

## Abstracts for BioDay 2007

### **Interactions between drought responses and phytochrome pathways.**

Drought sensing and response are crucial to optimizing fitness in weedy annuals. To further understand the pathways that mediate drought-response plasticity we examined interactions between phytochrome and drought stress with *Arabidopsis thaliana*. Previous studies have suggested that phytochrome may mediate germination pathways through abscisic acid, linking the light-sensing hormone to drought response. Phytochrome mutants were grown under moist and dry soil conditions in Percival chambers. Phytochromes A, B, and E influenced stomatal conductance of drought-stressed plants in a web of inhibition and promotion. It is clear that the three phytochromes are present in drought-response pathways and loss of phytochrome function can have a pronounced effect on the strength of drought response in *A. thaliana*. Phytochrome-mediated pathways reduce aboveground biomass in moist conditions and increase aboveground biomass in drought conditions, indicating that phytochrome may mediate drought-avoidance strategies. *PHYE* may actually confer negative fitness under drought stress but evolutionarily the gene was conserved, likely due to its importance to germination under cold conditions.

### **Activation and Transport Functions in the *Serratia*-type Hemolysin and Transporter of *Chromobacterium Violaceum*.**

The *Serratia*-type hemolysin and transporter are an example of a Two-Partner Secretion system in Gram<sup>-</sup> bacteria. The *Serratia* hemolysin has shown an absolute requirement for its cognate transporter for both activation and secretion across the outer membrane. Previous work [Brumbach, Eason, and Anderson, *FEMS Microbiol Lett*, **267** (2007) 243-250] established the presence of a *Serratia*-type hemolysin (ChlA) and transporter (ChlB) in *C. violaceum*, and a lytic phenotype for *E. coli* expressing ChlA in the absence of ChlB. We have examined this ChlB-independent lytic phenotypic by enzyme assay, complementation, and osmotic protection against lysis. Our results indicate that (a) in the absence of ChlB, accumulation of ChlA in the *E. coli* periplasm leads to leakage and lysis of the bacteria; (b) ChlA released in this manner forms pores in erythrocytes that are similar in size to those formed by ChlA that is secreted by ChlB; (c) ChlA released in this manner can be activated for more effective lysis by complementation with a ChlB-secreted activation domain (ChlA<sup>'</sup>); and (d) ChlB appears to allow egress of *E. coli* periplasmic enzymes, but only if it has transported ChlA.

## **What Nest Site Characteristics Influence The Frequency Of Nest Predation In Flammulated Owls?**

Several studies have shown that nest predation negatively affects avian fecundity and survival, and that it may constitute a major factor affecting the evolution of life histories. For many species of songbirds, nest predation also has influenced habitat selected for nesting. However, the importance of predation in affecting the selection of nesting habitat in raptors is less documented. Consequently, our research focused on determining the extent to which the selection of nesting habitat in Flammulated Owls (*Otus flammeolus*) is affected by predation by red squirrels (*Sciurus vulgaris*), which are the primary species of nest predator in this small, cavity-nesting raptor. During the summer of 2006, we quantified a total of 22 nest sites in the Pike National Forest in Colorado, comparing the habitat characteristics of 11 predated and 11 non predated sites. We expected to see that non predated nest sites would be higher from the ground and have smaller cavities, as well as fewer neighboring trees and lower overstory density. Our relatively small sample of data suggested that, compared to nests where predation occurred, nests that were not preyed upon had smaller nest cavity entrances ( $P=0.001$ ), were located higher off the ground ( $P=0.095$ ), and were located relatively farther from the closest large squirrel middens ( $P=0.051$ ). Conversely, squirrels showed no preference for nest sites in dense tree stands. These data provide telling evidence to what factors may play important roles in nest predation in Flammulated Owls. To further investigate these patterns, this preliminary data should be expanded upon to include quantification of more nest sites in both categories, as well as more characteristics of the sites for which the birds or squirrels may be selecting.

## **Transporter Independent Lysis of a *Serratia*-type Hemolysin of *Chromobacterium violaceum***

*Chromobacterium violaceum* is a Gram-negative opportunistic human pathogen and an inhabitant of tropical soils. It is primarily known for its synthesis of the pigment violacein. Recent genomic sequencing has also revealed several potential virulence factors within this microorganism. This study establishes the presence in *C. violaceum* of a *Serratia*-type hemolysin (ChlA) and its transporter (ChlB). We utilized amplification and cloning techniques to isolate these sequences and express them in *Escherichia coli*. We conducted hemolysis-in-culture assays to measure the extent of lytic activity and found evidence demonstrating transporter-independent lysis of ChlA.

**The attempted purification of the transcription Regulator protein *hilA*,** *HilA* is an important transcription regulator for bacteria in the *Salmonella* genus. This protein activates the transcription of genes vital to the Type Three Secretion System 1, which are located on the *Salmonella* pathogenicity island 1. While experimental data has given some idea about how this protein functions, its structure has never been solved. This gap in knowledge leaves much unknown about the mechanics of *hilA* function. Our research goal was to isolate functional, soluble *hilA* protein from plasmid-induced *Escherichia coli*, so that more information about its unique function could be determined. While we experienced some difficulties with equipment and supplies, we were able to determine the presence of *hilA* using SDS PAGE analysis and Dot blotting techniques. While this is a vital first step for this research, further study is necessary to complete the purification.

### **Environmental factors affecting riparian community dynamics and Tamarix distribution on the flood controlled and free flowing Arkansas River, Colorado**

Invasive species can change plant community composition by outcompeting local taxa, altering the local environment, or both; such species are effectively ecosystem engineers in that their life history and growth patterns in large part determine the type of environment they occupy. In fact, invasive species can directly alter environmental conditions to promote their establishment and persistence through time. *Tamarisk ramosissima* (the most common invasive species of the Tamaricaceae) is such a species; it has caused massive morphological changes to riparian ecosystems and their bank structures over the last century throughout the southwestern United States (Deloche et al., 2000). Growing as either small trees or dense stands of shoots, *Tamarisk* species displace or actively outcompete native species of willow (*Salix exigua*) and cottonwood (*Populus deltoides*) on the Arkansas River in CO.

Due to competition for light, *Tamarix* has been unable to invade many naturally flowing rivers (Sher and Marshall 2001). Under normal conditions for colonization by native species, *Tamarix* seedlings are at a great competitive disadvantage due to slow above ground biomass accumulation. However, damming and the resulting altered disturbance regime may give *Tamarix* an advantage over natives. Our study analyzed vegetation patterns using census, stem diameter and gas exchange physiology data to assess what factors affect the distribution and community structure of native species and *Tamarix* on the Arkansas River, CO.

**Changes in the composition of grassland communities within Indian Creek and Canyonlands National Park: Interdependence of cryptobiotic soil cover and cattle grazing on diversity and cover of vegetation species.**

The semi-arid grassland communities within Indian Creek and Canyonlands National Park, Utah, have evolved in the absence of large herds of ungulate grazers. However, since the early 1800's this region was actively grazed by cattle which lead to the reduction of cryptobiotic soil cover (a symbiotic grouping of keystone species) as well as changes in plant community composition. Starting in 1970, Canyonlands National Park was expanded and cattle grazing was removed from parts of Canyonlands, including the Indian Creek corridor. This removal of cattle afforded the opportunity to examine the interaction of cryptobiotic soil cover and cattle grazing activity on the composition of the grassland vegetation communities.

Ten study sites were analyzed in each of the following study regions; grazed/ high cryptobiotic soil cover, grazed/ low cryptobiotic soil cover, 35 years grazing cessation/ high cryptobiotic soil, and 35 years cessation/ low cryptobiotic soil. Regions with high cryptogram cover maintained a significantly higher percent cover and richness of native grass and forb species than regions with low cryptogram cover. Actively grazed regions had greater cover and richness of shrubs and cacti than regions where grazing has been removed over the last 35 years. Percent cover of *Acnatherum hymenoides* (Indian Ricegrass) and *Pleuraphus jamesii* (Galleta) was greatest in actively grazed sites and sites with high cryptobiotic soil. *Sporobolus cryptandruss* (Sand Dropseed) cover was significantly decreased in regions of low cryptogram cover when grazing was present. Exotic species *Bromus tectorum* (Cheat Grass), *Salsola pestifer* (Russian thistle), and *Chorispora tenella* (Purple Mustard) showed trends of higher percent cover and abundance in regions with low cryptobiotic soil cover. These results demonstrated that establishing high cryptobiotic soil cover is essential to maintaining high native species diversity. Interestingly, when cryptobiotic cover was low, the cessation of grazing caused a reduction in the abundance and cover of vegetation. In these vegetation communities it was thus demonstrated that grazing can increase the diversity within the community when grazing is maintained at a low level, supporting the intermediate disturbance hypothesis.

## **Pigment Epithelium-Derived Factor (PEDF): A Novel Tumor-Suppressor**

Pancreatic carcinoma (PC) is a devastating disease, with a near equal incidence and mortality. Ninety percent of PCs contain a mutation in the *Ras* gene. In fact, an activating mutation in *K-Ras* has been shown to be an initiating factor in cell proliferation and tumorigenic activities associated with PC. Understanding potential downstream targets of mutant K-Ras is critical in delineating the complex cell signaling pathways involved in the progression of this disease. One of the potential targets is PEDF, a non-inhibitory serpin expressed in retina, liver, testis, placenta, brain, ovaries, kidneys, prostate, and pancreas. Although PEDF has been extensively studied as an antiangiogenic factor in the eye, it has seen little attention as a potential tumor suppressor gene. Recently, however, it has been noted that nearly 75% of human pancreatic cancers showed significantly reduced PEDF expression. In our study, PEDF expression was assessed in an epithelial cell model. Cells stably expressing activated K-Ras showed a significant decrease in PEDF expression. Constitutive activation of Raf kinase, but not phosphatidylinositol-3-kinase, was sufficient to cause downregulation of PEDF expression, suggesting that Ras regulates PEDF expression by the Raf>MEK>ERK signaling pathway. Although, other Ras-effector pathways are involved because cells stably expressing activated K-Ras did not show a return of PEDF to normal levels when treated with a MEK inhibitor. Real time PCR and northern blot analyses showed that *PEDF* expression was reduced to various extents in several human pancreatic tumor-derived cell lines containing an activated *K-Ras* mutation or high levels of phospho-ERK. To further analyze the role of PEDF in Ras-mediated oncogenesis, we used a mouse model that contains a chronically active *K-Ras* gene. The phenotype of this mouse model is unique because it is normally representative of early disease, characterized by the development of ductal carcinoma *in situ* (comparable to human PanINs). These mice were crossed with *PEDF*-null mice. Fifty percent of the offspring developed infiltrating pancreatic adenocarcinoma at 13-15 months of age. Carcinoma cells and surrounding stroma expressed high levels of the proangiogenic factor, VEGF and lacked expression of the antiangiogenic factor, TSP-1. Additionally, gelatin zymography demonstrated an increase in MMP-9 (responsible ECM degradation). Together with the *in vitro* data, these findings are compelling evidence for an interaction between mutant K-Ras and PEDF, with implications of PEDF being a potential tumor suppressor gene and a promising therapeutic target.

## **Avian Response to Mechanical Forest Thinning in the Colorado Rocky Mountains**

Natural Selection has favored individuals whose habitat selection has resulted in higher reproduction and survival. Identifying habitat characteristics leading to higher reproduction and survival are critical in making sound management decisions. With ten million hectares of North American coniferous forest under extreme fire danger, robust fire mitigation is a necessity. These management strategies often include mechanical forest thinning which can lower the danger of large-scale fire, but previous research has shown varied consequences from thinning on the forest avifauna. I studied the response of the avian community to mechanical thinning in two areas west of Colorado Springs, CO. These two sites included a ponderosa pine (*Pinus ponderosa*) forest that had been subjected to rotary mastication in the winter of 2004 and a mixed conifer forest that had been selectively thinned during the 1980's. I used standard point count methodology (Bibby et al. 1997) to measure avian species richness and density during the breeding season of 2006. Thinning increased overall avian density and species richness in the ponderosa forest, but had no effect on the overall density or species richness in the mixed conifer forest. All species except swallows had higher densities in the thinned plot of the ponderosa forest. Ground nesting birds were found in higher densities in the thinned plot of both forests, largely corresponding to higher amounts of ground cover. Many factors contribute to the difference in avian response between locations including time since thinning, differing forest types, and different thinning intensities. These differences may imply that avian communities are either resistant to severe thinning treatments or have short term responses, and that each type of treatment has different affects on the avifauna. The results found in the ponderosa site are largely consistent with previous research conducted in North America showing positive responses to severe thinning treatments. As no study to date has looked at the affects of rotary mastication, these results provide valuable management recommendations for future fire mitigation strategies. Avian communities may respond favorably to these aggressive, yet necessary, fire mitigation treatments.

### **A phylogenetic/taxonomic assessment of the orchid *Laelia-Cattleya* alliance based on nuclear ribosomal internal transcribed spacer (ITS) sequences and inter-simple sequence repeats (ISSR)**

The *Laelia-Cattleya* alliance of orchids has recently been subjected to a phylogenetic analysis using nuclear and plastid DNA sequences. The analysis suggested some controversial taxonomic changes. This research was conducted to further examine these controversial taxa. The research utilized both internal transcribed spacer (ITS) sequences and inter-simple sequence repeat (ISSR) analysis to reevaluate previous classification. ITS sequencing required DNA extraction, PCR amplification and sequencing of ITS 1 and 2 DNA segments. The sequence was then used to create several different phylogenetic trees utilizing different grouping algorithms. These trees were then used to evaluate the evolution of species within the alliance and make recommendations for reclassification of some species. The trees corroborate previous research that supports the movement of the Brazilian *Laelias* (*L. alaorii*, *L. grandis*, *L. pumila*, *L. sincorana* and others) to *Sophranitis*. However, the results of this study do not support the creation of a new genus *Guarianthe*. The ISSR technique had too high a level of discrimination and was therefore unable to be used for analysis at the genus level. Future research should focus on the *Guarianthe* genus and its relationship to *Rhyncholaelia* and *C. bowringiana*. Additionally, the location of *C. maxima* within the *Cattleya* or *Laelia* genera needs addressing. This could be accomplished by pairing ITS 1 and 2 sequence with sequence from other genes or ITS spacer sequences, or by finding ISSR primers that were less specific.

### **Better Understanding the Austronesian Expansion: STR Analysis of Bai and Pumi Chinese Populations**

The peopling of Micronesia/Polynesia/Oceania, known as the Austronesian expansion, is known as the last great human migration dating back to 6000 years ago. To explain the origins of this expansion several hypotheses have been presented over the years. The hypothesis presently receiving the most support is entitled the 'slow boat' model presented by Kayser et al. This model identifies Southeast Asia as the origin of two major expansions, one towards Taiwan and the other towards Polynesia through Melanesia and the rest of island Southeast Asia. Our research was conducted through STR analysis of 67 samples from the Bai tribe and 64 samples from the Pumi tribe. The data from these two mainland Chinese populations was analyzed alongside STR data from other Southeast Asian populations which had been previously recorded. Centroid analysis, principle component analysis, and

maximum-likelihood tree analysis were run on all populations showing correlation between the Chinese and Melanesian tribes with the Taiwanese consistently isolated away from these tribes. These results add support to the ‘slow boat’ model and help to further increase understanding of the great expansion that occurred in this area.

### **Mutagenic Analysis of Residues Key to Intra- and Interdomain Interactions in XO4 HilA,**

HilA (after hyper-invasive locus A) is a 553 amino acid transcriptional activator for *Salmonella* SPI-1 invasion genes in response to integration of various environmental signals such as aeration, osmolarity, and pH. Though the N-terminal region possesses a high degree of similarity to the DNA binding and transcription activation domain of the OmpR family of transcriptional regulators, the role of the C-terminal region is less clear, though roles in regulation and activation have been suggested by deletion analysis. A series of motif swaps between *Salmonella typhimurium hila* and its *Chromobacterium violaceum* homolog were constructed via crossover PCR in Lamont Anderson's lab, and though either homolog is functional, the crossovers possess radically different phenotypes. In this study we employed a strategy of random mutagenesis on one particular crossover, XO4, which possesses the DNA binding domain from *Chromobacterium* and the TPR and TadD-like domains from *Salmonella*. A *lacZ* gene was placed under the control of a promoter known to be directly activated by HilA to detect HilA activity. We generated and screened a library of mutants on MacConkey-Lactose agar. Five mutations with distinct morphologies were detected and isolated from the screen and subsequently phenotypically characterized by  $\beta$ -galactosidase assays. Though sequencing has not yet been conclusive, analysis of mutants exhibiting a restoration of HilA activation may illuminate which residues are involved in C-terminal function and domain-domain interactions in HilA.