-State Mining District and the surrounding uncontaminated area. Pilot studies confirmed the presence of deer mice on both contaminated and reference sites, and we successfully collected mRNA of sufficient quality and quantity to proceed with further investigation. To identify and describe physiological pathways that have been altered due to heavy metal contamination *in situ*, we will use *Mus musculus* gene chips to compare gene expression levels in liver and kidney from *P. manicu/atus* that have three levels of contaminant exposure: 1) resident individuals trapped from sites contaminated with heavy metals for more than 100 years, 2) individuals that are naive to contamination and are exposed for 60 days, 3) individuals that are not exposed to contamination (Specific Aim 1). Although we found deer mice present on contaminated sites, it is unclear if these animals are truly resident to contaminated sites or are migrants into the site. To determine whether contaminated sites act as ecological sinks or as evolutionary islands, we will estimate levels and direction of gene flow, determine levels of population isolation and identify genetic signatures of population bottlenecks using micro satellite markers developed for *Peromyscus* (Specific Aim 2). These studies will provide critical insight into understanding how the disruption of physiological pathways affects ecological processes and the evolutionary trajectory of a population of small mammals. Ultimately, understanding the role of toxicological stress to induce ecological and evolutionary change will provide a mechanistic view of anthropogenic landscape modification.