





Ecological Genomics at Konza Prairie Loretta Johnson, Mike Herman Ecological Genomics Institute



What is Ecological Genomics?



Martin Feder, U of Chicago

- integrative field
- genetic mechanisms underlying responses of organisms to their natural environment.
- genome-enabled approaches
 - functions of single or multiple genes.
- biochemical, physiological, morphological, behavioral responses of adaptive significance
- "finding the genes that matter".



Conceptual Model

COLOGICA



Rationale for Ecological Genomics

- Why is an ecological context necessary?
 - Many genomes sequenced, but the function of many genes are not known
 - Several studies show completely different gene expression in parallel controlled growth chamber and field studies
- Why is a genetic/genomic context necessary?
 - Elucidates underlying mechanism for ecological response
 - Better understanding of phenomena
 - May provide ability to predict response

MC Ungerer, LC Johnson, M Herman 2007. "Ecological Genomics: Finding the genes that matter in the environment". Heredity

What are the cross-cutting questions?

- What is the genetic basis for ecological responses to the environment?
- What are the regulatory and genetic pathways involved in organismal responses to their environment?
- What is the ecological context necessary to understand gene expression within organisms?









Approach:Apply the genomic tools available for model organisms to native organisms growing under field conditions

Advantages

- Ecological relevance
- Organisms growing under field conditions
- Select ecologically dominant organisms

Approach:

 to use genome sequences of close relatives of model organisms to make inferences about non-model organisms

Microarrays and Gene Expression

- 2 growth conditions.
 e.g. high/low N, H₂O
- isolate mRNA → cDNA (complementary DNA)
- hybridize to "chip" with fragments of all the genes.
- readout of gene expressed only in each condition or both conditions.



From:

Konstantin V. Krutovskii and David B. Neale (2001) Food and Agriculture Organization of the United Nations, Forest Genetic Resources Working Papers

Applying the genome tools available for corn to big bluestem Big Bluestem Corn



Family: Poaceae

Subfamily: Panicoideae

Tribe: Andropogoneae

Genus: Andropogon

Species: Andropogon gerardii Vitman





New sequencing technology enables ecological discoveries

- Mass sequencing techniques (454, Solexa)
- Generates 20 million bases in 5 hours
- Produces 200,000 randomized 100bp reads
- Enables new kinds of microbial diversity studies
- Soon many more ecological relevant species can be sequenced

Ecological genomics of big bluestem





Linking genes and ecosystems: transcriptome and physiological responses to climate change



Karen Garrett, Jan Leach, Scot Hulbert, Steve Travers, Zhongwen Tang, Jianfa Bai, Amgad Saleh, Mendy Smith, Alan Knapp and Phil Fay

Goals:

- 1) Use microarrays to measure transcriptome of native grasses under ambient and proposed climate change conditions
- 2) determine candidate genes and functional groups for stress response to climate change,
- 3) link physiological phenotype to transcriptome patterns,
- 4) determine role of individual genes in ecosystem function

Conclusions



• there were statistically significant relationships between gene transcription levels and environmental temperature variables but not water availability variables

• functional analysis indicated big bluestem upregulates mostly genes associated with chaperone proteins, and protein biosynthesis in response to stress, while downregulating transcription factors







Big bluestem

Gene expression responses to delayed precipitation in RaMPs

Travers et al. 2007

Nematode Ecological Genomics

Ken Jones, Joe Coolon, John Blair, Tim Todd, Michael Herman

- Genetic basis of nematode community responses to environmental change
 - Nematodes respond quickly and are bioindicators of environmental disturbance
 - Define community change at the lowest level using molecular tools



- identify interesting native taxa to study genomic responses
- field studies of native soil nematodes
- Determine ecological drivers affecting taxon responses
- Identify interesting genes to study
 - laboratory-based studies using microarray analysis of a model nematode: C. elegans
- Determine expression levels of genes of interest in native study taxa on Konza prairie



Are changes in food resources (bacteria) causing the observed changes in the nematode community?

- Lab-based approach using a model organism
- Gene discovery using the lab nematode C. elegans
- Isolate 3 soil bacteria from Konza soils
- Grow C. elegans on Konza bacteria
- Use microarrays to discover genes differentially expressed in response to the different bacterial environments





Are changes in food resources (bacteria) causing the observed changes in the nematode community?

It may be related to nematode species ability to respond to pathogens

202 unique genes!



- Which genes are functionally important? Pathogen-related
- Disrupt gene function by mutation
- Assess function by measuring fitness of mutants in identified genes
 - life table analysis in the lab!
- Predict: removing important gene function will reduce fitness

Environmental and ecological controls on gene expression of root processes in prairie plants Loretta Johnson & Jyoti Shah, G. K. Surabhi, S. Kumar



Goal: Combine ecology and genomics to better understand root processes under field conditions

- -> Identify genes exhibiting altered expression in response to changing environments (abiotic and biotic stress) e.g., nitrogen, water, grazing
- -> Link processes controlling root growth in natural systems to regulation of gene expression in roots
- ->Take advantage of the genomic resources developed for corn, a close prairie grass relative

Why study roots?

- Root processes dominate in tallgrass prairie
- Root productivity exceeds aboveground NPP
- Grasslands contain 15% of the world's soil carbon, which is primarily derived from root inputs
- Yet, root processes are not well studied
- Candidate genes involved in root proliferation in response to N identified in Arabidopsis





PRELIMINARY MICROARRAY RESULTS big bluestem roots harvested 2 weeks after N fertilization



11530 features showed hybridization signals (60.05%) to corn features

- ~ 570 genes were significantly hybridized
- ~ 320 showed differential expression

2-fold or more up-regulated genes with N

■ Ribosomal protein ·8 7% ■ Histones :6.5% Growth regulation :3.2% □ DNA binding protein :1.0% Plant stress/ Defense :4.3% RNA metabolism :1.0% ■ Transporter genes :4.3% ← Amino acid metabolism :3.2% Glycolysis :3.2% □ Lignin biosynthesis :1.0% □ Lipid metabolism :1.0% □ Glycolysis 3.2% Pentose phosphate pathway :1.0% ■ Cell wall metabolism :2.2% Unknown proteins :19.5% Others :21.7% □ Transcription factor :4.3% □ Signaling :7.6% ← □ Root development :2.2%





■ Ribosomal protein :16.7% Histones :2.1% Growth harmones :2.1% □ DNA binding protein :3.6% ■ Plant stress/Defense :2.9% ■ RNA binding protein :2.1% ■ Transporter genes :1.4% \square Membrane associated :0.7% ■ Nitrogen metabolism :0.7% ■ Glycolysis :0.7% Amino acid metabolism :0.7% ■ Sulfate assimilation :0.7% ■ Lignin metabolism :2.1% Respiratory metabolism :0.7% ■ Unknown proteins :22.6% ■ Others :15.3% Regulatory proteins (n-metabolism) :3.6 □ Signaling :4.3% □ Transcription factor :5.8%

2-fold or more down regulated genes

Ecological genomics of big bluestem





Erin Frank Drought stress doubles disease severity in this context



Phytohormone responses to disease and drought stress in *Andropogon gerardii*



Erin Frank is comparing gene expression, phytohormone, and polar lipid responses to drought and pathogen stress

Functional role of root endophytes: Arabidopsis microarray dissection of root endophyte mutualism (Ari Jumponnen)

- Periconia endophytes were isolated from Konza LTER
- Colonize Arabidopsis thaliana
- Improve A. thaliana growth
- Induce defense pathways
- Affymetrix ATH1 arrays to resolve improved growth



A. Periconia in A. thaliana roots

B. Paired mock- and Periconiainoculated Arabidopsis

C. Arabidopsis growth responses to Periconia



Ecological genomics of big bluestem



Bacterial community response to nitrogen addition (Ken Jones et al)

- Tag bacterial 16s rDNA sequences by plot to assess community response to perturbations.
- Percent identity of a group of sequences reflects taxonomic level
 - e.g. 92% roughly corresponds to family; 97% to species.
- Nitrogen addition reduces richness and diversity
- Question: Is this the reason for the changes in the nematode community?



Massive parallel sequencing to test RaMP effects on soil microbial diversity (Ari Jumponnen) RaMPS microbe

1,200-1,800 sequences/sample saturate eukaryon but not bacterial richness in soil

- The temperature or rainfall manipulations did not affect community summary stats (richness, diversity...)
- Individual responsive OTUs (taxa) could be identified (*Trichoglossum, Mycosphaerella*)



diversitv

Richness by sample

Richness by treatment

P>0.05 for all terms.



Future directions for Ecogen within the context of Konza LTER



- Can organisms adjust in time and space to the unprecedented pace of environmental change?
- What are the genetic/molecular mechanisms underlying ecological response to environmental change?
- How can we use long-term experiments as an opportunity to investigate potential genetic differentiation over decades?
- Does genotypic diversity of the dominant plant species have cascading effects to higher trophic levels?



For more information, visit our website at www.ksu.edu/ecogen