ANALYSIS OF EXPRESSION OF SMALL REGULATORY RNAS IN BARLEY

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Small RNAs (sRNA) of 15-30 nucleotides regulate various processes in eukaryotes. Regulatory sRNAs are produced through the RNA interference (RNAi) pathway from double stranded RNA (dsRNA). Dicer or dicer-like enzyme processes the dsRNAs into 15-30 bp dsRNAs, which in turn bind to the RNA-induced silencing complex (RISC) to regulate gene expression. We previously cloned sRNAs from barley. In this experiment, we determined the expression pattern of these sRNAs in different organs of barley (lemma, palea, awn, seed, leaf, leaf sheath, stem, and rachis) using northern blotting. Total RNA was extracted using a Trizol-based method. The quality of the extracted RNA was determined spectrophotometrically and by agarose-formaldehyde gel electrophoresis. Thirty µg of total RNA was separated on a denaturing polyacrylamide gel for northern blotting. Results of the northern analysis will be presented at the poster session.

THE EFFECTS OF HYPERGRAVITY ON DEVELOPMENT OF THE HEART AND BEHAVIOR IN XENOPUS LAEVIS

Stacey Howes (Biology)
Dr. Darrell Wiens (Biology)

Every living organism on earth has developed and evolved in a 1G environment. It is likely that any deviation from Earth's standard gravity will influence development, particularly at early stages. Previous reports from this lab showed that body length is reduced and ventricle size increased during development at 7G. The objective was to study the effect of hypergravity on the development of the ventricular myocardial wall, neuromuscular responsiveness, and equilibrium organs of Xenopus laevis. At early gastrulation, (Nieuwkoop and Faber Stage 10) embryos were placed in a centrifuge simulating 7G until stage 45 (approximately 72 hours from initiation of gastrulation). Mortality did not differ from controls and averaged less than 10%. Immediately following centrifugation, embryos were stimulated to test neuromuscular responsiveness. Thirty percent of hypergravity embryos required more than a tail touch, compared to 8% in controls. A quarter of the embryos were fixed in paraformaldehyde and their body dimensions were measured. Overall body length in the 7G group was, on average, 7.7% shorter. Fixed embryos were sectioned and immunostained. The thickness of the ventricle wall and papillary muscles observed in heart cross sections showed a 5.3% and 24.8% increase, respectively, in 7G embryos. Remaining live embryos were saved and swimming behavior was observed for 5 minutes daily. Abnormal swimming behavior was found in 33% of 7G embryos and 2% of controls during this time. These embryos were later subjected to an orientation-swimming test. Hypergravity embryos required, on average, 9% longer to become oriented. The data demonstrates that hypergravity has significant effects on the development of the ventricular myocardial wall, neuromuscular responsiveness, and equilibrium organs. Specific mediators of these apparent effects on cardiac tissue and swimming behavior are being investigated.

CONNEXIN43 ROLE IN SECONDARY NEURULATION OF DEVELOPING CHICK EMBROY

Stacey Howes (Biology)
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The central nervous system forms mainly through the elevation, approach, and eventual fusion of a pair of neural folds in the dorsal outer surface layer of cells to create the neural tube that will later become the brain and spinal cord. However, the posterior segment (tail) of the spinal cord forms through a distinctive secondary neurulation process, which is similar in human and chick embryos. Secondary neurulation is in part the transition of the tail bud from a loose arrangement of undifferentiated stem cells (mesenchyme) into a neural epithelial tube. Connexin43 is

the vital channel protein used to establish gap junctions required for communication among cells during this transition. Such communication may be vital for cavitation of the tail bud and organization of the developing lumina which are later fused together to create the canal inside the posterior segment of the neural tube. Thioridazine is a psychotherapeutic drug known specifically to inhibit connexin43 based gap junctions in secondary nuerulation. Therefore, thioridazine can be used to determine the importance of gap junction communication in secondary neurulation. We tested the effects of thioridazine in chick embryos approaching the initiation of secondary neurulation. At this time, chick embryos are windowed and injected under the developing tail bud with a known effective concentration of thioridazine. Following 24-hour post-injection incubation, embryos are harvested and fixed in paraformaldehyde. Embryos are photographed for study of abnormal morphology, specifically in the spinal cord and tail bud. They are immunostained with a Connexin43 antibody and sectioned to reveal localization. Preliminary results suggest that thioridazine is an effective inhibitor in secondary neurulation. We have observed that in the presence of thioridizine, tail bud mesenchyme cells were disorganized with large clusters of both mesenchyme and epithelium and no characteristic lumina. Confirmation of these findings is being investigated.

THE EFFECT OF DIVERSITY ON THE EVOLUTION OF PHYSIOLOGY IN SWITCHGRASS (PANICUM VIRGATUM)

Lucas Joers (Biology)
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Diversity influences the invasibility and productivity of plant communities, but its effect on the evolution of species within those communities is poorly understood. In this study, we tested whether diversity influences natural selection on the physiology of switchgrass (Panicum virgatum), using four treatments of native vegetation: (1) switchgrass monoculture, (2) five warm-season grasses, (3) 16 grasses and forbs (Biomass Mix), and (4) 32 grasses and forbs (Prairie Mix). In each treatment plot (0.3 - 0.56 ha), we measured photosynthetic rate, stomatal conductance, chlorophyll content, flowering time and height on 100 systematically sampled individuals. Ultimately, we will estimate selection as the relationship between each of these traits and two fitness measures (aboveground biomass, total seed production). We replicated this experiment in two soil types with contrasting resource availability. Preliminary treatment differences show that plants are shortest in the warm season grass plots and tallest in the Biomass Mix. Further, plants in the warm season grass plots have the lowest photosynthetic rate and chlorophyll content, while plants in the Prairie Mix have the highest photosynthetic rate. We predict that plants will incur stronger selection for high photosynthetic function and faster growth in diverse prairie plots than in grass plots because of increased competition for light. Our selection results could provide new perspective on the long-term sustainability of these treatments as potential biofuel crops.

This research was supported by The University of Northern Iowa Department of Biology, Iowa EPSCoR, and the Tallgrass Prairie Center.

INVESTIGATING BACTERIOPHAGE STRAIN MAITENANCE IN BACILLUS CEREUS SPORES

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Sporulation occurs as part of the life cycle of Bacillus anthracis and other related varieties. Some bacteriophages (viruses of bacteria: 'phages') are able to remain encapsulated inside bacterial spores in nature. Bacterial spores are more resistant than phages to environmental conditions, such as temperature. We examined aspects of host spore long term storage of phages. Time course samples of soil cultures inoculated with B. cereus 569 UM20 provided data on phage burst timing, phage levels and spore presence in soil cultures under laboratory conditions. Spore samples were isolated from soil using heat treatment (65°C, 30 min). Spores containing phage left plaques (clearings) on host lawns when spores germinated, releasing phages. Spores and phages were triple-serial isolated and increased for characterization, including phage host range. Vegetative bacteria were inoculated with known phage isolates and forced

to sporulate in brain-heart infusion broth. As we observe phages isolated from naturally derived spores, we continue toward our goal of artificially reproducing phage encapsulation in spores in order to reliably store important phage isolates over extended periods of time.

OPTIMIZATION OF MICROSATELLITE POLYMERASE CHAIN REACTION FOR THE EXAMINATION OF THE POPULATION GENETIC STRUCTURE OF LONGNOSE DACE

Hayley Rinehart (Biology)
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Dr. Peter Berendzen (Biology)

Rhinichthys cataractae, longnose dace, is a non-game, native fish species found in northeast Iowa that is listed as a Species of Great Conservation Need in the Iowa Wildlife Action Plan. Its habitat is topographically distinct from the rest of Iowa, but like the rest of the state, is facing degradation from sources such as climate change, fragmentation, channelization, and agriculture. To focus conservation efforts, the genetic structure of populations found in northeast Iowa must be examined, thereby revealing levels of connectivity within and among river drainages. Microsatellites are genetic markers consisting of small repetitive nucleotide units that are commonly utilized in conservation genetics to assess the overall genetic diversity and health of a population. The purpose of this study was to complete a preliminary screening of microsatellite loci, a crucial step in choosing variable markers that will yield quality genotyping data. DNA was extracted from fin tissue samples originating from four river drainages in northeast Iowa. Microsatellite loci were amplified by polymerase chain reaction (PCR) using primers designed for Rhinichthys cataractae. The PCR products were visualized on both agarose and polyacrylamide gels. Three approaches were used to optimize PCR results. First, the primary annealing temperature was raised or lowered to fix problems such as stuttering and non-amplification. Second, the Type-it Microsatellite PCR Kit by Qiagen was used on loci insensitive to annealing temperature adjustment. Lastly, LCO primers (designed for another species) were implemented in a few trials to see if the results were more indicative of allele polymorphism than the loci specific to Rhinichthys cataractae. Annealing temperature adjustment yielded inconsistent results. The PCR Kit did not improve problematic amplifications, but a few LCO loci were amplified with clear results. Microsatellite optimization is ongoing, but after further trials, the LCO loci might be useful for systematic genotyping.

This research was supported by the State Wildlife Grants Program, Iowa Department of Natural Resources; IAF Research Award, UNI Office of the Executive Vice President and Provost; and the UNI College of Humanities, Arts and Sciences.

COMPARISON OF GENETIC VARIABILITY AND DEMOGRAPHICS OF TWO CODISTRIBUTED FISH IN NORTHEAST IOWA, THE ABUNDANT COMMON SHINER AND THE DECLINING LONGNOSE DACE

Erica Scullin (Biology)
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Dr. Peter Berendzen (Biology)

Population genetics are important in understanding the health of an ecosystem. Changing habitats and human stressors can affect a species by restricting gene flow and ultimately reducing genetic diversity. A lack of variability could prevent adaptations necessary for survival and lead to a greater risk of extinction. A useful tool in population genetics is the microsatellite, a sequence of nucleotide repeats at a specific locus in the genome. These microsatellite loci are markers that determine level of connectivity and gene flow within a species. An area of such research interests is the unique terrain of northeast Iowa; valleys and dense vegetation correlate with cooler water temperatures and uncommon streambed compositions. This results in a unique assemblage of fish species in the state. The Iowa Wildlife Action Plan has generated a list of recognized fishes of concern in this region. Possible causes for the decreasing population size include but are not limited to stream contamination, habitat loss, and population fragmentation. The common shiner, Luxilus cornutus, is an abundant native fish that is often found in the same streams as one species of

concern, the longnose dace, Rhinichthys cataractae. The overall goal is to compare the demographic patterns of the common shiner and longnose dace to determine similarities and differences in stress responses. Common shiner fin clip samples were collected from northeast Iowa river drainages where the two species coexist. DNA was extracted from 149 individuals. Six of the eight known microsatellite loci have been screened for the ability to amplify via PCR and variability in the northeast Iowa populations. Once the useful loci have been identified all 149 samples will be genotyped. Demographic analyses will be performed and the results will be compared to a study on the population genetics of the longnose dace being conducted in parallel in the lab.

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BUTTERFLY COMMUNITY DYNAMICS IN A RESTORED PRAIRIE USED FOR BIOFUEL PRODUCTION

Nicholas E. TeBockhorst (with Jarrett D. Pfrimmer) (Biology)
Dr. Mark C. Myers (Biology)

The conversion of native Midwestern tallgrass prairie to monoculture production of corn (Zea mays) and soybeans (Glycene max) for food and fuel has resulted in a significant decrease in habitat for insect pollinators, including butterflies. Compared to conventional biofuels such as corn based ethanol, prairie biomass produces greater energy yields while providing high quality wildlife habitat and protecting soil and water resources. My research took place at Cedar River Natural Resource Area in Black Hawk County Iowa, USA. In spring 2009, the University of Northern Iowa's Tallgrass Prairie Center seeded 48 research plots in conventional fields to one of four experimental treatments of native vegetation: 1) switchgrass monoculture, 2) warm-season grass mix (5 species), 3) biomass mix (8 forb and 8 grass species), or 4) prairie mix (20 forb, 3 sedge, and 9 grass species). During the summers of 2010, 2011, and 2012, our team conducted visual surveys of butterflies (class Lepidoptera) a group widely recognized as bioindicators of ecosystem health. I used these data to compare butterfly abundance, species richness, and composition among treatments over the three year study. I hypothesized that butterfly abundance and richness will increase over the three year period as well as that more diverse plantings will support a greater abundance and diversity level of butterflies. The conversion of marginal agricultural lands to areas of natural vegetation cultivated for biofuel production would be beneficial to increase abundance of butterflies through creation of habitat and providing a food source.

THE CYTOTOXIC EFFECTS OF METOLACHLOR ON HEPG2 CELLS

Macy Ubben (Biology)
Dr. Kavita R. Dhanwada (Biology)

Metolachlor is one of the most commonly used herbicides in the Midwestern United States; it is a frequent contaminant of ground and surface waters due to run-off into nearby water sources after spring application to corn and soybean fields. It inhibits protein synthesis as well as chlorophyll synthesis in plants. Recent studies have shown that metolachlor has a number of effects on non-target cells including genotoxicity in tadpoles and human lymphocytes as well as cytotoxic effects on lymphocytes, changes in protein activity, and slowed or decreased cell growth. Previous research from our lab has shown metolachlor exposure led to decreased levels of growth in human liver (HepG2) cells after 72 hour exposure to 50 ppb (parts per billion), and in normal human fibroblasts after 72 hour exposure (1.6 ppb). In the current study, we wanted to understand the mechanism for decreased cell growth after metolachlor exposure. Specifically, we wanted to determine if metolachlor exposure results in cytotoxicity or necrosis of HepG2 cells. We treated the human liver cells with increasing concentrations of herbicide (0-1000 ppb) for 24, 48, and 72 hours. Live/dead viability/cytotoxicity assays were performed to determine the ratio of treated dead and living cells to control cells. When treated cells were compared with non-treated (control) cells, a higher statistically significant level of cytotoxicity was only seen after a 72 hour metolachlor exposure of 1000 ppb. Lower concentrations of her-

bicide did not result in significant cytotoxicity at 24, 48 or 72 hours suggesting that decreased cell growth observed after metolachlor exposure may not be due to cytotoxic effects (necrosis) and other mechanisms such as apoptosis or altered cell cycle may be involved.

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DEVELOPMENT OF VEIN PATTERN IN COMPOUND LEAVES OF SOLANUM LYCOPERSICUM (TOMATO)

Layton Weishaar (Biology)
Dr. Julie Kang (Biology)

Much research has been done on the simple leaf morphology and vascular development in Arabidopsis thaliana. The interconnection between the formation of vein pattern and the morphology of compound leaves is enigmatic and continues to raise questions in plant developmental biology. This is the first study to map out vascular development in the compound leafed species Solanum lycopersicum (tomato) to determine the relationship between leaf shape and vein patterning. We used the molecular marker Arabidopsis thaliana Homeobox Gene-8 (AtHB-8::GUS), one of the earliest genetic markers of vascular development, to visualize the spatial and temporal patterns of vascular strand formation in tomato leaves. Imaging data shows that the midvein develops at early stages of leaf development and connects to stem vascular bundles. However, expression of AtHB-8::GUS was not detected in developing lobes at very early stages of leaf development. Our data suggests that leaf lobe development precedes vascular strand formation suggesting that these two developmental processes are separated temporally. Although the direction of vascular strand formation within developing lobes is unclear at the present time, closer examination of the vascular strands will shed light on this subject.

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THE EFFECT OF NUTRIENT AVAILABILITY ON THE EVOLUTION OF PHYSIOLOGY IN TWO TALLGRASS PRAIRIE SPECIES

Angela Wrage (Biology)
Dr. Mark Sherrard (Biology)

Species from fertile environments generally have faster growth rates and higher photosynthesis than those from infertile environments, suggesting that nutrient availability has shaped the evolution of these traits. In this study, we tested whether nutrient availability influences natural selection on the physiology of two species with contrasting ecologies: Heliopsis helianthoides (a competitive forb) and Desmodium canadense (a stress-tolerant legume). Using research plots established by the Tallgrass Prairie Center, we measured the relative growth rate, chlorophyll content, flowering time, and specific leaf area of 375 individuals of each species split among three soil types with contrasting nutrient availability (low - high: sandy loam, loam, clay loam). Ultimately, we will estimate selection as the relationship between these traits and fitness (assessed based on aboveground biomass and total seed production). Heliopsis plants in nutrient-rich soil grew faster, flowered earlier, and had higher chlorophyll content than those in nutrient-poor soil. Desmodium plants were equally tall in all soil types, and those in loam soil had the highest mortality. For Heliopsis, we predict that selection will favor plants with rapid growth, high chlorophyll content, and high specific leaf area in nutrient-rich soil. In contrast, we predict that these traits will not affect the fitness of Heliopsis plants in nutrient-poor soil, or of Desmodium plants in any soil type. This research provides new insight into whether rapid growth, high chlorophyll content, and high specific leaf area are adaptive in fertile environments.

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