

BioDay Student Abstracts 2008

Localizing chloroplast genes in the dinoflagellate *Karenia brevis*

Karenia brevis is a dinoflagellate which undergoes extensive blooms that cause harm to fish, marine mammals, and humans due to the production of a brevetoxin. *K. brevis* has a confusing evolutionary history that makes studying gene expression complicated. This study tries to find the location of the photosystem genes, as some dinoflagellates are known to have moved the photosystem genes out of the chloroplast and into the nuclei. We used density centrifugation in order to obtain pure isolates of chloroplast DNA and nuclear DNA. We used PCR to check for the presence of photosystem genes. The results were inconclusive, because we obtained positive PCR product upon amplification of both the chloroplast and the nuclear DNA.

Heat Stress Response and Nutrient Deficiencies in Early vs. Later Development in Cherry Tomato

The common tomato plant (*Lycopersicon lycopersicum*) is an integral agricultural commodity throughout the world. With an ever-changing global climate, environmental stresses that plants must undergo will become more severe, testing their hardiness and adaptability. Tomato plants are an important indicator of climate change because the plants accumulate heat shock proteins, which allow for physiological adaptation to extreme temperatures. Our experiment of tomato plants aimed to study the effects of heat shock and nutrient deprivation on seedlings in different stages of development. By measuring chlorophyll content, root to shoot ratio, biomass, and germination frequency, we were able to analyze hardiness and adaptability of seedlings on different nutrient media with varying levels of heat shock exposure. These data were compared to earlier data, which looked at seedling and mature plant exposure to heat shock in order to understand the interactions between life-history and heat stress. The timing, level of heat shock, and nutrient levels have an influence on plant responses and adaptability to heat stress. Plants with higher nutrient media with an earlier exposure to heat shock will be more able to adapt to extreme temperatures and potentially to global climate change.

Can *Vibrio fischeri* form biofilms on sand under laboratory conditions?

Vibrio fischeri is a bioluminescent marine bacterium found in oceans worldwide. Many aquatic bacterial species subsist primarily in biofilms, which are complex bacterial colonies that develop attached to abiotic or biotic substrates. I hypothesized that *V. fischeri* are able to form biofilms on sand under laboratory conditions. Most published procedures for dissecting the genetic basis of biofilm formation rely on unnatural substrates such as plastics, and development of a procedure for biofilm formation on sand

would allow study of biofilm formation under more natural conditions. Using a sand-attachment protocol for the freshwater bacteria *Pseudomonas aeruginosa* as a basis, I tested the hypothesis that *V. fischeri* grow as biofilms attached to sand, and I modified the biofilm formation assay to optimize growth for *V. fischeri*. I determined that free-living *V. fischeri* do, in fact, colonize sand as biofilms, and outlined a specific sand-attachment protocol for *V. fischeri* through modification of numerous variables of the *P. aeruginosa* protocol.

Assessment of the regulatory T cell (Treg) immunophenotype CD4⁺CD25⁺ constituting the CD4⁺FoxP3⁺ subset and the potential role of the balance between Treg and activated effector T cells in the induction of chronic Graft-versus-Host Disease

Naturally arising regulatory T cells (Tregs) expressing the cell surface proteins CD4 and CD25 have proven essential in the prevention of autoimmune diseases in mice. Since its discovery in 1995 by Sakaguchi et al., interest has grown considerably in the therapeutic potential of CD4⁺CD25⁺ Tregs in humans. Research efforts with Treg in mind have particularly focused on developing immunosuppressant treatments for the post-transplant, *alloimmune* condition Graft-versus-Host Disease (GvHD). In order to better utilize Tregs in immunotherapy, efficient methods of isolating these cells from blood samples must be devised. This research utilized both flow cytometric and statistical analyses to determine the accuracy of CD4⁺CD25⁺ surface expression as a marker for the Treg subset expressing the transcription factor FoxP3. Since FoxP3 expression in human Tregs is associated with strong immunoregulation, CD4 T cells expressing FoxP3 were considered the overall Treg population. Lymphocytes from patients following blood stem cell transplantation were stained with fluorescent antibodies to measure their surface and intracellular protein expression. In addition to a CD25/FoxP3 comparison, the resultant immune cell counts—accompanied by data compiled in 2007 by Cutler et al.—were used to retrospectively compare the T cell subpopulations *in vivo* of patients with chronic GvHD with those that showed no symptoms of GvHD. The results of this study corroborate previous research done in 2006 by Liu et al. refuting the correlation between CD25 and FoxP3 expression. Our results also suggest that lower but significant amounts of FoxP3⁺ Treg in fact do not express CD25. Efforts to correlate GvHD incidence with different T cell subpopulations showed a noticeable but statistically insignificant trend in the balance of Treg and activated effector T cells. These findings suggest that potentially significant amounts of functional Treg are left unaccounted for in the use of CD25 as a Treg identifier. Though the method of using CD25 antibody provides a higher concentration of functional Tregs, this study suggests that it results in less Tregs overall isolated in a given blood sample. Future research should focus on finding more effective surface markers,

including the IL-7 receptor CD127, to enable broader isolation of functional Tregs.

A More Detailed Phylogenetic Analysis of the *Pleurothallis* Clade from Pridgeon and Chase (2001), with Emphasis on *Pleurothallis* Subsection Macrophyllae-Fasciculatae, Using Nuclear ITS DNA Sequencing.

Recently, morphologically-based revisions of the genus *Pleurothallis* have been proposed. Szlachetko and Margonska (2001) proposed the genus *Zosterophyllum* for *Pleurothallis* subsection Macrophyllae-Fasciculatae (M-F), and Luer (2005) subsequently proposed the alternate genus *Acronia* by uniting *Pleurothallis* subsection M-F with subsections *Acronia* and *Amphygiae*. The molecular phylogenetic studies by Pridgeon, Solano and Chase (2001) and Pridgeon and Chase (2001), however, hinted that these taxonomic revisions might not be justified. Here we report a preliminary analysis of a more detailed study of the *Pleurothallis* clade from Pridgeon and Chase (2001), with emphasis on *Pleurothallis* subsection M-F. While most bootstrap values in the phylogenetic trees are relatively low, we can draw some preliminary conclusions. The *P. cobriformis*-*P. excavata*-*P. dorotheae* clade is closely related to *P. ruscifolia* and only distantly related to subsection M-F and, therefore, transfer to *Acronia* or *Zosterophyllum* is not supported. The *P. allenii*-*P. rowleei* clade is closely related to *P. ruscifolia* and only distantly related to subsection M-F and, therefore, transfer to *Acronia* or *Zosterophyllum* is not supported. Species of *Pleurothallis* subsection M-F form a discrete but poorly supported clade, sister to but apart from the clade containing *P. ruscifolia*, which could conceivably form part of a more narrowly defined genus *Acronia* or *Zosterophyllum*.

Reversed Sexual Size Dimorphism in Flammulated Owls (*Otus flammeolus*)

In most vertebrate species male individuals have a larger body size than their female counterparts. However, in many raptorial species this pattern is reversed. Hypothesis attempting to explain evolutionary origins of this trait, known as reversed sexual size dimorphism, fall into three general categories: differential niche utilization, incubation and feeding advantages, and sex-related behavior. The degree to which dimorphism is expressed in raptors is positively correlated with mobility and agility of prey. Size dimorphism data for the flammulated owl (*Otus flammeolus*) has not been closely studied. We wished to determine if the reversed sexual size dimorphism patterns in flammulated owls are consistent with other raptorial species. We analyzed the mass, wing cord length, primary feather length and rectrix (tail) length of 93 males and 120 females captured on the Manitou Experimental Forest in Colorado from 1981-2007. Since previous studies have found

reversed sexual size dimorphism to be less pronounced in insectivorous species, such as flammulated owls, we expected the morphological parameters of females to be only slightly larger than males in all areas. We found that female flammulated owls do indeed have a larger mass than males; however, males have longer wing cords. There is also a trend towards longer rectrices in the males. The lighter body mass, longer wings and longer tails of males may increase their maneuverability, resulting in increased foraging efficiency. This morphology in males may function to reduce intersexual competition, although data on this are currently lacking. In females, a larger body size likely is beneficial during the egg laying and incubation stages when energy demands are high.

A Molecular Reconsideration of the Orchid Sub-tribe Pleurothallidinae: Phylogenetic Analysis and DNA Barcoding

Recently attempts have been made to reorganize the Pleurothallidinae sub-tribe by the creation of new genera (*Acronia* by Luer, 2005 and *Zosterophyllanthos* by Szlachetko and Margonska, 2001), which relocate select species within *Pleurothallis* based on morphological data. DNA sequencing technology utilized in other phylogenetics studies (Pridgeon et al., 2001; Pridgeon and Chase, 2001; Pridgeon and Chase, 2005) challenges these conclusions. The phylogenetic analysis presented in this paper analyzed internally transcribed DNA spacer (ITS) sequences from 78 individuals of *Pleurothallis*, representing 58 different species. Based upon the trees constructed, these preliminary data do not support the creation of the new genera *Acronia* or *Zosterophyllanthos*. Pending further research and inclusion of supplemental data such as additional samples and sequences, it is possible that enough statistical support may be generated to support creating a new genus by elevating certain members of sub-section Macrophyllae-Fasiculatae. The clade containing *P. excavata* was well supported and appears to vary from *P. ruscifolia* in a strict consensus. Therefore this grouping could be elevated to the level of genus, or distinguished as its own sub-section or sub-genus. The plastid genes *rpoC1* and *rpoB2* were also investigated for their potential use in both phylogenetic and DNA barcoding studies of *Pleurothallis*. Neither gene, however, was found to provide an adequate signal to distinguish between species at the species level.

An Analysis of Ten *hilA-XO4* Gain-of-Function Mutants

HilA is a transcription factor in *Salmonella*. It activates the *invF* and *prgH* promoters through direct binding, which ultimately induces all of the genes needed for *Salmonella* to invade intestinal epithelial cells. A series of motif swaps between *Salmonella* HilA and its *Chromobacterium violaceum* homolog were constructed by crossover PCR. One of these chimeras, called

hilA-XO4, activates transcription at low levels. We ran a series of Beta-galactosidase assays and sequencing analysis on ten *invF* and *prgH hilA-XO4* gain-of-function mutants. Mutants A9, A12, A13, and A17 had the highest fold activation, while mutants A1 and A13 appeared to have changes in their amino acid sequences when compared to *philA-XO4*.

Establishing a robust system for infecting *Caenorhabditis elegans* with recombinant *Salmonella typhimurium*

Caenorhabditis elegans are used as a model host for understanding bacterial pathogenesis. In our study, we developed methods for infecting the nematodes with *Salmonella* in order to test the effects of specific *Salmonella* mutations on infection in an animal model. The *Salmonella* protein we study, HilA, turns on genes known to be important for invasion of epithelial cells and evasion of innate immunity, which the worms and mammals have in common. The worms must be maintained at low temperatures (25°C), which is too low to allow strong expression of *hilA* in normal *S. typhimurium*. We created recombinant *S. typhimurium* that can express *hilA* at this low temperature when they are exposed to arabinose. We established reproducible procedures for infecting *C. elegans* with these engineered *S. typhimurium*. Our research will enable two future goals: 1) use of *C. elegans* and *S. typhimurium* in classroom laboratories and 2) use of this system to investigate the impact of different mutations in *hilA* on pathogenesis.

Complex phytochrome interactions promote a drought escape strategy of root-to-shoot ratio and stomatal conductance in *A. thaliana*

The ability to sense and respond effectively to drought stress can be important for plant fitness. Plastic responses to drought stress are likely mediated by several genes, including the five-gene family encoding the photoreceptor phytochrome. Here, phytochrome loss-of-function mutations from two *Arabidopsis thaliana* ecotypes, Landsberg *erecta* and Columbia, were employed in order to examine the role of three phytochromes (phyA, phyB, and phyE) in drought stress response. Phytochrome mutant lines were subjected to drought and moist treatments in two identical drought stress experiments. Drought response was measured in stomatal conductance (g_{ST}), fluorescence, root to shoot ratio (R:S) and abscisic acid (ABA) concentration. Overall, drought treatment plants had greater R:S and less g_{ST} than moist treatment plants. However, wild-type (WT) lines had less pronounced responses to the drought treatment than phytochrome mutants, suggesting a role of phytochrome in suppressing drought tolerance. Gene interactions took a variety of forms, depending on the parameter under study. In promoting stomatal drought response, phyB appeared to interact antagonistically with phyA and phyE. While all three played an additive role

in inhibiting R:S increases as a drought response, phyB seemed to act less directly on R:S than phyA or phyE. The models proposed here provide a hint at the complexity surrounding the genetic mechanisms behind plastic responses to drought stress.

Environmental factors affecting riparian community dynamics and *Tamarix* distribution: Arkansas River, CO

Invasive species can change plant community composition by outcompeting local taxa, altering the environment, or both; such species are effective ecosystem engineers because their life history and growth patterns in large part determine the type of environment they occupy. *Tamarix ramosissima*, the most common invasive species of the Tamaricaceae, has caused massive morphological changes to riparian ecosystems and their bank structures over the last century, locally, the Arkansas River. Growing either as dense stands of shoots or small trees, *Tamarix* species displace or actively outcompete native species of willow (*Salix exigua*) and cottonwood (*Populus deltoides*). Under normal low light conditions establishment is difficult for *Tamarix*. However, damming and the resulting altered flooding regimes and soil moisture availability may give *Tamarix* an advantage over natives. Our study analyzes community structure and how *Tamarix* competes with natives above and below dam sites.

IS711 insertion sequence elements and the *omp2a* gene as distinctive genetic markers in cetacean and pinniped *Brucella* isolates

Two new species names (*B. cetaceae* and *B. pinnipediae*) for the gram-negative bacteria identified in marine mammals as *Brucella* have been previously proposed based on DNA polymorphism at several loci distinct from the six classically recognized *Brucella* species found in terrestrial mammals. Specificity of genetic elements among marine mammal *Brucella* isolates was assessed to evaluate possible classification of these strains. PCR amplification and sequence analysis was performed on IS711 DNA insertion sequence elements because they have more copies in marine mammal isolates than in terrestrial species. Locus-specific PCR primers for an IS711 sequence and flanking region and an IS711 sequence located downstream of the *bp26* (*omp28*) gene, common among marine isolates, were used to determine that the specific copies of IS711 could be used as genetic markers for marine mammal isolates as a group. Differentiation between cetacean and pinniped isolates utilized amplification from established primers and sequence analysis of four DNA fragments (I, II, III, IV). Fragment I was previously described as pinniped-specific, having no homology with any nucleotide sequences in *Brucella* species; Fragments II, III, and IV were established as cetacean-specific and contained portions of IS711 insertion sequence elements. A 519 base-pair region of the *omp2a*

gene was also amplified in the marine mammal *Brucella* isolates, and was submitted for sequencing after undergoing cloning quantification and purification. The purified sequences were then compared to published *Brucella* biovars and species for phylogenetic classification, and more taxonomic research should be conducted in this area. Our research did not confirm the literature supporting the distinction between cetacean and pinniped *Brucella* isolates and further investigations could focus on validating fragments I, II, III, IV as appropriate for distinction, or locating new genetic elements specific to either cetacean or pinniped isolates.

Effects of the Hayman Fire After Five Years on Water Quality and Benthic Macroinvertebrates of the South Platte River, Colorado

Little is known about the length of time required for the recovery of aquatic systems which have suffered the effects of nearby forest fire. The Hayman Fire of 2002, the largest fire in Colorado history, allowed a long-term study to be conducted to investigate the timeline of recovery of lotic systems. The indirect effects of fire on lotic systems include decreased water quality, increased erosion and sedimentation, and decreased leaf litter cover, all of which adversely affect benthic macroinvertebrate communities. In October of both 2004 and 2007, benthic macroinvertebrate density, richness and composition, as well as water quality, leaf litter cover and silt deposition were sampled on areas of the South Platte River. During each year, eight sites were sampled in an area moderately affected by the fire, and another eight were sampled in a nearby control area. In the burned area between 2004 and 2007, benthic macroinvertebrate density increased 211%, and morphological richness increased 88%. However, in 2007 density was still 46% lower in the burned area than the control, and morphological richness 42% lower. Interestingly, the density of the order Diptera which can thrive in a variety of conditions and habitats, increased 2143% in the burned area. The density of Ephemeroptera, an order more sensitive to water quality and sedimentation, did not change in the burned area, but decreased 38% in the control area. Water quality as measured by dissolved oxygen, temperature, and pH improved somewhat in the burned area, but not significantly. Leaf litter cover and silt deposition did not change significantly in this time period, and in 2007 there was still 147% more silt in the burned area than the control area. Results indicate that 5 years after the fire water quality has not improved noticeably, but the benthic macroinvertebrate community has begun to recover in density and richness. Water quality continues to effect the composition of different groups of macroinvertebrates The system should continue to be monitored to better define the succession of different groups of macroinvertebrates, and overall recovery time of lotic systems from fire.

An Experimental Procedure for Performing β -galactosidase Assays in 96-Well Plates

HilA is a transcription factor that activates the transcription of specific pathogenic genes in *Salmonella*. Previous research has focused on understanding the structure and function of HilA and its relationship to the structure and function of the promoter region on DNA to which it binds. Prior methods used test tubes for cultures of various *Salmonella* and *E. coli* strains to better understand the properties of HilA and the promoter regions for the *prgH* and *invF-1* genes. Using a *lacZ* reporter, β -galactosidase assays are used to quantify the activation of the target genes by HilA. Recently, a small plastic plate with 96 300 μ L wells became available for laboratory use. The plates greatly increase the efficiency of testing multiple bacterial strains in many environmental conditions. Unfortunately the methods used in the test tubes do not work well in the 96-well plates because of their different sizes, layouts, and limitations. My research focused on revising the test tube appropriate methods to make them work in the new plates. I varied the previous methods for the inoculation of the desired strains to test, arabinose induction, and the β -galactosidase assay, until they showed promising results in the new plates. I found that it is important to separate each different strain by at least one well to avoid cross contamination as well as using small amounts of chloroform to lyse the cells instead of a multi component mixture used before. The new methods produced the desired results 2 out of 3 times indicating a need for further refinement before they can be confidently used to test gene activation. Future experimentation on the lysis procedure as well as finding the best layout for the arabinose induction would be greatly beneficial for this work.

The release of periplasmic β -lactamase from *E. coli* is due to a secondary transport phenomenon associated with *Chromobacterium violaceum* hemolysin, and not outer membrane disruption.

Chromobacterium violaceum is a Gram-negative opportunistic human pathogen native to tropical and sub-tropical river ecosystems. Analysis of the *C. violaceum* genome sequence revealed possible virulence factors, including several potential cytolytic toxins. A recent study confirmed one of these putative cytolytins to be a Two-Partner Secretion (TPS) system consisting of a hemolysin (ChlA) and transporter (ChlB), a system similar to that observed in *Serratia marcescens*. During investigation of *C. violaceum* two-partner secretion in *E. coli*, strains expressing the hemolysin and transporter proteins also released periplasmic β -lactamase into culture supernatants. Two mechanisms of lactamase release were proposed: 1) the transport-induced leakage of enzyme due to a generally permeabilized outer membrane or 2) a secondary transport phenomenon involving the hemolysin and transporter. Minimum Inhibitory Concentration (MIC) assays, with and without the membrane permeabilizing agent polymixin B-nonapeptide, were

conducted in order to measure changes in antibiotic sensitivity that would reflect comparative disruption of outer membrane integrity. A correlation of MIC data with lactamase release experiments from previous studies suggested that the outer membrane remains relatively intact, under conditions where lactamase was found in the culture supernatant. These results support the secondary transport hypothesis of lactamase release in *E. coli* strains that also secrete the *C. violaceum* hemolysin.

Climatic Controls on Radial Growth of Mountain Big Sagebrush

Mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*) is common in arid regions throughout the Intermountain West. Climate change may affect both growth rate and elevational distribution which have the potential to impact nearby grasses and forbs as well as net carbon flux. Studies relating annual growth rings with climate data provide a method to predict future changes in growth rates based on regional climate models. Within the Gunnison Basin we examined three sites along an elevation gradient from 2,500 m to 3,300 m. At the mid- and high-elevation sites missing rings prevented cross-dating so climatic relationships could not be tested. At the lower limit of mountain big sagebrush we were able to show a strong positive correlation with the amount of winter precipitation ($r^2=0.37$) and a strong negative correlation with summer temperature ($r^2=0.43$). Given climate model predictions for significantly increased temperatures and uncertain changes in precipitation, it is most likely that mountain big sagebrush will show decreased growth with future climate changes. Such changes have the potential to decrease the density of grasses and forbs as well as decrease net carbon uptake. More studies need to be done to examine the factors affecting radial growth at higher elevations.

Genetic Analysis of the *Salmonella* transcription factor HilA.

All species of *Salmonella* rely on genes on the Pathogenicity Island I (SPI1) to carry out infection in a mammalian host. Chief among these genes is *hilA*, a gene encoding a transcription factor HilA. This transcription factor plays an essential role in regulation of the cascade that allows the formation of a Type III secretion system and its subsequent effector molecules. HilA has been proven to bind to two promoters on SPI1, P_{invF} and P_{prgH} . However, beyond identifying the domain of HilA that binds to these promoters, not much is known about the functions of other domains of the protein. By mutating the *hilA* gene in an *E. coli* model and analyzing mutants with β -galactosidase assays, we hoped to identify mutants with significantly reduced HilA function. Once these mutants were isolated and sequenced, further studies could be completed to analyze the result of point mutations in the *hilA* gene and consequently, the functions of various domains of the protein could be deduced.

Dispersal of two failed cliff swallow (*Petrochelidonpyrrhonota*) colonies in Nebraska: group or individual?

Cliff Swallows, *Petrochelidonpyrrhonota*, are a highly social passerine that breeds in colonies throughout the Platte River Valley, NE, every summer. When the swallows return to breed, they choose a breeding colony based on ectoparasite load and colony size. This study looks at evidence for group movement in the swallows from two high-failure 2006 colonies, 18 and 88, in making their decision on breeding colony in 2007. Mark-recapture data from 2006 and 2007 was used to determine movement of swallows between the two summers. Sixty-six birds from colony 18 were recaptured in 2007, and 213 swallows from colony 88 were recaptured. Five models were used that predicted how the birds would move if they chose colonies individually according to distance, colony size, distance and colony size, equal distribution of recaptured birds, and proportion of colony size when compared to whole colony group. The colony groups were determined by the type of structure the birds used to nest – a bridge over a canal (“canal colony”), or a culvert under the railroad (“railroad colony”). Of the birds that nested in colony 18 (a canal colony) in 2006, 92.42% of those returned to nest in a canal colony, and 91.08% of birds that nested in colony 88, a railroad colony, nested in a railroad colony in 2007. Using the expected values generated from the models, chi-square analyses were run. When the fumigated colonies were included, the birds did not follow any of the models; however, when the fumigated colonies were excluded, the swallows in the railroad colonies tended to choose the closer colonies, while the canal colonies tended to choose the larger colonies. Large numbers of swallows were seen to move from one colony to another together, but whether this is due to a group decision is not determinable at this point. More studies of this kind, as well as migrational studies, need to be done before any conclusions can be made about group movement.

Methods of inhibiting HIV: Targeting stages in the virus life cycle

Acquired Human Immune Deficiency Syndrome (AIDS) is perhaps the most devastating disease to strike the modern world. Worldwide, 14,000 new infections occur every day, bringing the number of infected people to about 40 million (Altmeyer 2004). Many new drugs have been developed since AZT in 1987 and just as many are in clinical trials today. In a brief overview of the HIV lifecycle, it is evident that we are only in the middle of the battle against HIV and the development of new drugs. Once an HIV virus has been exposed to a T-cell, its lifecycle begins and can be broken down into eight stages: attachment, fusion, uncoating, reverse transcription, integration, transcription, translation and maturation. Currently, there are five classes of antiretroviral drugs on the market to target five specific parts of the HIV lifecycle. These classes of drugs are 1) fusion inhibitors 2) CCR5 antagonists 3) Reverse Transcriptase Inhibitors 4) Protease Inhibitors and 5)

Integrase Strand Transfer Inhibitors. Every class of drug works in a specific way to inhibit the HIV life cycle and for every drug, resistant strains of HIV have been found. In this paper, the complex molecular biology of the HIV virus is explored, as well as how different drugs interfere on a molecular level with viral processes. These complicated factors have led to amazing research in this field of medicine and incredible advances in treatment for HIV/AIDS. Nevertheless, science is far from finding a cure for AIDS.