

Ecological Genomics Institute

**Abstracts of Research Projects
Funded in 2006**

Jeremy L. Marshall (PI) and Kun Yan Zhu (Co-PI)

Genomics of adaptive phenotypic plasticity in a cricket – a new approach to an old question

SUMMARY

Dissecting the underlying genomics of complex phenotypes such as life-history traits and behavior has consumed the careers of many biologists for more than three decades. Over this time span, real progress in identifying the genes of complex phenotypes has only been made in a handful of model systems (e.g., *Arabidopsis*, and *Drosophila*). For most other organisms researchers have used a quantitative trait locus (QTL) approach to identify the number and interaction of genomic regions that contribute to the phenotype of interest. However, in this genomic era, these types of results are becoming increasingly unsatisfying as we appear to be getting no closer to the underlying genes. So, we are left with the question, “are there approaches that could target genes of interest in non-model systems?” Here, we propose a “proof-of-concept” project that will combine a new mass sequencing technology (pyrosequencing) with a transcriptome-wide marker analysis (cDNA-AFLPs) to assess the genomics of plasticity in embryonic diapause in the cricket, *Allonemobius socius*. In brief, we will take advantage of heritable variation in diapause plasticity within a single population to generate low and high plasticity individuals. We will then utilize two replicates per plasticity type in our pyrosequencing cDNA-AFLP approach. This will entail generating double-stranded cDNA for all four individuals, conducting AFLP analysis on each sample with eight primer combinations, and pyrosequencing the resulting 32 samples using the services provided by 454 Life Sciences. These data will at a minimum provide insights into gene expression differences between treatments and a database of brain transcripts for future functional/comparative genomic questions. The potentially exciting, and proof-of-concept, portion of this approach is the ability to simultaneously evaluate allelic variation associated with the phenotype. If successful, this would provide a relatively quick, cost effective strategy for identifying allelic and gene expression variation underlying complex phenotypes.

Theodore J Morgan

ASSOCIATION MAPPING OF COLD TOLERANCE GENES IN DROSPHILA

SUMMARY

This project is focused on identifying the naturally occurring functional alleles that underlie adaptive phenotypic variation in nature. This project will address this question using cold tolerance in *Drosophila melanogaster* as a model system for adaptive-trait evolution. Cold tolerance is a very interesting adaptive phenotype in *Drosophila* as it exhibits pronounced latitudinal geographic variation in cold tolerance scores among populations and among species. Although, there has been substantial interest in the cold tolerance phenotypes over the last 10 years, we have been limited in our ability to ask questions about the molecular basis of phenotypic variation in nature because the candidate loci underlying these phenotypes have been unknown. This project will expand on this previous work that has documented substantial phenotypic variation in cold tolerance within natural populations of *Drosophila melanogaster*. This natural variation will be genetically dissected via linkage disequilibrium association mapping of individual cold-inducible candidate genes. These candidate loci have been identified and implicated in the expression of cold-tolerance phenotypes via previous QTL analyses, mutational screens, and microarray analyses of laboratory and artificially selected lines. The immediate goals of this project period are: 1) To identify a panel single nucleotide polymorphisms (SNPs) within cold-tolerance candidate loci via full sequencing of 24 naturally derived fly lines. 2) To genotype these polymorphisms within a larger cohort of naturally derived fly lines. 3) To perform a robust assessment of associations between this molecular variation and natural phenotypic variation in cold tolerance. This project will facilitate the identification of functionally and ecologically important allelic variation and will represent the first study to characterize the nature and the frequency of molecular polymorphisms affecting natural variation in cold tolerance. These results will ultimately shed light on long-standing evolutionary questions about the mechanisms maintaining standing variation for adaptive traits in nature.

Mark C. Ungerer

CBF/DREB1 expression variation and temperature adaptation in *Arabidopsis thaliana*

Summary

Understanding the genetic basis of adaptive phenotypic variation remains a central goal of ecological and evolutionary genomics. Historically, greater emphasis has been placed on variation in gene sequence (as opposed to variation in gene expression) in explaining adaptive phenotypic differences among populations and/or species. Natural variation in gene expression, however, is increasingly recognized as a potent form of standing genetic diversity upon which natural selection can act. Expression of the CBF/DREB1 gene family plays a critical role in the ability of *Arabidopsis thaliana* plants to acclimate to low temperatures and withstand subsequent freezing stress. Preliminary data from my laboratory indicate that natural accessions of *A. thaliana* exhibit adaptive variation in freezing tolerance that follows a latitudinal gradient. Moreover, accessions at opposite extremes of freezing tolerance ability exhibit striking differences in rates of induction and maximum expression levels of members of the CBF/DREB1 gene family. Three inter-related projects are proposed: (1) we will determine the extent to which expression of the three CBF/DREB1 genes (i.e., CBF1/DREB1B, CBF2/DREB1C, and CBF3/DREB1A) is correlated with differences in freezing tolerance in a panel of *A. thaliana* accessions from diverse regions of the species' native range, (2) in the same panel of accessions, we will examine levels and patterns of genetic polymorphism in each CBF/DREB1 5' upstream and protein-coding region; the analysis of CBF/DREB1 5' upstream regions will be conducted in light of the positions of previously identified CBF/DREB1 regulatory sequences, and (3) we will test whether differences in CBF/DREB1 expression patterns among natural accessions are attributable to cis-regulatory differences. These experiments will provide a more comprehensive understanding of the molecular basis of variation in gene expression and how this variation can influence an adaptive phenotype in nature.

Ruth Welti

Plant adaptation to elevated carbon dioxide: Using a lipidomic approach to identify alterations in lipid metabolism and signaling in *Arabidopsis thaliana*

Summary

Intellectual Merit:

Lipids form the structure of membranes, are fuels for metabolism, and make up the largest, and arguably the most important, group of signaling compounds and precursors of signaling compounds. Although lipid species have been demonstrated to play important roles in plant response to many environmental phenomena, the effect of elevated carbon dioxide on lipids, and particularly on lipid species involved in signaling, has not been examined in detail. In the proposed work, the effect of elevated carbon dioxide on the levels of over 300 lipid molecular species from 20 genotypes of *Arabidopsis thaliana* will be determined, utilizing rapid and comprehensive lipidomics technology. The genotypes include (1) plants from varying locations in North America, Europe, and the Cape Verde Islands and (2) in plants that have been selected at elevated CO₂. The following hypotheses will be tested:

1. CO₂ treatment (380 or 700 ppm) during plant growth will affect the levels of specific lipid species.
2. The levels of particular lipid species will be influenced by genotype and in-lab selection at elevated CO₂.
3. There will be significant correlations between specific lipid species, gene expression, other metabolite levels, including carbohydrate concentration and leaf nitrogen level, and physiological parameters, including time of flowering, biomass at the time of flowering, and photosynthetic rate.

Our **long term goals** are to obtain NSF funding to investigate the genetic basis for, efficacy of, and roles of alterations in lipid metabolism that occur when plants adapt to elevated CO₂ or when plant development is altered by selection at elevated CO₂.

Broader Impacts

The proposed work will provide training for a postdoctoral fellow and a graduate student. The work will also provide data on the variation in lipid molecular species composition in *Arabidopsis* as a function of geographical variation. These data will be deposited in LipidomeDB, an online-accessible database, and will serve as baseline data for other researchers who plan to examine the interplay between genotype and environmental factors on lipid composition.

Development of a Multi-applicable Insect cDNA Microarray for Assessing Environmental Health in Aquatic Systems

Kun Yan Zhu, Ph.D.

Summary

Oxygen depletion, a phenomenon that occurs in aquatic environments when dissolved oxygen concentrations decrease to a point detrimental to aquatic organisms, is a growing global concern. However, very little is known about hypoxic (low oxygen) stress-induced changes in metabolism and response to other environmental stressors in aquatic organisms. We propose to develop a multi-applicable cDNA microarray to examine the transcriptional response to hypoxic stress and other environmental stressors (e.g., pesticides, heavy metals, nutrients) in the aquatic midge, *Chironomus tentans*, an ecologically important bioindicator species. Our long-term goal is to elucidate the regulatory mechanisms controlling an organism's responses to environmental challenges. The specific hypothesis behind this project is that hypoxic stress can affect an organism's metabolism, leading to alterations of numerous genes' expression. This hypothesis is mainly based on: 1) oxygen is an essential molecule in energy production and various biochemical reactions; and 2) hypoxic stress has been found to significantly affect the metabolism of mammals and fish. In this pilot project, our specific aims are to: 1) construct a cDNA library and develop a cDNA microarray of *C. tentans*, and 2) examine genomic responses of *C. tentans* to hypoxia using the microarray. Our ultimate goal of this research is to develop valuable genomic resources (i.e., cDNA library and microarray) and generate preliminary data for submitting a comprehensive proposal to a federal funding agency with an emphasis on metabolic responses to hypoxic stress. The microarray can also be used to examine genomic responses of the organism to other environmental stressors in aquatic systems. Further characterization of the stress-responsive genes can eventually help us: 1) understand the mechanism of stressor's effects, and cellular and molecular responses to the stressor; 2) develop stress-specific marker genes for assessing environmental quality; and 3) identify effects at higher levels of biological organization, such as communities and ecosystems.