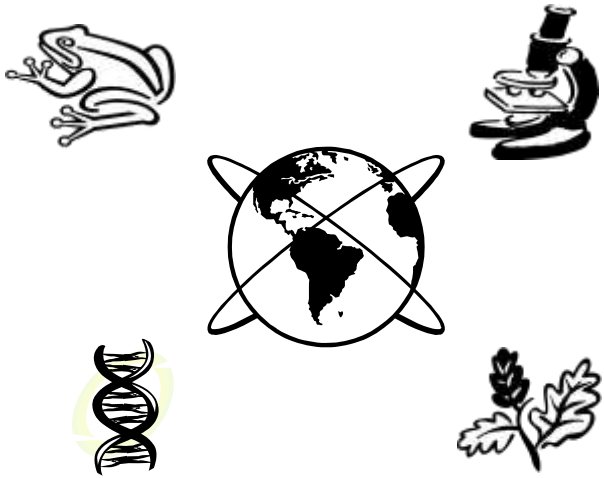
Phi Sigma

Research Symposium

2011



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SCHEDULE OF EVENTS

Friday, April 15th, 2011

8:30 – 9:15 AM	**Registration**
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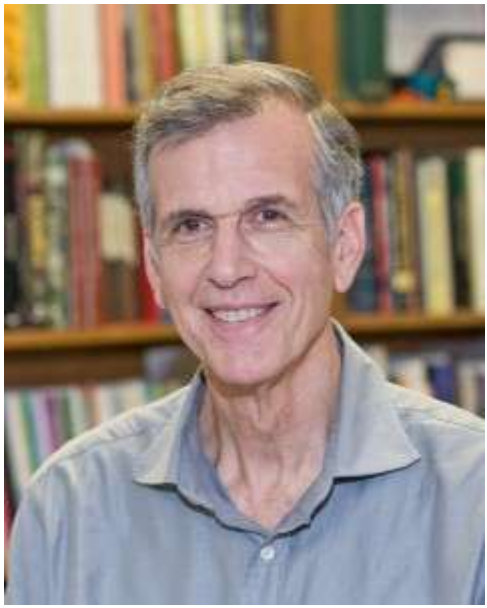
9:30 – 10:30 AM	**Oral Session 1** (SLB 121) Keith Bowers Darcy Gordon Brian Grebliunas Nick Tardi
10:30-11:30 AM	**Poster Session 1** (SLB Atrium)
11:30-12:30PM	**Lunch**
12:30-1:30 PM	**Poster Session 2** (SLB Atrium)
1:30-2:30 PM	**Oral Session 2** (SLB 121) Sandrine Clairardin Joe Fader Geoff Ower Ebony Murrell
3:00-4:00 PM	**Keynote Address** (SCHROEDER 130) Dr. Robert Ricklefs University of Missouri at St. Louis

KEYNOTE ADDRESS

Dr. Robert E. Ricklefs
Curators' Professor of Biology
Department of Biology, University of Missouri at St. Louis



"Regional perspectives on ecological communities"

Schroeder Hall, Room 130 at 3:00 P.M.

ORAL PRESENTATION ABSTRACTS

* participating in award competition

Sex allocation in a polygamous bird: the house wren (Troglodytes aedon)

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In most animal taxa, males compete with one another for access to females. Thus, intra-sexual competition over mates increases variance in the reproductive success of males relative to females, whose reproductive success is limited by the number of eggs they can produce. This should favor mothers that optimally invest in sons and daughters to maximize their fitness return. Typically, mothers should preferentially invest in sons when they can produce high-quality offspring of high reproductive value and daughters when they can only produce average- or low-quality offspring, thus avoiding investment in low-quality sons of low reproductive value.
In altricial birds, many maternal and environmental effects influence the reproductive potential of individual offspring. Therefore, because the ability of a female to raise high-quality offspring often varies in space and time, the question of whether females invest differently in sons and daughters should take into account such spatial and temporal environmental heterogeneity. I will present data describing how maternal house wrens (*Troglodytes aedon*) invest in sons and daughters across varying ecological contexts and how individual females alter their investment strategies across reproductive attempts.

Effects of Bisphenol-A on larval development of container-dwelling mosquitoes: one more reason to hate mosquitoes

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Endocrine disrupting compounds (EDCs) have been implicated in a variety of biological problems from population decline to various cancers. Bisphenol-A (BPA) is one such endocrine disruptor that is widely used in manufacturing and is nearly ubiquitous in the environment. BPA has been shown to have estrogenic (feminizing) and toxic effects in developing organisms from a wide range of vertebrate taxa; however, little is known effects of this compound on invertebrate development. To study the effects of BPA on invertebrate development, larvae two species of container-dwelling mosquitoes, *Aedes aegypti* and *Aedes albopictus*, were reared individually in one of eight concentrations of BPA (serial dilution; 100ppm-0.01ppb) or in a control lacking BPA. To test for effects on development we recorded time to adulthood, survival, mass and sex. We found that BPA did not affect sex ratios, time to adulthood or mass in either species; however there was a significant negative effect of BPA on survival in both species. As these species utilize man-made containers which often contain BPA, understanding how this compound effects survival may provide important information for methods of controlling mosquito populations. Further, this information can be used in comparative approaches in understanding conservation or control of other invertebrate species with similar life histories. As these species do not develop in isolation in nature, future studies will focus on successively higher levels of ecological organization by investigating effects of BPA on interspecific and intraspecific interactions during larval development and how these effects are manifested in the adult populations.

How many eggs per basket? A direct comparison of clutch-size differences between container-dwelling mosquitoes *Aedes albopictus* and *A. aegypti*.

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For organisms that colonize discrete habitats (e.g., rotting fruit, water containers), competition experienced by an individual depends on occupants in the same patch. The distribution of individuals across patches therefore influences intraspecific and interspecific competition and the possibility of coexistence between competing species. For example, if a superior competitor is nonrandomly clumped in a fraction of available patches (intraspecific aggregation), intraspecific competition increases relative to interspecific competition and coexistence may be possible. This is the aggregation model of coexistence and we investigated it as a potential mechanism explaining coexistence between container-dwelling mosquitoes in southern Florida. *Aedes albopictus* has displaced resident *A. aegypti* in much of the southeast US however they coexist in some areas. *A. albopictus* is known as a superior competitor to *A. aegypti* therefore possible coexistence mechanisms have received much attention. Evidence suggests that *A. albopictus* spreads eggs across patches less than *A. aegypti* which would increase intraspecific aggregation of *A. albopictus* and facilitate coexistence via the aggregation model described above. No direct comparison of clutch-laying behavior of these species has been made however and the contribution of this mechanism to coexistence is unknown. We compared clutch-laying behavior of *A. albopictus* and *A. aegypti* in the laboratory. Gravid females were placed singly in greenhouses with four containers of water for oviposition. Containers were spaced close or far apart and eggs were collected after 48 hours. *A. albopictus* aggregated eggs significantly more across containers than *A. aegypti* ($p = 0.0002$) which is consistent with the aggregation hypothesis of coexistence.

Is the way to her heart through her stomach? An investigation of the refractory-inducing effects of nuptial food gifts in decorated crickets.

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Sexual conflict over female re-mating behavior has been implicated in the evolution and maintenance of nuptial food gifts in insects. Recent evidence suggests that the spermatophylax provided by male decorated crickets, *G. sigillatus*, a gelatinous adjunct to a male's spermatophore that is consumed by the female after mating, contains substances that inhibit female sexual receptivity. The identity of these substances remains unknown, but a significant component of the solid fraction of the gifts is comprised of free amino acids, primarily glycine and proline. We tested the hypothesis that glycine is the refractory-inducing substance by: 1) injecting female *A. domesticus* with solutions containing glycine, proline, or a saline control and 2) feeding female *A. domesticus* experimental gels containing glycine, proline, a mixture of both, or a saline control. The results of these experiments suggest that ingestion, but not injection, of glycine leads to a delay in remounting and re-mating by female *A. domesticus*. The absence of an effect of glycine injected into the haemocoel of the female suggests a mechanism as to how glycine inhibits female sexual receptivity: glycine may be stimulating taste neurons that have downstream neurological effects on female behaviour.

5 **Grebliunas, Brian** (Graduate) - Talk*

Enhancing denitrification in constructed wetlands through manipulation of nutrient stoichiometry

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Agricultural tile drains transmit water laden with heavy nitrate (NO_3-N) loads and relatively lower levels of phosphorus (PO_4-P) and carbon (C). Reducing nitrate loading to streams using wetlands may be limited by tile water stoichiometry. We examined how nutrient stoichiometry of tile drainage affects denitrification rates. If successful this may provide a new method for enhancing wetland efficiency. Within our study site, each of the three wetland complexes receive tile drainage from a single agricultural field, however denitrification varies significantly between each complex. NO_3-N availability limits denitrification, but limitation by PO_4-P and C may further limit denitrification rates. Using a combination of laboratory and replicate treatment wetlands as an experimental system, we can test the effects of nutrient stoichiometry on denitrification potential in the laboratory and then apply the results to replicate experimental wetland systems. A preliminary study using several ratios of PO_4-P in relation to NO_3-N has shown the addition of P to enhance denitrification rates significantly. If PO_4-P or C is found to be limiting, managing for the stoichiometric requirements of denitrifiers may ensure enhanced NO_3-N removal.

Ecological succession in container-dwelling Diptera: does predation resistance trade off with colonization ability, competitive ability, or both?

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Previous succession studies have established a linear tradeoff between colonization and competitive abilities among colonizing species. However, in container systems this colonization/competition relationship among larval Diptera is non-linear, suggesting that an additional factor may influence succession in this system. Competitive ability has previously been demonstrated to be inversely related to predation resistance, but this tradeoff has never been applied to succession. The major predator in container systems is the larva of the mosquito *Toxorhynchites rutilus*. Due to its size, voracity, and delayed arrival, *T. rutilus* has a strong, but timed effect on community composition; therefore, resistance to predation by *T. rutilus* is a likely tradeoff that contributes to the succession patterns we observe. However, it is unknown whether species that colonize before *T. rutilus* are resistant to predation (since they are poor competitors), or if they are at least as vulnerable to *T. rutilus* predation as the superior competitors that supplant them. To test these alternative hypotheses, I placed 80 water-filled containers in an oak-hickory forest for 7 weeks. I removed superior competitors from half of the containers, and allowed the other half to be colonized naturally. Removal of the superior competitors had no significant effect on *T. rutilus* colonization time nor *T. rutilus* abundance, but *T. rutilus* abundance was negatively correlated with prey abundance in both treatments. These data support that predation resistance in container Diptera is not simply an inverse function of competitive ability, but rather trades off with both competitive and colonization abilities.

7 **Ower, Geoff** (Graduate) - Talk*

Sexual selection of male song in free-living sagebrush crickets, *Cyphoderris strepitans*

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In crickets, acoustic signaling by males plays an important role in mate attraction and is thought to impose high energetic costs upon males, potentially making song an honest indicator of male quality. The objective of this study was to determine which acoustical features of male song in the sagebrush cricket, *Cyphoderris strepitans* (Orthoptera: Haglidae), are attractive to females and to measure the strength and shape of sexual selection acting upon these characters. While mating, female sagebrush crickets feed on the hind wings of males, which allows the mating status of males to be ascertained by simply inspecting their hind wings for the wounds inflicted by females. Populations in Grand Teton National Park were regularly censused throughout the breeding season and once they had reached a ratio of approximately 50:50 virgin to mated males, males were captured and their songs were recorded. Several acoustical characters were measured (pulse duration, interpulse duration, dominant frequency, train duration, and intertrain duration) and their selection surfaces analyzed using a dichotomous fitness measure (virgin = 0, non-virgin = 1). Multivariate selection analysis revealed significant linear and nonlinear selection on male song, with each of the five measured song characters contributing to male attractiveness.

8 **Tardi, Nicholas** (Graduate) - Talk*

Exploring the role of a FERM-PTP protein in controlling tissue size; Is Pez the missing link in Yorkie's chain?

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The Hippo pathway is a recently discovered tumor suppressor system that plays a key role in controlling organ size through regulation of cell proliferation and apoptosis in both vertebrates and invertebrates alike. Coordination between cell division and programmed cell death is indispensable for growth and development, reproduction, and basic homeostasis. With each cell choosing when and where to move, grow, divide, differentiate, communicate with other cells, or die, it is essential to properly regulate the mechanisms that control these decisions. Mis-regulation of these mechanisms is evident in the case of tumor formation and metastasis, in which a cells genetic material is damaged and evades apoptosis, ignoring its ?instinct? to make a self sacrifice for the benefit of the organism. Recently, we found that Pez binds to two Hippo pathway components: the recent addition to the pathway, Kibra, a tumor suppressor that binds Merlin, Expanded, and Hippo, and the transcriptional coactivator Yorkie. We propose that Pez acts as a scaffold protein by providing a platform for assembly of the Hippo/Warts kinase complex, and sequesters Yorkie at the membrane to keep it inactive. Regulation of this kinase cascade may be the key to controlling the balance between tissue growth and death.

POSTER PRESENTATION ABSTRACTS

* participating in award competition

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101 **Amport, Jeff** (Undergraduate) - Poster*

Role of Ankyrin in Na,K-ATPase Trafficking

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The sodium potassium pump (Na,K-ATPase) is a heterodimer, whose primary function is to carry out the coupled active transport of Na and K ions using the energy derived from ATP hydrolysis. The α-subunit (~110 kDa) of Na,K-ATPase contains a sequence of 25 amino acids (Ser142-Val166) known as the minimum ankyrin binding domain (MAB) and forms a tight association with ankyrin (Ank-G) in vitro. Ankyrin is a cytoskeletal protein responsible for mediating basolateral distribution of the Na,K-ATPase in epithelial cells that is critical for maintaining cell polarity. Phenotypes with defective ankyrin are the underlying cause of many heredity disorders, and are attributed to ankyrin's role in facilitating proteins to the plasma membrane. Although the interaction between the MAB and ankyrin has been shown in vitro, the role that the MAB plays in Na pump membrane delivery remains to be elucidated. These initial experiments are designed to determine the minimum sequence requirement for the MAB to interact with ankyrin. Wild-type MAB will be compared to an MAB mutated at three residues suggested to be crucial for ankyrin binding. Affinity purified wt-MAB and mutant MAB will be used as "bait" in GST pull-down experiments with ankyrin. GFP-Ankyrin expressed in HEK-293 cells. After determining that mutant MAB does not bind ankyrin, the equivalent mutations will be constructed in full length GFP-alpha. If ankyrin binding to the MAB is essential for Na,K-ATPase trafficking, the mutated GFP-alpha should fail to escape the endoplasmic reticulum in transfected HEK-293 cells.

Phenological niche separation from native species increases reproductive success of an invasive species: *Alliaria petiolata* (Brassicaceae) – garlic mustard

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Successful biological invasion requires correspondence between invader functional traits and their adaptability in novel environments. We focused on specific phenological and ecophysiological characteristics of the herbaceous biennial, *Alliaria petiolata,* related to its successful invasion of deciduous forest groundlayers in eastern North American. We tested for phenological separation between *A. petiolata* and native groundlayer species during spring of its second year, when the plant accumulates 91% of its total biomass, and assessed importance of availability of high irradiance before tree canopy closure on growth and reproductive output. We experimentally shaded plants *in situ* during three intervals: (1) before native groundlayer was well developed (March 3 - April 20), (2) after April 20 to tree canopy closure (May 18), and (3) after canopy closure to May 29. We measured maximum photosynthetic rates (A_{max}) in early (April 13-14) and late (May 22-26) spring. *A. petiolata* began rapid growth and reached maximum cover earlier than most native groundlayer species. Shading effect on plant growth and resource allocation to vegetative growth and reproduction varied depending upon timing and duration of shading. Comparison of treatments differing by being shaded or unshaded in only one of three intervals showed that unshaded plants consistently had significantly higher production than shaded plants only during the first interval. Greatest A_{max} occurred in early spring (April 13-14), when groundlayer irradiance was high. Success of *A. petiolata* in invading this community is likely related to phenological niche separation and temporal availability of resources not available to most native species in early spring.

103 **Breaux, Jennifer** (Graduate) - Poster*

Lingering effects of a hard childhood: larval competition and adult immunity in *Aedes aegypti*

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For insects, stress experienced in the juvenile stage may produce negative effects that carry over into the adult stage. Container-dwelling Aedes mosquitoes are an excellent system for investigating cross-stage effects. Larvae develop in a discrete environment where detritus input to containers determines nutrient content and, with density, determines per capita food availability. Competition for limited resources prolongs development, increases larval mortality, and reduces adult size, fecundity, and longevity. Previous studies also suggest that competitively stressed Aedes may be more susceptible than non-stressed individuals to dengue virus infection and dissemination. I postulated that larval competition may render stressed individuals more susceptible to vector-borne metazoan parasites via inhibition of pathogen-induced immune responses. When adult Aedes aegypti from larvae raised at multiple experimental densities were challenged with mixed bacterial inoculation, Cecropin A (a broad-spectrum antimicrobial and antiparasitic peptide) transcription was greater in density-stressed than in non-stressed individuals ($P<0.006$), suggesting a compensatory immune response in stressed females. A similar response was absent for transcription of the immune peptide Defensin A. Using A. aegypti (Liverpool) and Brugia pahangi as a model system, we tested whether different larval densities yield adults differing in susceptibility to B. pahangi infection. We found no effect of larval density stress on intensity of B. pahangi infection of adults. These preliminary results suggest that effects of competition among larvae on immunity and infection may be complex.

104 **Eisner-Pryor, Leah** (Graduate) - Poster*

Ectoparasites, Immunue Function, and Development in the European Starling

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We carried out an experimental study of the effects of nestling ectoparasite load on growth, immunity, circulating corticosterone levels, begging and parental provisioning in European starlings. We tested adaptive immunity by randomly priming half of the nestlings in the study with PHA or saline on day 5 followed by a wing web injection of PHA on day 16. We measured the elicited primary or secondary swelling response on day 17 to assess cutaneous immunity and immune memory formation characteristic of such adaptive immunity. We tested innate immunity by performing bactericidal assays on days 5, 10 and 15 of development. Parental provisioning and nestling begging were digitally recorded on days 11 and 14. Despite treatment to reduce ectoparasites in experimental nests, control and treated nests did not differ in nestling ectoparasite burdens. We found no significant variation between day 17 primary and secondary swelling responses to PHA yet found significant increases in bacterial killing capacity with nestling age as well as significant within- and between-nest variation at all three ages. In order to explain variation both within and between nests, we analyzed the relationship between bactericidal ability and nestling mass, blood glucose, hematocrit, and parental provisioning. None of these variables showed significant correlation with bactericidal activity. Continuing analyses will measure fecal and plasma corticosteroids, nestling begging behavior and ectoparasite loads in effort to further explain the observed variation.

105 **Engle, Staci** (Undergraduate) - Poster*

Measuring Intestinal Secretory IgA to Track Changes in the Mucosal Immune Response with Age

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Antibody production is an important response of the immune system upon contact with a foreign antigen. When the intestinal mucosal surface comes into contact with antigens, they are taken up into lymphoid tissues called Peyer?s patches (PP) and isolated lymphoid follicles through microfold cells of the mucosal epithelium. The presence of antigens activates the B cells in the lymphoid tissues to enter circulation and mature to effector plasma cells. They home back to the intestinal mucosal tissue where they secrete large amounts of IgA antibodies. By measuring the amount of IgA secreted we will be able to examine the intestinal B cell immune response of young versus old mice and look at the impact of aging. In our lab we use genetically engineered mice that produce identical IgA antibodies that recognize nitrophenyl (NP), an inert chemical. An ELISA protocol was developed to detect anti-NP IgA antibodies in the serum and fecal material. In our transgenic QM mice, low levels of specific IgA were detected. In order to stimulate the antibody production, we immunized mice via oral gavage. Oral gavage with NP-CTB showed no significant increase in IgA levels, so we substituted a stronger adjuvant, NP-CT, to get a stronger response. This method of oral gavage immunization and IgA detection will be very useful for our labs studies on PP response and the effect of aging on mucosal immunity.

106 **Frankenberger, Edward** (Undergraduate) - Poster*

Characterization of *csoR*, a novel regulator of copper homeostasis in *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive bacterium that is notorious as a causative agent of a wide range of infections. It is part of the normal microflora of the nose and skin in about 20% of the population. It is the most prevalent nosocomial bacterial pathogen isolated from patients. *S. aureus* is capable of growing in a wide range of diverse environmental conditions, such as heat, high salt levels and in the presence of heavy metals. The bacteria require special strategies to adjust to these growth-restricting conditions. Signals from the environment are used to control the bacterial cellular physiology, mediating a specific response to ensure persistence and growth. When colonizing a host, pathogenic bacteria compete with the host for the available nutrients. In addition to the various macro elements, various inorganic ions are also needed in trace amounts by the bacteria. These inorganic ions (Fe, Zn, Cu, Co, Ni, etc) serve as cofactors in many vital physiological processes. However, concentration of these ions above physiological levels is toxic for cells, and hence their intracellular concentrations must be regulated. Genes involved in homeostasis of iron, zinc and cobalt in *S. aureus* have been identified. However, little is known about copper resistance, tolerance and homeostasis in *S. aureus*. The aim of this research was to characterize csoR, a putative negative regulator of copper homeostasis using cloning, over-expression and qRT PCR under various growth conditions. It was observed that under high concentration of copper csoR is significantly induced, emphasizing its role in copper tolerance.

107 **Hammond, Anthony** (Undergraduate) – Poster*

Characterization of the binding capacity of Na,K-ATPase external and internal binding sites to organic cations and alkali metals

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In the Na,K-ATPase catalytic cycle, three intramembranous sodium cations are exchanged for two extramembranous potassium cations across cellular compartments of animal cells. To accomplish this, the Na,K-ATPase alters between two structural conformations, E1 and E2, in a ping-pong mechanism. In a physiological state, the phosphorylated form of E2, with external-facing ion binding sites, selects extramembranous potassium cations despite more than a 10-fold greater extramembranous sodium cations. Conversely, the E1 form, with internal-facing binding sites, selects intramembranous sodium cations despite over 10-fold greater intramembranous potassium cations. The mechanisms entailing cation binding selection by the ion binding sites (site-I and site-II shown to bind sodium or potassium cations, site-III shown to bind only sodium cations) are weakly understood. Previous studies from our lab showed guanidinium behaves like sodium cations on the cystolic-facing, cation binding sites of the Na,K-ATPase. I propose to investigate the binding constraints of the internal- and external- facing ion binding sites of Na,K-ATPase utilizing structurally related organic cations, and to investigate the ion binding site capacities of cesium and lithium (within the same elemental group as sodium and potassium). I will extend our prior observations to determine whether the analytes are capable of mimicking either potassium or sodium cations at the extramembranous-facing or intramembranous-facing ion binding sites, respectively. Specifically, I will characterize and quantify the effect of guanidinium, formadinium, acetamidinium, lithium, and cesium on the Na,K-ATPase. Ouabain-sensitive ATPase activity from purified sheep renal Na,K-ATPase will be used to kinetically determine the mode of interaction by these analytes.

Chemoreception as a mode of predator detection in the red-eared slider, *Trachemys scripta elegans*

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Chemoreception, the detection of and response to chemical cues present in air or water, has been shown to be a viable form of predator detection in various aquatic vertebrates; however, this has yet to be shown in aquatic reptiles. Due to the typically murky habitat in which freshwater turtles reside, it is reasonable to suggest that turtles rely on chemoreception more heavily than vision as a sensory mode when below the surface of the water. To evaluate this, subadult red-eared sliders were exposed to 20 mL of water conditioned with cues from the common snapping turtle, a known predator of juvenile sliders. Pre-stimulus administration behavior and post-stimulus administration behavior was scored for rate of movement, amount of time spent within a refuge, and amount of time spent displaying sensory detection behavior. Analysis revealed no significant differences in treatments, though an overall effect of disturbance of the water's surface during the administration of the stimulus may be a confounding factor. To address this, future studies will not only attempt to evaluate the relative importance of both vision and chemoreception in predator detection while more closely controlling for this surface disturbance, but also directly evaluate the importance of responding to such a stimulus in the context of a terrestrial or aerial predator attack.

109 **Holton, Nathaniel** (Graduate) - Poster*

The Role of Activation Induced Cytidine Deaminase in Breast Cancer

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Activated B-cells express Activation Induced Cytidine Deaminase (AID) in order to diversify the immunoglobulin (Ig) gene sequence and structure. This allows for greater antigen affinity, via point mutations within the variable region of the antibody. This is accomplished by AID deaminating cytidines to uracil. Generally, a uracil in genomic DNA will be repaired faithfully, however at the Ig locus the repair results in mutations altering antigen affinity and cell proliferation. Recently AID has been shown to be expressed in colon, rectal, breast cancer models. This suggests that the misexpression of AID in cells other than B-cells can lead to genomic instability and can have disastrous effects. In this seminar AID expression in MCF-7, a human breast cancer cell line, will be discussed as I am attempting to determine its role this cancer model.

110 **Hoover, Nathan ; Jordan, Corey** (Undergraduate) – Poster*

The Paradox between Density Dependent Effects on Mosquito Larvae and Adult Production

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Mosquitoes are a health concern as vectors of human pathogens such as West Nile,, LaCrosse, and Dengue viruses. Chemical or biological control targeting larvae can produce a paradoxical result: production of surviving adults may be as great or greater when control agents are used on larvae and induce measurable mortality of larvae. These situations are referred to as “compensatory” or “over-compensatory” mortality, respectively, and are believed to arise because extrinsic mortality due to control agents reduces larval density, and so reduces density-dependent effects on larvae, resulting in greater larval growth, survival, and development, thereby increasing production of adult mosquitoes. Key to understanding these effects are data on effects of density on daily survival, growth, and development of mosquito larvae across a range of densities. We raised three species of mosquitoes (*Aedes albopictus*, *Aedes triseriatus*, and *Aedes aegypti*) at single-species densities of 40 to 120 in 200 ml water, with a standard amount of detritus resources. Survivorship and larval developmental stage were recorded daily. Cumulative survivorship was significantly affected by density and species, but not by interaction of density and species. Thus, survivorship of all three species responds to density in similar ways. Daily mortality rate increased with density in all species. Developmental progress also showed significant effects of density and species but no significant interaction. Density effects on survival and development are thus similar for all three species, suggesting that extrinsic mortality of larvae is likely to cause compensatory or over-compensatory mortality in all three of these species.

111 **Javeed, Naureen** (Graduate) - Poster

Phenotypic protein trapping with a Minos-based element: Effects of genomic target size

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We recently developed a protein trapping system termed Hostile takeover (Hto), with the goal of identifying disruptive proteins or protein fragments. Hto carries a UAS/promoter sequence followed by an exon encoding FLAG-tagged mCherry red fluorescent protein (RFP). Hto is mobilized throughout the genome, and then its expression is induced using a GAL4 driver. The RFP exon splices to the next downstream exon in the genome to yield an RFP fusion protein. Flies with GAL4-dependent phenotypes are selected and used to establish lines. We find the Hto system seems to be biased toward tagging transcription factor (TFs), and here we consider three possible reasons for this. 1) Genes encoding tissue specific TFs tend to have expansive upstream regions and their start codons are often surrounded by very large introns; this characteristic structure presents an ideal target for tagging by Hto. Indeed, target sizes of Hto lines are more than double those of a set of random genes. 2) TFs can trigger misexpression phenotypes even at low levels of expression. Non-TF genes with large target sizes may not be expressed well enough to trigger phenotypes. 3) TF fusion proteins can disrupt development via several mechanisms, including dominant negative, neomorphic, and hypermorphic behaviors. We show evidence for a dominant negative mechanism in at least one case, and also evaluate the DNA binding ability of the fusion proteins by imaging RFP on polytene spreads. Overall, the Hto lines generated should facilitate both in vivo and biochemical analyses of TFs as well as cytoplasmic cell regulators.

112 **Lothery, Cassie** (Graduate) - Poster*

Do food supplements reduce stress in incubating female house wrens (*Troglodytes aedon*)?

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Corticosterone (CORT) is a glucocorticoid hormone that has primarily metabolic functions in birds, and is known as the "avian stress hormone." During times of chronic or acute stress (e.g., low food availability or presence of a predator, respectively), plasma CORT concentration increases resulting in a shift toward self-maintenance behaviors that ensure survival (e.g., foraging or nest abandonment). The house wren (*Troglodytes aedon*) is an ideal species in which to study parental resource allocation and its effects on stress because female house wrens are solely responsible for the incubation of eggs, which requires that they spend a great deal of time applying heat to regulate egg temperature. Thus, female wrens are challenged with the problem during incubation of how to allocate enough resources to care for the embryos developing in their eggs while obtaining enough food to meet their own metabolic demands. We tested the hypothesis that female house wrens engage in a trade-off between self-maintenance (foraging for food) and caring for embryos, and that this trade-off results in increased stress levels during incubation. We predicted that food-supplemented females would be less chronically stressed than unsupplemented controls, resulting in lower baseline CORT. Shortly after hatching, we obtained a blood sample from control and experimental females and conducted an enzyme immunoassay to determine plasma CORT concentrations. The results are not consistent with the hypothesis that females are stressed during incubation as there was no difference in mean CORT concentrations between treatments.

Mucosal Immunity: Optimization of Immunofluorescence protocol for visualization of Peyer's Patches

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The mammalian intestine is a site of continuous antigenic challenge due to the presence of ingested pathogens. Organized lymphoid nodules called Peyer's Patches (PP), covered by M cells, deliver antigens to B cells resulting in production secretory IgA which protects against pathogens and maintains normal microflora. Age advancement is believed to bring immunosenescence, which is strongly correlated with the increased incidence inflammatory bowel disease and colon cancer in the elderly. Our laboratory uses genetically engineered mouse models to investigate the effect of aging on mucosal immunity. In order to visualize antigen-specific PP B cells, we developed an immunofluorescence staining protocol. First, we designed a method to mount the PP to consistently section them with a cryostat. Next, we determined the concentration of anti-B cell dye to use. However, unstained samples showed a high level of autofluoresence, particularly along the border. To address this problem, we tried washing with sodium borohydride ($NaBH_4$). Post-fixation, tissues were treated with freshly prepared $NaBH_4$ solution before antibody incubation. Treatment significantly reduced fluorescence of unstained samples. In the stained samples, we noticed high non-specific staining, therefore, we tried replacing the fixative paraformaldehyde, with acetone. This change allowed us to observe specific PP staining with our B cell dye. We have now included a second dye that specifically stains our transgenic B cells and have successfully visualized dual stained cells. In the future, we plan to compare PP composition among young and aged QM mice to address the effect of aging in mucosal immunity.

114 **Morrison, Annie ; Rogers, Jasmine** (Undergraduate) - Poster

Migration of Aged vs Young B Cells to Mucosal Peyer's Patches

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The digestive system is exposed to many pathogenic microorganisms. B cells are a key component of the immune system that protect from disease by producing antibodies. Peyer's Patches (PP) are small areas in the mucosa of the small intestine where B cells are triggered to produce antibodies during an infection. Other studies have suggested that while systemic immunity is impaired with age, mucosal immunity may be preserved. Thus, our lab is interested in PP B cell number/ function in old versus young individuals. To study B cell responses, we use a mouse model with genetically engineered B cells. Small numbers of transgenic (Tg) donor B cells are transplanted into normal recipient mice. Since B cells normally circulate throughout the body, we first determined if transplanted B cells were detectable in the PP of recipients. We found that a week after transplant, small percentages of the donor B cells were present in the recipients' PP. Next, we compared the ability of young versus aged donor B cells to migrate to the PP. We found that B cells from the young donors tended to be more prevalent in the PP than cells from the aged, suggesting a defect in the ability of the aged cells to migrate properly. Finally, we examined the function/activation of transferred PP B cells by immunizing the mice with antigen via oral gavage. PP were harvested and activation protein expression was determined by flow cytometry.

115 **O'Neal, Paul** (Graduate) - Poster

Impacts of seasonal variation in desiccation and resource input on interspecific competition between *Aedes albopictus* and *Aedes aegypti*

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The mosquitoes *Aedes albopictus* and *Aedes aegypti* often co-occur as larvae in container communities in southern Florida, despite evidence that *Ae. albopictus* is a superior resource competitor to *Ae. aegypti*. Laboratory studies suggest that *Ae. aegypti* eggs are more desiccation-resistant than *Ae. albopictus* eggs and that the outcome of competition is reversed by habitat drying. We tested whether seasonal changes in environmental conditions in the field affect the competitive interactions of these species. We exposed seven density combinations of *Ae. albopictus* and *Ae. aegypti* eggs to three desiccation environments [ambient with no hatching, ambient with natural hatching, high humidity with no hatching] for two weeks during both the Florida wet season (summer) and dry season (spring). Eggs were then induced to hatch and larvae allowed to compete until emergence as adults. We incorporated seasonal differences in resource input by collecting leaf and animal detritus from proxy containers weekly in each season, and uniformly distributing pooled detritus among experimental containers. Survival to adulthood was significantly greater for both species in the dry season than the wet season. Competition was evident in the wet season, but much reduced in the dry season, and was not affected by the egg environment, suggesting that differential egg mortality between seasons had little effect on this interaction. Significantly more litter accumulated in proxy cups during the dry season than during the wet season, suggesting that greater resource inputs during the dry season may ameliorate effects of resource competition between *Ae. albopictus* and *Ae. aegypti*.

116 **Phutane, Megha** (Graduate) - Poster*

Characterization of Putative Monolignol Biosynthesis Genes in the Model Grass Brachypodium distachyon

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Liquid fuels derived from petroleum have propelled transportation in the United States for over a century. However, tight supplies, increasing world demand, and geopolitical instability in petroleum producing countries are contributing to volatile pricing that threatens U.S. and world economies. To break our dependence on foreign oil, scientists are working to develop alternative transportation fuels, including those derived from plant biomass. One method of biofuels generation involves the enzymatic extraction and fermentation of sugars derived from thermochemically pretreated lignocellulosic biomass. One hurdle to making this process economically attractive is the recalcitrance of lignin in the plant cell walls. Lignin is highly resistant to degradation and blocks hydrolytic enzyme access to cell wall polysaccharides. More than 10 enzymes are involved in synthesizing lignin monomers, which get incorporated into heterogeneous lignin polymers via oxidation reactions in the cell wall. Given that lignin is essential for plant survival, reducing plant lignin levels too much through genetic manipulation results in unhealthy plants susceptible to pathogen attack. Therefore, we are taking a combinatorial approach in studying ways to modestly lower lignin content and at the same time alter lignin composition and structure to allow for more efficient biomass deconstruction. As part of these efforts, we are employing the new model grass *Brachypodium distachyon* (Brachypodium). We have found that RNA interference (RNAi) constructs targeting knockdown of putative PAL (Phenylalanine Ammonia Lyase) and CAD (Cinnamyl Alcohol Dehydrogenase) genes resulted in reduced and altered lignin composition along with increased sugar release upon hydrolytic enzyme treatment. This is one of the first reports of altering lignin in Brachypodium, and this work provides important tools for studying the effects that more complex and novel cell wall alterations have on biomass deconstruction and plant fitness.

117 **Rockwell, Tim** (Undergraduate) - Poster

Cell division in the charophycean green alga Entransia fimbriata

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Differential interference contrast light microscopy and transmission electron microscopy were used to study cell division in *Entransia fimbriata*, a member of the Klebsormidiales. Cells contained a single large vacuole and a single large parietal chloroplast, each of which extended the length of the cell. The nucleus resided next to the chloroplast at the midpoint. The first indication of cell division was cleavage of the chloroplast, which divided completely or almost completely prior to mitosis. Initiation of a septum occurred after chloroplast division and before or during prophase, but the septum did not continue to develop until later. The late prophase nucleus was diamond shaped, and the nucleolus was present until just before metaphase. Though the telophase nuclei initially formed near the division plane, they had moved to opposite ends of the cell and were no longer aligned with each other during cytokinesis. Septum formation was centripetal. *Entransia*, like *Chlorokybus*, exhibits chloroplast division and septum initiation before prophase. These processes are reported to occur later in *Klebsormidium*. Cell division in *Entransia* is consistent with that in other early divergent charophycean algae.

Cause of Bat Mortality at Wind Farms: Barotrauma vs. Collision

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Wind farms are often considered to be an environmentally friendlier industrial method of energy production than traditional sources, and such facilities are increasing worldwide. However, the wind turbines of many large wind farms in the U.S. cause the death of seemingly large quantities of migrating bats, primarily hoary (*Lasiurus cinereus*), eastern red (*Lasiurus borealis*), and silver-haired (*Lasionycteris noctivagans*). Using bats salvaged at a large wind farm (Twin Groves Wind Farm, McLean County, IL) and at a large building (McCormick Place, Chicago, IL), we are testing the two leading hypotheses regarding bat fatalities at wind farms: barotrauma vs. collision. Barotrauma refers to mortality caused by organ damage due solely to entering the low pressure zone of a turning wind turbine blade and collision refers to blunt trauma from physically hitting a moving or stationary structure. We are using histopathology techniques, physical examination, and x-rays to measure lung, eardrum and skeletal damage that can be linked to either barotrauma or collision as the cause of mortality. This project is the first to assess bat mortality using indicators of auditory barotrauma and the second to use histochemical and x-ray techniques as barotrauma vs. collision fatality markers. Determining the cause of bat mortality should facilitate cost-effective changes in the way turbines are designed or operated.

119 **Sawa, Ashley** (Undergraduate) – Poster*

Estradiol metabolism during embryonic development in red-eared slider *(Trachemys scripta)*

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In vertebrates, the presence of estradiol during embryonic development influences numerous traits of the developing embryo. Recent work in oviparous vertebrates indicates that embryos have the ability to metabolize maternal estradiol present in eggs. This study characterized estradiol metabolism by red-eared slider (*Trachemys scripta*) embryos throughout embryonic development. Tritiated estradiol was applied to eggs at oviposition and we then measured the amount of estradiol and estradiol metabolites in various egg components across embryonic development. Results indicate that the majority of the estradiol was metabolized to several estrogen sulfates, including estriol sulfate, estradiol sulfate and estrone sulfate. Also, metabolite levels in the yolk and albumen decreased throughout development. The developmental decrease in the yolk and albumen samples is consistent with the transfer of metabolites to the embryo as later stages of egg development occur. Characterization of the metabolites present in the embryo is currently ongoing.

120 **Sengupta, Mrittika** (Graduate) - Poster*

Chromatin Immuno-Precipitation Identifies Genes Under Direct VraSR Regulation in *Staphylococcus aureus*

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Transcriptional profiling of *Staphylococcus aureus* treated with cell wall-active antibiotics identified the two component system, VraSR, as one of the key players in response to antibiotic stress. Although it has been shown that a number of genes are regulated by the VraSR system, it has not been shown which genes are under direct VraSR regulation and which genes are not. In this study Chromatin Immuno-Precipitation (ChIP) techniques were used to identify the genes under direct VraSR control. The results showed that cell wall biosynthesis associated genes like *pbp2, murZ* and *sgtB* are under the direct regulation of the VraSR system. Increased expression of VraR was observed both in Methicillin susceptible *S. aureus* (MSSA) as well as in Methicillin resistant *S. aureus* (MRSA) strains in response to oxacillin stress in a growth phase dependent manner. Real time PCR analysis further confirmed these observations. This work showed for the first time the *in vivo* regulatory mechanism of the VraSR regulon.

121 **Smith, Rebecca** (Undergraduate) - Poster*

Don't talk to strangers: Female Association Preference between Novel and Familiar Males in the Madagascar Hissing Cockroach (*Gromphadorhina Portentosa*)

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The ability to identify friend from foe in many animal species is crucial to survival; determining conspecifics from enemies is vital in mating and reproduction. The purpose of this experiment was to determine if there are any association preferences between novel and familiar males in the female Madagascar Hissing Cockroach, *Gromphadorhina portentosa*. In this study, seven colonies were set up at Illinois State University containing one male and four females. Colonies used in this experiment were established five weeks prior to the start of the experiment. Novel and familiar males were tethered at separate ends within a container; females were allowed ten minutes acclamation time before the start of the experiment. Data was collected every minute for twenty minutes using a point sampling technique. A one-tailed t-test was used to analyze the proportion of time females spent with in association with novel over familiar males, with a significance level of α=0.05. Familiar and novel males were compared within each trial for female association preference. Assuming that males with an established territory do in fact protect the females and nymphs within that territory, we hypothesized that females would choose a familiar male over a novel male. Indeed, we found that females spent significantly more time in association with the familiar male than the novel male. These results support our hypothesis on why females may choose to associate with a familiar male. Findings here suggest that association preference of female Madagascar Hissing Cockroaches may be influenced by prior adult experience, which may lead to mate preference and adaptive benefits for female fitness. Territory holders are often strong and aggressive males capable of having many mates. Female preference for such traits in males may guarantee her overall fitness.

Scent of a female: cuticular hydrocarbons facilitate chemosensory self-referencing in decorated crickets.

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Female decorated crickets, *Gryllodes sigillatus*, gain genetic benefits by mating polyandrously, and females preferentially mate with novel males over previous mates. Further evidence suggests that females "tag" males with their own unique chemical cues at mating, and are later able to recognize these cues in subsequent encounters via chemosensory self-referencing. For such a mechanism to be reliable, females must possess unique chemical signatures. Analyses of cuticular hydrocarbon (CHC) profiles of females from nine inbred lines via gas chromatography/mass spectrometry have identified 15 hydrocarbons that show substantial additive genetic variance. The homogeneity of CHC profiles within inbred lines demonstrates that these genetically unique chemicals can provide reliable cues for female chemosensory self-referencing. In the present study, we tested the hypothesis that females use CHCs as the underlying self-referent chemical cues used in mate recognition. We experimentally manipulated the chemical cues of males by externally applying female-derived CHCs, establishing two groups of males: 'novel' males bearing the cues of unrelated inbred females; and 'familiar' males bearing the cues of inbred sisters of the focal female. We then performed mate-choice trials in which focal females were allowed to choose between males from each treatment group. We found that focal females mated significantly more often with 'novel' than with 'familiar' males. The results of this behavioral assay provide further evidence that CHCs provide the proximate basis for chemosensory self-referencing. Self-referencing via cuticular hydrocarbons may be a ubiquitous mechanism in arthropods to facilitate mate recognition and maximize the benefits of polyandry.

123 **Williams, Jonathan** (Graduate) - Poster

MutS dependent responses to viral G4 DNA

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DNA secondary structure is common, especially in single stranded DNA. These secondary structures have been shown to influence genomic stability by effecting recombination. One of these DNA secondary structures that is found in a diverse range of organisms is G quadruplex or G4 DNA. G4 DNA is formed from Guanine rich DNA sequences. While G4 forming sequences are widespread, functional roles in the genome are not established. Even so, G4 structures are often found in recombination hot spots such as telomeres, antibody gene switch regions. We have found that the DNA repair protein, MutS, recognizes G4 with high affinity and binding is independent of known repair roles. I hypothesized that MutS has repair independent function affiliated with G4 bindings. I will test this model using an experimental system based filimentes phage, M13, harboring sequences that are capable or not capable of forming G4 DNA. I predict that MutS will have the ability to recognize and metabolize G4 forming DNA that is independent of the mismatch repair pathway or may even inhibit the repair pathway of the enzyme.

124 **Zimmerman, Laura** (Graduate) – Poster*

Age-related changes in antibodies of the red-eared slider, *Trachemys scripta*

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In humans, specific antibody responses decrease with age while natural antibodies (NAbs) increase. This reduction in specific antibody responses has been viewed as a contributor to the increase of mortality and morbidity with increasing age. Like humans, reptiles show an age-specific increase in NAbs, but unlike humans, reptiles of all ages have a less robust specific antibody response. Consequently, NAbs are viewed as a key component of immune defense in reptiles. Given the comparatively lesser reliance on the specific antibody response in reptiles, the age-specific increase in NAbs may constitute an improvement in immune defense. However, little is known about the quality of antibodies produced with age in reptiles. In order to investigate this, blood samples were taken from male and female adult red-eared slider turtles, Trachemys scripta, throughout the active season. Because red-eared sliders grow throughout their lifetime, plastron length was measured as a proxy for age. Affinity of antibodies to lipopolysaccharides (LPS), an antigen likely encountered by turtles, and keyhole limpet hemocyanin (KLH), a novel antigen, was determined using a competitive ELISA. Preliminary results suggest that affinity to either antigen did not vary seasonally or with sex. Affinity to LPS increased with age while affinity to KLH did not. This suggests that while affinity may not improve in the short term with immunization, affinity may improve with repeated exposure over time. Therefore, in reptiles, unlike in humans, changes in humoral responses may not cause impairment in immune defense with age.

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