

Abstracts for BioDay 2006

Is there a long-term trend towards earlier breeding in Flammulated Owls?

Climate change has become both a global and regional issue in recent years. While several studies have focused on how temperature change may be affecting avian species in Europe, very few studies have looked at North American birds in this context. The purpose of this study is to determine if there is a long-term trend toward earlier initiation of incubation in Flammulated Owls (*Otus flammeolus*) in south-central Colorado and if regional climate change could be a contributing factor. From 1981-2005, we found that the initiation of incubation advanced 13.6 days. This translates into a mean Julian date of incubation initiation of 145 in 2005, compared to 156 in 1981. This data correlates with a mean May temperature increase of 1.7 °C during the study period. While there are competing hypotheses that may account for advancement of breeding, the strong positive trend of regional climate change is notable. This is especially so given that these trends parallel results of a number of European studies that address the causal effect of global warming on breeding chronologies of avian species. The results of this study join other investigations in suggesting that climate change may have dramatic effects in the breeding ecology of organisms worldwide.

PM1 cells yield HIV replication rates comparable to those in peripheral blood mononuclear cells

The progression towards AIDS is highly dependent on the replication rates of the HIV-1 strain. Replication assays have previously been conducted on peripheral blood mononuclear cells (PBMC) to compare the rates of virus production and patient disease progression. PBMCs must be isolated from a single seronegative donor to critically compare replication rates. Not only is it difficult to isolate PBMCs from a single donor for assays conducted over a long period of time, but the preparation and maintenance of the cells is inefficient and costly. The PM1 cell line is a derivative of the Hut-78 T-cell line, expresses all coreceptors necessary to facilitate viral entry, and shows promise as an alternate system in which to assay HIV-1 kinetics. This study investigated the PM1 system and optimized a protocol to assay replication rates of varying HIV-1 strains. A target cell count of 4×10^5 cells and a viral inoculum of 2000 tissue culture infectious doses (TCID₅₀; yielding a multiplicity of infection of 0.005) showed optimal viral replication and cell density in culture. Viral replication assays were conducted using strains isolated from six clinical isolates and two laboratory stock strains. Cultures were initially conducted in the presence of human interleukin-2 (hIL-2) but

were later performed in the absence of hIL-2 because hIL-2 caused a decrease in viral uptake. Two clinical isolates showed no initial infection into the PM1 cells while two others did; those which showed positive infection, as well as one stock strain, yielded replication rates similar to those found in PBMC cultures. After the withdrawal of hIL-2, no isolates produced virus except for one stock strain whose replication rate decreased dramatically. Flow cytometry was conducted on both PM1 cell cultures grown in media supplemented with hIL-2 as well as PM1 cells grown without hIL-2. The results showed continued expression of both the CXCR4 and the CCR5 coreceptor; however, the coreceptors were expressed at slightly lower levels in cells grown in the presence of hIL-2. Those viruses able to infect the PM1 cells initially showed correlative replication rates among PBMC and PM1 cultures suggesting that the PM1 replication assay might prove to be a more efficient and less costly means of assessing viral kinetics. Replication assays of HIV-1 strains must be repeated on a freshly thawed batch of hIL-2-naive PM1 to verify viral susceptibility before the PM1 assay can be used for comparative and competitive assays in the HIV-1 laboratories.

Environmental and Genetic Regulators of the Invasion Gene *hilA*, in *Salmonella bongori*

hilA encodes a transcriptional activator in *Salmonella*, which regulates other virulence genes in the *Salmonella* pathogenicity island 1 (SPI1). These genes encode components of a type three secretion system, used by the bacterium to invade host cells. Environmental and genetic components contribute to the regulation of HilA activity in *Salmonella bongori*. *S. bongori* is a sub species that is unique as it has not been studied in depth, but also generally infects only reptiles and amphibians. Research was conducted to determine how environmental conditions regulate HilA activity in *S. bongori* as compared to the better understood *S. typhimurium*. A plasmid-reporter (pJB24) bearing a *prgH::lacZY* reporter fusion, was transformed into two strains of *S. bongori*, which served to indicate when HilA was active. Cells were grown at different temperatures, salt concentrations and pH levels and -galactosidase assays were performed to measure the activity of HilA. HilA was more active in *S. bongori* under high salt, slightly basic conditions at 25 degrees Celsius. This was similar to the control, *Salmonella typhimurium* with reporter *prgH::lacZY*, except that *S. typhimurium* had more HilA activity at 37 degrees Celsius rather than 25 degrees. Further research was conducted to identify any genetic regulators or signal transduction pathways potentially affecting expression of SPI1 genes. *S. bongori* strains were mutagenized with a transposon to identify

any mutants lacking HilA activity within reporter strains. From physical evidence of colony color on indicator plates, the level of HilA activity was measured and observed. Among the 1586 mutant colonies grown none were deficient in HilA activity.

A model of HilA Activation in *Salmonella enterica* sv. *Typhimurium* Based on Co-Expression Strain Assays

HilA is a transcriptional activator of the *inv* and *prg* operons which encode essential constituents of the Type III Secretion System found on *Salmonella* Pathogenicity Island 1. HilA is a 553 amino acid protein, containing a DNA-binding N-terminus as well as a large C-terminus of unknown function. Activation and binding abilities of several different HilA variants, including *Salmonella* HilA deletions and domain exchanges between *Salmonella* and *Chromobacterium violaceum* HilA proteins, were investigated using β -galactosidase assays. Data gathered from these assays included evidence suggesting that the HilA N-terminus repressed activity of intact HilA in a co-expression strain, but this result was exclusive to the *invF* promoter. These observations, in conjunction with previous data gathered on the *prgH* promoter, lend support to the idea that HilA exhibits different modes of activation at P_{prgH} and P_{invF-1} , a possibility that has been suggested in the literature. The data also suggest a tentative role for the HilA C-terminus in oligomerization of HilA, the status of which may yield differential activation at the *inv* and *prg* operons.

Biofilm formation and motility characterization of 8 *Vibrio fischeri* strains.

Vibrio fischeri are important symbionts of the Hawaiian Bobtail squid *Euprymna scolopes* and the mutualistic relationship between the two is a complex one. It is believed that biofilm formation is a crucial stage of *V. fischeri* colonization. Following biofilm formation, the bacteria use quorum sensing to emit light, effectively disguising the squid in the water column during the night. The classic model of bacterial biofilm formation involves two types of motility: swimming and twitching. Bacteria utilize swimming motility to approach a surface and then twitching motility to attach and move out along the surface. In this study we observed and characterized motility of eight strains of *V. fischeri* as well as their ability to form biofilms. Biofilms were formed for all strains over 72 hours as well as 24 hours. Some strains showed 24 hour biofilm formation that was significantly different from the 72 hour formation. Strains ES191 and VLS2 showed 24 hour biofilms significantly diminished from the 72 hour trial while strains SR5 and CG101 showed 24 hour biofilms significantly greater. WH1 was

identified as a robust twitching strain as well as a consistently strong biofilm forming one. The original *V. fischeri* isolate from the Bobtail squid, ES114, consistently did not twitch well and did not form strong biofilms compared to the other 7 strains. To investigate whether one type of motility is more crucial to biofilm formation, twitching or swimming, we used an ultraviolet mutagenesis to create motility mutants. We screened for and identified five motility mutants of the isolate WH1. Four displayed twitching motility phenotypes different from wild type but normal swimming motility. One mutant, F20, showed both altered twitching and swimming motility from wild type. Only F20 was shown to be significantly defective in biofilm formation from wild type at a 95% level. The other four mutants however were shown to be significantly defective at the 90% level. No specific type of motility, however, was shown to be more crucial to biofilm formation than another. We offer further support of the traditional biofilm model in which both twitching and swimming motility are crucial.

Female Mate Choice in Collared Lizards: Use of Chemical or Visual Signals in Detection of Chigger Mite Ectoparasites on Males

To avoid parasitism, female collared lizards (*Crotaphytus collaris*) may rely on chemical and/or visual information about available males when choosing a mate. My study attempted to determine whether female collared lizards could detect chigger mites (*Eutrombicula cinnabaris*) on male lizards by scent, and whether chemical or visual cues (red patches formed by congregations of mites) were more important to females in choosing a mate. Lizards (N = 11 females, 22 males) were captured at Konza Prairie Biological Station and Tuttle Creek Reservoir near Manhattan, Kansas. Male lizards were paired by similarity of physical characteristics, and mites were removed from a randomly chosen male of each pair. Male scents were collected by housing each on filter paper for 24 hours. Filter paper containing each male's scent was placed on either side of an observation tank and a randomly chosen female was introduced to the center of the chamber. The trial was ended when a female had clearly spent more than 50% of her time (minimum time was 30 minutes) on one male's side and that male was considered to be preferred. Of 11 females tested, 9 preferred the "no chigger" scent (chi-square $p = 0.03$). Females also spent a higher percentage of their time on the side of the male who did not have chigger mites ($p = 0.075$). The preferred male in the scent test was then painted with a red spot to simulate the presence of chigger mites and the rejected male was painted with a more camouflaged green spot. The two males were tethered on either side of the observation tank, with each male on the side where his scent had been located in the first trial. The same female was then

reintroduced to the chamber and observed until she spent more than 50% of her time on one male's side of the tank. In the second, visual test, females did not choose one male over the other. My results indicate that female collared lizards are able to detect the presence of chiggers on a male through chemical cues. However, visual cues that contradict chemical signals appear to either reinforce the choice made on scent alone or cause the female to alter her decision. While the presence of ectoparasites on a potential mate may be important indicators for choice, females may base their final decision on a combination of other factors as well.

Seasonal Diet Variation of Mountain Lion (*Puma concolor*) in the Atascosa Range

Mountain lion (*Puma concolor*) occupy a large and diverse range throughout the Western hemisphere. While numerous studies have analyzed the total diet of mountain lion within an ecosystem, seasonal affects on prey availability and therefore predatory behavior have remained understudied. Our study site in the Atascosa Range in Southern Arizona aimed to understand mountain lion seasonal diet variation through the employment of non-invasive techniques, namely scatological analysis. In analyzing 110 mountain lion scats at the macroscopic and microscopic level we were able to gain valuable insight into primary prey species selection within the region as well as seasonal variation of prey selection amongst eastern cottontail (*Sylvilagus floridanus*), javelina (*Pecari tajacu*), agriculturally employed cow (*Bos taurus*), and canids. In addition to giving us behavioral insight of mountain lion activities, seasonal diet selection information aids in management techniques, and this is of particular interest due to the recently discovered presence of jaguar (*Panthera onca*) within the region. Known sympatric association between mountain lion and jaguar encourage greater understanding of prey species in the region to guarantee effective management plans for the survival of both predators and all associated species.

Testing Affinity for Sebaceous vs Apocrine Derived Human Chemosensory Stimuli in *Glossina Swynnertoni* in Tarangire National Park, Tanzania

Trypanosomiasis is a systematic disease caused by parasites in the genus *Trypanosoma*. Tsetse flies (genus: *Glossina*) are widely studied as a result of their role as vectors of several species of trypanosome in sub-Saharan Africa. Tsetse flies select their hosts based on visual and chemosensory stimuli (Colvin and Gibson, 1995), but a study by Mohammed- Amed (1998) demonstrated that chemosensory cues play a larger role.

Excretions of the sweat glands and their microbial break down products create the body-odor profile of vertebrates. The two different types of human sweat gland secretions have different chemical compositions. Human sebaceous glands secrete free glycerols and apocrine glands secrete steroids.

The goal of this study was to compare the affinity of *Glossina swyertoni* for human sebaceous and apocrine derived sweat. To determine affinity identical traps (Ndgeva and Minok, 1999) were baited with swabbings from the upper lip and axillae. Catch numbers were used to determine affinity. It was found that *Glossina* species of Tarangire showed a statistically significant greater affinity for sebaceous derived sweat. It was also found that subject sex, origin, and blood type of human host had no significant effect on affinity of tsetse flies.

Flammulated Owl nest site preferences in burned and unburned forests in Colorado.

In response to a need for more research concerning faunal responses to wildfire and habitat usage patterns in a post-fire environment, I compared characteristics of Flammulated Owl (*Otus flammeolus*) nest sites in the Hayman Fire burn scar and on the Manitou Experimental Forest (unburned) in central Colorado to available but unused sites. Flammulated Owls are a small, insectivorous forest raptor associated with mature montane forests for foraging, roosting and territorial defense. As an obligate secondary cavity nester, owl territories must contain at least some suitable cavities. Used cavities in the burned and unburned study areas were both higher above the ground and had larger entrance diameters than unused cavities. In the Hayman Fire used sites contained a significantly higher percentage of dead trees than unused sites. Also, both used and unused sites in the Hayman Fire contained a significantly higher percentage of dead trees than did used and unused sites on the Manitou Experimental Forest. Dead trees occurring in used sites showed a greater degree of decay than dead trees in unused sites. Finally, quaking aspen (*Populus tremuloides*) was overrepresented in used sites while thin leaf alder (*Alnus tenuifolia*) was underrepresented. These data suggest that nest site selection in Flammulated Owls is driven by predator avoidance, specifically avoiding depredation by Red Squirrels. Owls appear to be employing several predation avoidance strategies by: 1) selecting sites that are surrounded by other potential nest sites, thus reducing the search efficacy of predators; 2) concealing nests; and, 3) selecting habitat that is of sub-optimal quality for squirrels.

Alpine Revegetation using Plug Transplants: A New Technique for Restoring Social Trails on 14,000-foot Peaks Across Colorado

Alpine plant life has been highly degraded on 14,000 ft peaks across Colorado due to hikers using unplanned social trails. The Colorado Fourteeners Initiative, Rocky Mountain Field Institute and the US Forest Service utilize many techniques such as seeding, turf transplants and plug transplants to restore this damage. While the success of turf has been documented and seedlings have been studied, plugs of transplanted, intact vegetation have never been monitored for survival or effectiveness. I hypothesized that (1) survival of plugs in wet and moist communities would be higher than in dry communities, and (2) species richness across restored and undisturbed sites would be maintained. This study took place during the summer of 2005 on Mt. Bierstadt, Quandary Peak, Wetterhorn Peak, Mt. Sneffels and Mt. Harvard. The plugs in the study ranged from one year old on Wetterhorn Peak to five years old on Mt. Harvard.

Individual plugs were deemed successful when the majority of the plants within them were either alive and healthy or vigorous and sexually reproducing. Overall, 63 % of the plugs in the study were successful. Plug success was 57% higher in wet and moist communities than in dry communities. Total vegetation cover was 10% higher in restored communities than in unrestored communities, and species richness was maintained. Plug success was not significantly different among willow carr and moist meadow communities on Mt. Bierstadt due to similar soil moistures between the two communities. The largest difference in plug survival between communities was apparent on Quandary Peak where wet and dry communities were compared. Mt. Harvard had the highest rate of plug success, where all plugs had plants reproducing in a wet meadow. On Mt. Sneffels three different restoration techniques testing the affects of erosion matting and native mulch did not appear to effect plug success. However, due to increased litter in sites where erosion matting was present, seedlings may be more successful, and long term results could show higher vegetative cover in these sites. Results show that plugs are a highly successful means of restoration, and that the success of plugs is increased in wet and moist microsites. Vegetation cover and species richness data display the positive effects of restoration on degraded alpine vegetation.

Seed viability and germinability of alpine species: Spatial variability in sites located throughout the Colorado Rocky Mountains

Approximately one-tenth of alpine ecosystems in the U.S. require restoration from human disturbance. One technique used in alpine restoration, seeding with native, locally collected seeds, introduces diversity into disturbed areas and requires few resources and little labor compared to other restoration techniques. However, little is known about spatial variation in alpine seed viability and germinability, which are critical in seeding success. I collected seeds of 13 species of perennial grasses and forbs from sites located on four mountain ranges in Colorado and tested 50 seeds each for viability (with tetrazolium) and germinability. All samples had relatively high viability, 40 to 70%. Germinability ranged from 0% to 100%, with 75% of samples (both grasses and forbs) having germinability over 50%. Most samples showed no variation in viability among sites while nearly all samples displayed a substantial amount of variation in germinability among sites. No variation was observed between wet and dry microsites on the same mountain for viability or germinability. The lack of variation in viability and between wet and dry microsites could be due to favorable conditions for plant growth and reproduction across all study areas during 2004 and 2005. This would have led to overall high viability, which could lessen differences among sites. The lack of patterns in variation of germinability among sites suggests that many factors may interact with the life history traits of each species to determine germinability. My results show that, at least in favorable years, viability and germinability do not limit success in seeding restoration for most species.