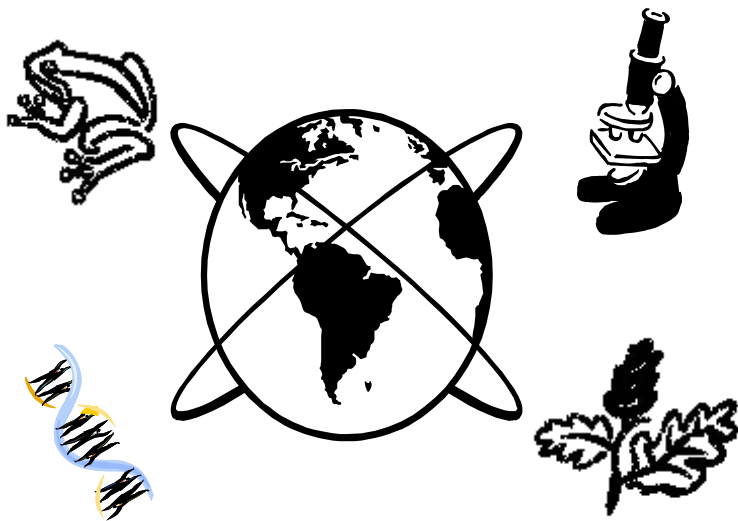


**13th Annual  
Phi Sigma - Biological Sciences  
Research Symposium**



**Illinois State University  
17 February 2012**

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## **SCHEDULE OF EVENTS**

**Friday, February 17th, 2012**

<b>8:30 – 9:00 AM</b>	<b>Registration</b>  Continental Breakfast 1 <sup>st</sup> floor of Science Laboratory Building
<b>9:00 – 9:15 AM</b>	<b>Opening Remarks (121 SLB)</b>
<b>9:15-10:30 AM</b>	<b>Oral Session 1 (121 SLB)</b> Lauren Beal Chris Howard John Robinson Dan Covey Brian Grebliunas
<b>10:30-11:30 AM</b>	<b>Poster Session 1 (even numbers)</b>
<b>11:00-12:30 PM</b>	<b>Lunch Break</b>
<b>12:30-1:30 PM</b>	<b>Poster Session 2 (odd numbers)</b>
<b>2:00-3:15 PM</b>	<b>Oral Session 2 (121 SLB)</b> Charitha Galva Keith Bowers Katie Westby Jen Breaux Laura Zimmerman
<b>3:15-4:15 PM</b>	<b>Keynote Address (Shroeder 138)</b> Jackie Bickenbach



# **KEYNOTE ADDRESS**

**Jackie Bickenbach**

University of Iowa

"Expression of Oct4 is sufficient to reprogram human skin keratinocytes into alternative cell types with potential for cell therapy"

**3:15pm**

**Schroeder 138**

## **ORAL PRESENTATION ABSTRACTS**

**\* participating in award competition**



## **Inhibition of Cattail Germination by Interspecific and Intraspecific Cattail Root Extracts**

Lauren M. Beal, Bradley J. Cook

Three species of cattails are common in the upper Midwestern USA. *Typha latifolia* is native to North America, *T. angustifolia* is an exotic species introduced from Eurasia, and *T. x glauca* is a hybrid between the native and exotic. *Typha angustifolia* and *T. x glauca* are invasive species that are reducing the biological diversity of many wetlands. Individuals of each species produce ~250,000 seeds each year but preliminary genetic profiles of cattail communities suggest that each species primarily reproduces clonally through rhizomes. Therefore, seeds are not effective locally and invasive clonal reproduction suggests that a strong competitive mechanism is at work. Some research suggests that *T. latifolia* releases auto-toxic root exudates that inhibit germination of conspecifics and *T. angustifolia* has a similar allelopathic effect on native heterospecifics. Our objective was to determine if aqueous root extracts from the three cattail species inhibit cattail seed germination. We tested the effects of cattail root extracts on cattail germination for all interspecific and intraspecific combinations and used deionized water as a control in bioassays. Our results among all species by extract combinations show there were no allelopathic effects. However, there were 4 facilitative effects. The germination of *T. angustifolia* seeds was facilitated by root extracts from all three species and *T. latifolia* root extracts facilitated the germination of *T. x glauca* seeds. The autotoxic feedback of *T. latifolia* and the positive effects of its extract on *T. angustifolia* and *T. x glauca* may contribute to the invasiveness of the exotic and hybrid cattails.

## **The effects of neurotoxic doses of methamphetamine on dopamine signaling in the striatum**

Christopher Howard, Paul Garris

Methamphetamine (METH) is a highly addictive and widely abused psychostimulant. Unfortunately, METH is toxic to many brain systems and METH abuse is associated with substantial depletions of the neurotransmitter dopamine in the striatum. These dopamine depletions have been associated with impairments of learning and memory processes in METH abusers, but exactly how METH-induced dopamine depletions alter physiological dopamine signaling is not fully understood. Here, I will discuss the ramifications of partial dopamine loss on rapid dopamine signaling events that are essential to reward learning. Dopamine signaling was assessed by implanting a microsensor in the striatum of awake, behaving rats pretreated with either METH or saline. METH-pretreatment resulted in significant reductions in tissue DA content that were associated with deficits in dopamine signaling. While the exact implications of altered DA signals is not known, future studies will attempt to characterize these changes as a mechanism involved in METH-induced impairments in cognitive functioning.

## **Characterization of the effect of Methamphetamine-induced neurotoxicity on pharmacologically elicited dopamine transients**

John Robinson, Paul Garris

Methamphetamine (METH) is a psychostimulant drug that is highly abused in many parts of the world, including America. With its use come many health concerns: tachycardia, hypertension, suicidal thoughts and acute psychosis, to name a few. Accompanying these short-term dangers, METH is also known to cause significant depletions to the neurotransmitter dopamine (DA), via neurotoxic mechanisms in the brain region known as the striatum. DA depletions of this type have been directly linked to functional impairments of learning and memory processes; however, the direct effects on normal DAergic signaling have not been fully elucidated. My goal has been to characterize the effects that a neurotoxic regimen of METH has on phasic DAergic signaling, a type of DA signaling that is required for associative learning and involved in synaptic plasticity. These signals are transient increases in DA concentration that are typically elicited by primary rewards (food, sex, etc.) and cues predicting these rewards, but in my experiments the transients were pharmacologically elicited. Transients were monitored by a voltammetric technique at a carbon fiber microelectrode in the striatum of anesthetized rats that had been pretreated with either METH or saline, a METH dose known to cause striatal DA depletions. Related studies suggest that phasic signals should be decreased in amplitude and frequency. This study is designed to help explain how METH abuse leads to cognitive impairments, contributing to the knowledge needed to advance our strategies for treatment of METH users.

## Stimulation-dependent and regional effects of AMPH action

Dan Covey, Paul Garris

Amphetamine (AMPH) is proposed to disrupt dopamine neurotransmission by depleting vesicular dopamine stores. However, direct measures of AMPH's effects on electrically evoked dopamine levels vary, with some studies showing an increase but others reporting a decrease. The decrease is consistent with *in vitro* experiments and the postulate of vesicular depletion, but the increase suggests a more complex drug action in the live animal. In the present work we tested the hypothesis that AMPH's effects on electrically evoked dopamine levels *in vivo* vary according to stimulation parameters and brain subregion. Dopamine was simultaneously measured in both the dorsal and ventral striatum of the anesthetized rat at an implanted microsensor. Dopamine neurons were electrically stimulated using three different stimulation intensities: (1) mild (0.4 s) (2) moderate (2 s) and intense (10 s). Each stimulation was applied before and after administration of AMPH (1 or 10 mg/kg), cocaine (40 mg/kg), or saline. AMPH's effects on evoked dopamine levels varied in the dorsal striatum by stimulation parameters, with an increase observed with the mild stimulus and decreases observed with both moderate and intense stimuli. In contrast, an increase in evoked dopamine levels was observed in the ventral striatum with all stimulation parameters. Cocaine, in contrast, elevated dopamine levels evoked by all stimulations in all regions. Taken together, these results have important pharmacological implications for AMPH's unique actions, and indicate selection of stimulation parameters is crucial for investigating drug effects on exocytotic dopamine release.

## **The Importance of Nutrient Stoichiometry on Denitrification in Wetlands Receiving Agricultural Tile Drainage**

Brian Grebliunas, Bill Perry

Nutrient stoichiometry within a watershed is dictated by the surrounding land use, and can play a significant role in the cycling of nutrients of concern, specifically nitrate. Tile drained agricultural watersheds experience extremely elevated inputs of nitrate and low inputs of phosphorus and carbon compared to natural wetlands. Either carbon, phosphorus, or both may limit denitrification in this type of system. We examined the effect of altered C:N:P ratios on denitrification rates within constructed wetland sediments over time in laboratory incubations (0, 5, 10, and 20 days). Both nutrient ratio ( $F_{(15)}=80.44$ ,  $p < 0.0001$ ) and incubation length ( $F_{(2)}=20.29$ ,  $p < 0.0001$ ) had a significant effect on denitrification. Denitrification rates responded positively to the presence of carbon, and the addition of carbon proved to be critical in maintaining denitrification at all nitrate concentrations (2 and 20 mg/L) over time. However, denitrification was not phosphorus limited, which suggests either inputs from sediment remobilization or tile inputs are adequate. Agricultural wetlands may be carbon limited due to low inputs and low retention in wetlands during elevated flow events.

**A potential intracellular physiological role for Na,K-ATPase:  
Maintenance of nucleoplasmic calcium.**

Charitha Galva, Craig Gatto

Na,K-ATPase (NKA) is an integral membrane protein responsible for establishing and maintaining the  $\text{Na}^+$  electrochemical gradient across most animal cells by actively exporting  $3\text{Na}^+$  ions in exchange for  $2\text{K}^+$  ions. Thus, NKA has been studied predominately with respect to its transporter function in the plasma membrane. Recently, NKA has been reported to have a non-transporter function as a signaling receptor for the Src pathway. In this report, we suggest that NKA may have an additional intracellular function. Specifically, extending our earlier observation that NKA functions within the Endoplasmic Reticulum (Gatto et al., *AJP-cell phys.*, **281**, C982-C992, 2001), we have now observed that NKA localizes to the nuclear envelope (NE). Immunocytochemistry with commercially available alpha and beta antibodies revealed that NKA was located on the nuclear envelope. Moreover, we observed an overlap between nuclear NKA and NCX (Na/Ca-exchanger) localization when the same nuclei were immunostained with NCX antibody. Given that NCX is driven by a  $\text{Na}^+$  gradient, we propose that the intracellular NKA function is utilized by NCX to extrude  $\text{Ca}^{2+}$  from the nucleus. We measured NKA function in isolated HEK cell nuclei via CoroNa-Green fluorescence. We observed an ATP-dependent, strophanthidin-sensitive  $\text{Na}^+$  accumulation in the nuclear envelope loaded with the Na-sensitive dye. Analogous experiments utilizing Fluo-5N, a  $\text{Ca}^{2+}$  sensitive dye, also revealed an ATP-dependent, strophanthidin-sensitive  $\text{Ca}^{2+}$  accumulation in the nuclear envelope of Thapsigargin treated and Fluo-5N loaded HEK293 nuclei. This work is supported by NIH grant DK083859.

**Sex-biased terminal investment in offspring induced by maternal immune challenge in the house wren (*Troglodytes aedon*)**

Keith Bowers, Charles Thompson, Scott Sakaluk

The reproductive costs associated with the up-regulation of immunity have been well-documented and constitute a fundamental trade-off between reproduction and self-maintenance. However, recent experimental work activating the immune system of breeding individuals suggests that parents may increase their reproductive effort following immunostimulation as a form of terminal parental investment as prospects for future reproduction decline. We tested the trade-off and terminal investment hypotheses in a wild population of house wrens (*Troglodytes aedon*) by challenging the immune system of breeding females with lipopolysaccharide, a potent but non-lethal antigen. Immunized females showed no evidence of reproductive costs associated with the immunostimulation; instead, they produced offspring of higher phenotypic quality, but in a sex-specific manner. Relative to control offspring, sons of immunized females had increased body mass and their sisters exhibited higher cutaneous immune responsiveness to phytohaemagglutinin injection, constituting an adaptive strategy of sex-biased allocation by immune-challenged females to enhance the reproductive value of their offspring. Thus, results are consistent with the terminal investment hypothesis, and suggest that maternal immunization can induce pronounced transgenerational effects on offspring phenotypes.

## Habitat Characteristics and Larval Abundances of Medically Important Mosquito Species in Central Illinois

Katie Westby, Steve Juliano

Female mosquitoes of the genus *Aedes* oviposit in a range of natural and artificial water holding containers. Three common species, *Aedes triseriatus*, *Aedes albopictus*, and *Aedes japonicus* are known, or suspected, vectors of arboviruses such as West Nile and La Crosse that are important causes of human disease. The containers they inhabit have low primary productivity and their productivity depends on input from terrestrial flora and fauna. Field and laboratory studies show that both inter and intra-specific resource competition have important context-dependent effects on these species that may translate into physiological, immunological, and life history characteristics that impact vector competence. I conducted field sampling of these species in container habitats in central Illinois to quantify: 1) important habitat characteristics; 2) characteristic abundances of immature mosquitoes in those habitats; and 3) habitat quality. All contents were removed from tires, vases, and tree-holes that were positive for mosquitoes. Environmental variables such as canopy cover and water volume were recorded and mosquitoes were identified to species and counted. Habitat quality was assessed by quantifying type and amount of detritus and by a bioassay quantifying success of the three *Aedes* species reared in standard volumes of water from the container. Multivariate analysis of variance suggests that these species differ in their distributions among, and life history responses to type of container, water volume, and abundance of fine detritus. Thus container characteristics may be important determinants of adult vector competence in these species.



## **Life history trade-offs influence host-pathogen dynamics in mosquitoes**

Jen Breux, Steve Juliano

For insects, stress experienced in the juvenile stage may produce negative effects that carry over into the adult stage. Container-breeding *Aedes* mosquitoes are an excellent system for investigating such 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. Reduced food availability prolongs development, increases larval mortality, and reduces adult size, fecundity, and longevity. Previous studies also suggest that food-stressed *Aedes* may be more susceptible than non-stressed individuals to arboviral infection, with a higher proportion of stressed individuals becoming physiologically competent vectors of disease. I postulated that the mechanism by which larval competition may render stressed individuals more susceptible to parasites is via inhibition of immunity due to physiological stress associated with density-dependent food reduction. When mosquito 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 adults ( $P < 0.006$ ), suggesting a compensatory immune response in stressed individuals. A similar response was absent for transcription of the immune peptide Defensin A. To determine if the observed effects are the result of food limitation or other factors associated with larval crowding, our current studies aim to measure post-challenge immune responses in larvae reared under (a) constant food and varying density or (b) constant density and varying food levels. We are also testing the relationship between larval rearing density and susceptibility to infection with the parasitic nematode *Brugia pahangi*, focusing on potential correlations between transcription of immune-related genes and parameters related to infection intensity.

Laura Zimmerman, Glen Borchert, Ryan Paitz, Rachel Bowden

The vertebrate immune system is comprised of innate and adaptive compartments that act to protect an individual from pathogens. The innate branch serves as a non-specific primary defense, while the adaptive branch produces specific, longer-term protection through the generation of memory. The immune system of turtles is poorly characterized, and while both innate and adaptive compartments are present, a deeper understanding of immune functioning is hampered by the paucity of information available. A unique sequence database was generated consisting of ~3000 immune genes from human, mouse, rat, chicken, Fugu, Medaka, Anole, Stickleback, Turkey, Xenopus, Tetraodon, Zebrafish and Sea Lamprey. Annotations were determined using in-house BLASTs comparing query sequences to the genome assembly, cDNA assembly and unassembled sequencing reads at both the nucleotide level and as reciprocal best BLAST hits using homologous protein sequences. Here we define 100 immune-related genes of the painted turtle, and identify light chain, constant, and variable regions of immunoglobulin loci IgM and IgG, toll-like receptors (TLRs), major histocompatibility loci (MHC I and II), and important components of immune pathways (Table 1). We were unable to define either IgA or IgD, both of which have been hypothesized to be present in reptiles, nor did we find CD4, indicating these immune genes are either poorly conserved or may not be present in the painted turtle. Our analysis demonstrates that the painted turtle has a unique repertoire of TLRs, comprised of those found in amphibians, fish, birds, and mammals, including a TLR15-like receptor that has previously only been defined in birds (Table 2). With this new information on the chelonian immune system it may now be possible to develop reagents to elucidate immune properties, as well as better understand the evolution of the vertebrate immune system.

## **POSTER PRESENTATION ABSTRACTS**

**\* participating in award competition**

#1

**Tardi, Nick (Graduate) - Poster\***

**Efficient phenotypic analysis using unfixed, uncoated adult  
*Drosophila* for scanning electron microscopy.**

Nicholas Tardi, Kevin Edwards.

Projects featuring repetitive phenotypic analysis of insects, such as mutant screens, quantitative genetics, and taxonomic studies, could be greatly facilitated by a simpler approach to SEM. Here, we have applied Low Vacuum SEM to wild type and mutant *Drosophila*, and demonstrate that high quality ultrastructure data can be obtained easily using minimal preparation. Adult flies, frozen live for storage, were mounted on EM stubs with carbon cement and directly imaged with no chemical treatment or sputter coating. The key imaging parameters were identified and optimized, including pressure, spot size, accelerating voltage, working distance, and exposure time. Potential artifacts, including apparent water droplets, variations in chamber pressure, charging, and sample dehydration were investigated. We conclude that our optimized protocol is well suited to large scale imaging of eyes, wings, bristles, and other adult structures.

**Self-maintenance versus reproduction: effect of experimentally increased food availability on female incubation behavior, chronic stress levels, and offspring condition in house wrens**

Cassie Lothery, Charles Thompson, Scott Sakaluk

Corticosterone (CORT), the “avian stress hormone,” increases in the plasma during times of chronic or acute stress (e.g., low food availability or presence of a predator, respectively), and can mediate a shift toward self-maintenance behaviors that ensure survival (e.g., foraging or nest abandonment). Birds incubating eggs face the potentially stressful problem of how to allocate their time and energy between maintaining egg temperature and obtaining enough food to meet their own metabolic demands.

We tested the hypothesis that female house wrens (*Troglodytes aedon*), which incubate their eggs without male help, face a trade-off during incubation between self-maintenance (e.g., leaving the nest to forage for food) and warming their eggs, and that this trade-off results in increased levels of chronic stress. We predicted that food-supplemented females would (i) spend more time incubating their eggs, (ii) have lower CORT levels, and (iii) produce offspring in better condition than control females. As predicted, food-supplemented females spent more time incubating their eggs than control females. Although CORT significantly increased over the incubation period, food-supplemented females did not have significantly lower CORT levels than controls nor were their offspring in better condition than those of control females.

## **Understanding Endocrine Disruption by Bisphenol-A Through Mechanistic Studies**

Sandrine Clairardin, Rachel Bowden

Many man-made chemicals are released into the environment every day. Some of these chemicals, called endocrine disrupting compounds (EDCs), can affect the functioning of an organism's endocrine system and have been shown to have a wide range of negative, largely estrogenic, effects; however, little is understood about how these chemicals produce their effects. This project tests the effects of Bisphenol-A (BPA), an EDC commonly found in plastics, on steroid regulation as a possible mechanism. To understand this mechanism, we investigate steroid and BPA metabolism using two life stages, embryonic development and early post-hatch, of the red-eared slider turtle, *T. scripta*. Together, these data show that BPA may interfere with estradiol metabolism, which may lead to the estrogenic effects that are seen in many exposure studies.

## Subregional Differences in the Mechanism of Amphetamine Action in the Rat Striatum

Alicia Avelar, Paul Garris

Amphetamine (AMPH), a psychostimulant recognized for its abuse potential and clinical efficacy in treating attention-deficit hyperactivity disorder and narcolepsy, exerts many of its effects by elevating extracellular dopamine (DA) concentrations in the striatum. AMPH increases DA levels through a variety of actions, including activation of DA synthesis, decreased degradation of DA by inhibition of monoamine oxidase (MAO), and competitive blockade of the DA transporter (DAT). The mechanism of AMPH is considered unique, because of its ability to deplete vesicular DA stores leading to diminished exocytotic DA release and to promote non-exocytotic DA release. Recent work by our laboratory has suggested that AMPH, under some *in vivo* conditions, actually increases electrically evoked DA levels by a combination of up-regulated exocytotic DA release and decreased neuronal DA uptake. The goal of this study was to determine how AMPH augments exocytotic DA release. To assess AMPH action, we modified an experimental design that was used to demonstrate that the psychostimulant cocaine promotes exocytotic DA release *in vivo* through mobilization of vesicular storage pools (Venton et al., 2006). Electrically evoked DA levels were monitored in the dorsal and ventral striatum of the urethane-anesthetized rat using fast-scan cyclic voltammetry at a carbon fiber microelectrode. We show that AMPH alone increases evoked DA levels in both dorsal and ventral striata. Surprisingly, blocking DA synthesis and degradation prevented this increase in the dorsal striatum, while the ventral striatum was unaffected. These results suggest distinct mechanisms of AMPH on exocytotic DA release in the dorsal and ventral striatum.

## **Chronic Mite Infestation and its Effects on Nest Success, Immunity, and Development in European Starling Nestlings**

Leah Eisner Pryor, Joseph Casto

We experimentally assessed the effects of chronic haematophagous mite infestation on nest success, egg spottiness, growth, and immune function in European Starling nestlings. We did this by spraying half the nests with the common agricultural insecticide permethrin to reduce ectoparasite number and inoculating the other half of the nests with approximately 200 adult northern fowl mites during incubation. We measured innate immunity by performing bactericidal assays on days 10 and 15 post hatch. We analyzed cell mediated immunity by measuring lymphocyte proliferation in response to lipopolysaccharide and concanavalin A at 17 days post hatch and we measured wing length, tarsus length, and mass to assess growth. We found a significant effect of mite infestation on nest success. Significantly more nests failed before hatch in the mite-enhanced group. We also found a significant treatment effect on egg spottiness. Post-treatment, eggs in mite-enhanced nests were significantly more spotted than eggs in permethrin treated nests. There was no effect of treatment on growth. We found a significant effect of mite load on innate immunity. Nestling bactericidal ability was significantly lower in mite-enhanced nests at day 10 but significantly higher at day 15. We also found a significant effect of day on innate immunity, in that nestlings on day 15 had significantly higher bactericidal ability than nestlings on day 10 in both treatment groups. We found no effect of treatment on lymphocyte proliferation. Further comparison to lymphocyte proliferation in adult starlings should allow us to determine whether nestlings have generally reduced proliferative ability or are capable of producing adult-like proliferation in response to the mitogens tested.



**Antipredator behavior of red-eared slider hatchlings in response to visual and chemical predator cues**

Justin W. Hicke, Rachel M. Bowden

Vision and olfaction are common channels of predator detection in aquatic vertebrates, though research on predator detection by aquatic reptiles is relatively limited. Young red-eared slider turtles are prey for a wide variety of predators, including many avian species and other reptiles such as snapping turtles. In preliminary work done to characterize an antipredator response, hatchling red-eared sliders (*Trachemys scripta*) were exposed to a series of visual and chemical stimuli. Individuals were presented with visual cues in the form of a sudden overhead movement to simulate an aerial predator as well as chemical alarm cues in the form of filtered conspecific tissue homogenate to simulate an aquatic predation event. Initial analysis indicated that hatchlings tended to respond by ceasing movement and submerging. To further explore these responses, hatchlings were exposed to two similar tests. First, hatchlings were presented with a model of an aerial predator. Second, to assess the use of chemical cues, individuals were exposed to aquatic predator cues and conspecific alarm cues, both separately and simultaneously. For all trials, pre-stimulus and post-stimulus behavior was scored and included the time spent moving or motionless, and the time spent above or below the surface of the water. These studies provide a greater understanding of how turtle hatchlings respond to the presence of predators in their environment and may allow for a comparison of how responses differ depending upon the type of predation threat.

## **Water Velocity Effects on Rusty Crayfish Size and Shape and the Relationship with Performance in Elevated Water Velocity**

Fiorenza, D, Young, M, Kuhnke, R, Jacquemin, SJ, Jacks, AM, and Perry, WL

Lakes and stream invasions of rusty crayfish, *Orconectes rusticus* (OR), provide an opportunity to examine invasions in contrasting habitats. Success of OR in lakes has been attributed to larger body and chelae size. We examined populations of male OR (Form I and II) from lakes (LA) and stream reaches of high velocity (HVS [ $>50\text{cm}\cdot\text{sec}^{-1}$ ]), mid velocity (MVS [ $30\text{cm}\cdot\text{sec}^{-1}$ ]), and low velocity (LVS [ $<20\text{cm}\cdot\text{sec}^{-1}$ ]) to test the hypothesis that size and shape are related to water velocity. Chelae and body size and shape were analyzed using geometric morphometrics. Body size and shape differed significantly between HVS and LVS and LA in form I but not form II crayfish. Left and right chelae were significantly smaller in HVS compared to LVS and LA. HVS Chelae (Form I and II) were significantly shorter and broader than LVS and LA crayfish. Shape of chelae was also significantly correlated with performance under elevated flow in a laboratory mesocosm. We suggest that phenotypic plasticity may affect OR invasion dynamics across variable hydrologic regimes.

## **Fitness-related costs of increasing egg production in female house wrens (*Troglodytes aedon*)**

Christine Hodges, Charles Thompson, Scott Sakaluk

Understanding the relationship between reproductive effort and parental fitness has long been a focus of life-history theory. Trade-offs of resource allocation between parents and their offspring can directly influence current and future reproductive effort. David Lack first hypothesized that the clutch size of altricial birds is shaped evolutionary by the demands of chick rearing, and that birds are selected to produce the maximum number of young for which they can provide food. However, Lack's hypothesis has been shown not to apply in many cases, and attention has now shifted to investigating the possibility that clutch size is limited during the egg production and incubation stages of the breeding cycle. We tested the hypothesis that increased reproductive effort in all three stages of the reproductive cycle limits the clutch size of female house wrens. We predicted that experimental females that were induced to produce 3-4 extra eggs would (i) produce fewer surviving offspring or offspring of poorer quality, (ii) be less likely to produce a second brood, and (iii) if they did produce a second brood, have lower reproductive success than unmanipulated females (controls). Our results are consistent with the hypothesis as experimental females fledged significantly fewer offspring in both their first and second broods compared with controls. Although experimental females were equally as likely to produce a second brood as control females, they produced fewer eggs in their second clutch than control females.

**Non Watson-Crick base pairing and its effect on genome stability**

Jonathan Williams, Erik Larson

DNA secondary structure is common in single stranded DNA. These secondary structures have been shown to greatly influence genomic stability. One of these DNA secondary structures found in a diverse range of organisms, including humans, is G quadruplex or G4 DNA. G4 DNA is formed from a stretch of guanine rich repeat DNA sequences. G4 forming sequences are widespread in the genome, but their functional roles and effect in the genome are not established. Even so, G4 DNA is found in recombination hot spots such as telomeres, immunoglobulin heavy-chain switch regions, rDNA, and in the promoters of several proto-oncogenes. We have previously found that the DNA mismatch repair protein, MutS, recognizes G4 with high affinity and its binding is independent of known mismatch repair roles. We hypothesize that the repair of a G/T nucleotide mismatch will be inhibited by presence of G4 forming sequences flanking and surrounding the mismatch. This hypothesis will be tested by an in-vitro mismatch repair assay using human Ramos cell nuclear extract on test constructs that allow the percent repair to be quantified. The test constructs are nicked circular plasmids that contain a G/T mismatch with no G4 forming sequence, a mismatch upstream of the G4 forming sequence, and a mismatch in the middle of the G4 forming sequence. With this assay I will be able to determine the effect of G4 forming sequences on the repair ability of the mismatch repair pathway.

## **Investigation of fast voltage-sensitive dyes and their application in the crustacean stomatogastric nervous system**

Stephanie Preuss, Wolfgang Stein

The recording of neuronal activity relies on the measurement of membrane potential changes in neurons. Traditionally, these changes are detected by successively impaling neurons with glass microelectrodes, a time-consuming and potentially harmful procedure for the cells. The bath-application of fast voltage-sensitive dyes (VSDs), in contrast, not only enables easy access to small neurons but also allows the simultaneous recording of multiple neurons without the need to impale them. VSDs report membrane potential changes by changing their fluorescent characteristics. As optical recording is a cutting-edge technique the dyes used still need further investigation. To test their characteristics and qualities we are using a well-established system for neuronal pattern generation, the stomatogastric nervous system of the crab. The pyloric central pattern generator in the stomatogastric ganglion (STG) controls the pyloric filtering of food in the animal's foregut and consists of only 12 neurons that generate a stereotyped triphasic activity pattern. Here, we are testing how well the VSDs Di-4-ANEPPS and RH795 stain STG neurons and how fast the staining bleaches. We record changes in the motor pattern to assess dye toxicity and phototoxicity caused by illumination. Our results indicate that both dyes reliably stain all neuronal membranes in a concentration-dependent manner. The Di-4-ANEPPS staining was always stronger than the RH795 staining, but also showed quicker bleaching. Toxic effects increased with concentration for both dyes used, but they were stronger for Di-4-ANEPPS. We are currently testing whether bright staining also improves signal-to-noise ratio during optical recordings.

**Interspecific vs. intraspecific competition effects on the prairie plant  
*Chamaecrista fasciculata***

Nate Gibbs, Dawn Giovanetto, Raechel Pink and Diane Byers

Tallgrass prairies are a very species-rich ecosystem. For plants, the majority of these species are perennials. These perennials grow and accumulate significant biomass for multiple years before reproduction. The few annual species, such as *Chamaecrista fasciculata* (partridge pea), need to successfully produce seeds within one growing season. These annual species have to reproduce starting from seed while competing with the already established perennials. If dispersal is limited, annual species will be in dense patches with potentially significant intraspecific competition. We ask if interspecific, intraspecific or a mixture of the types of competition have the greatest impact on the success of *C. fasciculata*. We propose that interspecific competition by the perennial grass; *Schizachyrium scoparium* (little bluestem) will have a greater impact on *C. fasciculata* than intraspecific competition. *Schizachyrium scoparium* commonly occurs with *C. fasciculata* and with its well-developed root system it is expected to be a superior competitor. Using a greenhouse experiment, we grew one *C. fasciculata* plant with no competitors, two, four or eight competitors. When competitors were present they were all *C. fasciculata*, all *S. scoparium*, or a mixture, allowing for the test of different types and levels of competition. *Schizachyrium scoparium* was established in the pots before planting the *C. fasciculata* seeds. We quantified the days to flowering for the focal *C. fasciculata*. We will be measuring the biomass of this focal plant as well as the competitors. Results from this study can be applied to management and restoration of prairies where preservation of biodiversity is a goal.

## Human Activation Induced Cytidine Deaminase is Regulated by miR-93 and miR155

Nate Holton, Erk Larson

Activation Induced cytidine Deaminase (AID) targets immunoglobulin genes of B cells, where its activity leads to mutations that result in antibody gene diversification and improved immunity. While essential for proper immune responses, misregulated AID activity promotes genome rearrangements that cause cancer. This is classically illustrated in Burkitt's lymphoma, which is characterized by AID-induced *c-MYC* oncogene translocations. AID protein has also been observed in several epithelial cell cancers, suggesting it may contribute to mutator phenotypes in non-B cell cancers. Aberrant AID expression is likely repressed by specific molecular regulators, but these are not fully defined. Our analysis of the human *AID* mRNA transcript has revealed an extensive and uncharacterized 3' UTR encoding both miR-93 and miR-155 microRNA recognition sites. Co-transfection of *AID*-negative HEK 293 cells with *AID*-3'UTR luciferase reporter fusions and either miR-93 or miR-155 expression constructs resulted in strong translational repression of luciferase. In contrast, control luciferase reporter fusions were not repressed by either miR-93 or miR-155. Both miR-93 and miR-155 are present in the Burkitt's lymphoma, Ramos, and in the *AID*-expressing human breast carcinoma MCF-7. Consistent with a capacity for *AID* translational regulation, we demonstrate that over-expression of miR-93 and miR-155 in MCF-7 cells significantly reduces AID protein levels. Our results support a model whereby miR-93 and miR-155 function to reduce the potential for AID-induced genome instability. Considering that the intronic miR-93 is expressed along with the *MCM7* replication gene, this microRNA may act as a ubiquitous genome sentry that prevents mutagenesis through the repression of errant *AID* translation.

## **Finding the spike-initiation zone of a sensory neuron using voltage-sensitive dye imaging**

Carola Städele, Peter Andras, Wolfgang Stein

The ability to generate adequate behaviors at any given time is one of the most amazing features of the nervous system. Processing of sensory information is a prerequisite for this, as well as flexibility of the underlying network. Flexibility is often caused by the actions of neuromodulators, which alter intrinsic neuronal properties and modify action potential generation [1]. We are studying action potential generation using the crustacean stomatogastric nervous system - a well-characterized model system for neuromodulator actions. The sensory neuron AGR (anterior gastric receptor, a muscle tendon organ) is a bipolar neuron, which possesses two spike initiation zones. One of them generates functionally relevant tonic spike activity and is modulated by Octopamine [2]. The location of the spike-initiation zone in the axon, however, is unknown and can only be estimated with electrophysiological methods. Here we use optical imaging for tracking AGR action potential propagation in order to determine the site of action potential initiation. We show that after bath application of the voltage-sensitive dye di-4-ANEPPS (5 $\mu$ M) all neuronal membranes were stained, including those of the AGR axon. We tracked AGR action potential conduction along a nerve containing a total of  $\sim$ 45 axons by measuring changes in the fluorescence of the dye using a high-speed CCD camera (Scimedia). Although extensive averaging was necessary, we were able to reliably isolate AGR's action potentials from those of other neurons. We are currently testing whether we can track action potential propagation along the nerve in order to determine the AGR's spike initiation zone.

[1] Marder & Bucher, *Ann Rev Physiol*, 2007

[2] Daur, Nadim & Stein, *Europ J Neurosci*, 2009



**Tradeoffs Among Colonization Ability, Competitive Ability, and Predation Resistance Govern Succession in an Aquatic Insect Community**

Ebony Murrell, Steve Juliano

Evidence for colonization-competition tradeoffs has been documented in an increasing number of communities, and may be a key tradeoff that drives ecological succession in multiple systems. However, the role of additional, broad ecological tradeoffs in succession (such as a competition/predation tradeoff) has been less explored, particularly in regards to empirical studies. We propose two alternative 3-way tradeoff models that demonstrate how succession patterns can be determined by simultaneous tradeoffs among colonization ability, competitive ability, and vulnerability of species to a single keystone predator. We then assess these three niche characteristics among species of larval Diptera that colonize artificial containers, and are subject to predation by a single predator species, *Toxorhynchites rutilus*. Regression analyses demonstrate a quadratic relationship between colonization time and competitive ability, with both early- and late- colonizing species inferior as competitors. However, there were no significant tradeoffs between competitive ability and predator vulnerability or between predator vulnerability and colonization time. While the data do not fully support either of the models, the non-linear relationship between colonization and competitive abilities suggests that additional tradeoffs may govern succession in container systems. We discuss some additional factors that may lead to tradeoffs, particularly in late-colonizing species.

**Isolated Lymphoid Follicles in Mucosal Immunity During Aging**

Samrat Moitra, Laura Vogel

The Gastrointestinal tract is the best studied mucus membrane. This site receives a continuous challenge as there is a continual import of air and food borne pathogens. As a result, lymphoid cells are found in Gut Associated Lymphoid tissues (GALT) and organized lymphoid follicles. Specialized epithelial cells called Microfold Cells or M cells help to promote the immune response by delivering small fragments of pathogens from lumen to the underlying GALT. ILF which are dynamic structures in intestine, produced from follicular B2 cells, produce more phenotypically active IgA than PP. ILF are thought to be formed in response to intestinal flora and can change in size and number throughout life. IgA is produced in a T-cell independent manner in ILF. An alteration of the intestinal microbiota acts as stimuli which trigger the development of ILFs post birth from the crypts (CP) or nascent lymphoid tissues. The number of CP are much more in number than PPs in the mice intestine which indicates the contribution of ILFs in mucosal immunity. It also functions for the production of antigen specific IgA in response to the local inflammations. Immunohistochemistry and flow cytometric analysis are useful tools in analyzing functionalities of ILF in aging process.

Tyler Malone, Steve Juliano

We tested the Resource Concentration and Resource Dilution hypotheses for plant herbivore load by analyzing patterns of attack by two gall-making insects on Common Boneset (*Eupatorium perfoliatum*). We posed the following questions: 1. Are gall makers aggregated, uniform, or random in distribution among host plants? 2. Is the distribution of gall makers correlated with host plant density? 3. Is the distribution of gall makers correlated with the size of the host plant? We sampled plants at two prairies in Central Illinois. Total number of branches, insect galls, and insect larvae/gall were recorded for each plant. Our analysis consisted of regressions of frequency (proportion of parasitized plants) and intensity (galls/plant) vs. density (Boneset stems/quadrat) and vs. size of Boneset (number of branches/plant). We found that both gall makers were aggregated. *Neolasioptera perfoliata* showed a significant positive correlation between the frequency of galls and plant host size, but there was no significant correlation between frequency of galls and plant host density. *Rhagoletis sp.* showed no significant correlation between frequency of galls and plant host size or density. In addition, the intensity of *N. perfoliata* galls increased significantly with plant host size, but not with plant host density. The intensity of *Rhagoletis sp.* galls increased significantly with plant host size, but not with plant host density. In conclusion, analysis of the two gall makers in this study shows that attack of Boneset is most affected by the size of the plant host. Our results are most consistent with the Resource Dilution hypothesis.

**Effect of Aging on Mucosal B Cell Migration**

Jasmine Rogers, Annie Morrison, Laura Vogel

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 distribution and function in old versus young individuals. To study B cell responses, we use a mouse model with genetically engineered antigen-specific B cells, the quasi-monoclonal mouse. Small numbers of transgenic (Tg) donor B cells (reactive to nitrophenyl, NP) are transplanted into normal young recipient mice. 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.

**Pulsed Radiofrequency Attenuates Inflammatory Gene Expression Following a Peripheral Injury**

Dana Tilley, Laura Vogel

Pulsed radiofrequency (PRF) is a relatively well used therapy for a variety of patients whom exhibit chronic pain. However, very little of the mechanism is understood. Our research induced an injury model whose mechanical hypersensitivity was alleviated upon application of PRF. A variety of well characterized genes known to play a role in pain perception were further assessed via real-time PCR to determine changes in gene expression arising from PRF therapy. We observed significant changes in gene expression at the site of injury, proximally at the dorsal root ganglia, and centrally in the spinal cord for multiple genes in each tissue. Several genes that were significantly down regulated include Interleukin-1beta, Interleukin-6, and Tumor necrosis factor-alpha, all of which are prominent pro-inflammatory agents. We believe that understanding what happens on a molecular level will help enhance the field of pain management and make PRF a more effective therapy.

## Effect of temperature on hatch timing in Eastern Lubber grasshoppers

Amanda Smith, Ashley Nowak, Patrick Wagner, Rebekah Yates, Elise Janci, Ryan Bernales, Taelor Dietz, Alex Earhart, Ariel Fogle, Nigel Fullerton, Kyle Gromer, Brien Kliver, Jessica Ludwikowski, Tony Martini, Joe McGrath, Alex Polino, Molly Schumacher, Shannon Weick, J. Casto, D. Whitman

Biological rhythms temporally coordinate both individual and population-level life history events. In the Eastern Lubber grasshopper (*Romalea guttata*), hatching appears to be under such rhythmic control: in the Florida Everglades, Eastern Lubbers tend to hatch *en masse* at approximately mid-morning. Given the largely synchronous hatching in the field, we investigated the effect of temperature as a possible cue for the timing of hatching in the lab. We hypothesized that a cycling thermoperiod of disparate temperatures acts as a population-level cue, and predicted that egg pods maintained on a 12:12h 15:26°C thermoperiod would preferentially hatch during the warmer part of the temperature cycle, resulting in inter- and intra-pod hatch synchrony. Conversely, we predicted that egg pods maintained at a constant 26°C would hatch sporadically throughout the entire thermoperiod. The egg pods were monitored hourly, and the number of hatchlings from each pod was recorded for each hour throughout the 24-h thermocycle. As we predicted, significantly more hatches occurred during the 26°C portion of the cycling thermoperiod, however, more hatches also occurred during the equivalent time period in the constant incubator. The implications of this are further discussed.

Deb Petrik, John Sedbrook

Due to its small stature, relatively short life cycle, and fully sequenced genome, *Brachypodium distachyon* is a useful model for studying modification of lignin content and structure. We are interested in reducing lignin content and in modifying its structure such that it is easier to harvest cellulose from plant secondary cell walls, for breakdown and fermentation of its sugar components into ethanol. The gene FMT from *Angelica senensis* has been identified to code for the FA transferase enzyme, responsible for conversion of coniferyl alcohol plus ferulate to coniferyl ferulate. Coniferyl ferulate conjugates allow easier delignification under milder conditions (lower temperatures), thus requiring less energy input during the delignification process. For this reason, it is desired to introduce the FMT gene into switchgrass. Problematically, there is an enzyme in *Brachypodium* and switchgrass called PMT which competes for the coniferyl alcohol substrate. Therefore, our approach is to overexpress FMT while simultaneously knocking out or down the level of PMT gene expression. Genetic and phenotypic analysis is being conducted on transgenic lines carrying either overexpression of FMT or knockdown of PMT. Once transgenic lines of each have been verified, the two will be crossed and progeny which inherited both genetic modifications will be selected.

## Anti-predator Behavior of Larval Mosquitoes: Effects of food and differences between the sexes

Jillian Chamberlain

Aquatic insect larvae often change their behavior in the presence of water-borne predator cues, exhibiting fewer risky behaviors. The conflict between anti-predator behavior and hunger has been investigated in tadpoles, sex differences in anti-predator behavior are not uncommon in animals, but neither phenomenon has been established in mosquito larvae. The native mosquito *Aedes triseriatus* and the invasive *Aedes albopictus* were used with the native predator *Corethrella appendiculata* to test the hypothesis that larvae display a facultative response to predator cues based on hunger and sex. Larval behaviors associated with greater risk of predation have been established in prior studies of these species. Behavior of second instar *A. triseriatus* and *A. albopictus* was recorded in water that previously held either (1) ten second instar larvae of the same species (i.e. conspecifics alone) or (2) a *C. appendiculata* larva fed ten larvae of the same species daily for five days (conspecifics + predation cues). Second instar larvae were held for 8-10 hours in containers with three levels of food availability and video recorded for thirty-five minutes. Position and activity were recorded each minute starting at minute five. Larvae were allowed to mature in order to be sexed. There were significant behavioral differences among food treatments, with larvae in the low food treatment shifting behavior from thrashing to browsing. The predator treatment showed the same behavioral shift, inconsistent with past studies which showed that predator cues induced larvae to spend more time resting at the surface. Sexes differed, with females spending more time browsing and filtering than thrashing, and males spending more time thrashing. *A. triseriatus* was less active than *A. albopictus*, spending more time resting at the surface and filtering and less time thrashing. The two species reacted differently to food treatments, with *A. albopictus* showing a greater hunger-induced behavioral shift.



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**13<sup>th</sup> Annual  
Phi Sigma - Biological Sciences  
Research Symposium**

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Illinois State University  
17<sup>th</sup> February, 2012

*Department of  
Biological Sciences*



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**A Special Thanks to the Judges for the Best Student Presentation Awards:**

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Vickie Borowicz

Rachel Bowden

Alan Katz