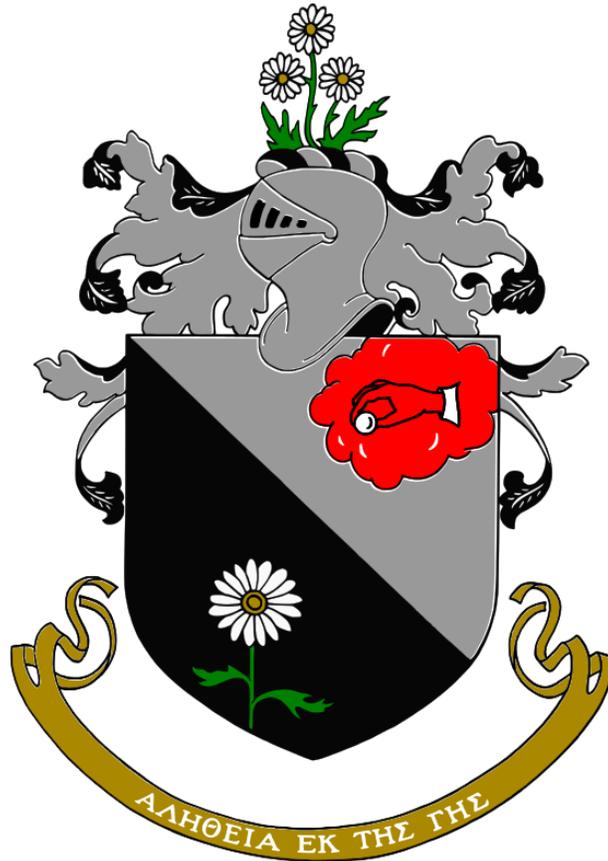


15th Annual Research Symposium Phi Sigma Biological Society



**4 April 2014
Illinois State University**

Thanks to Our Supporters at Illinois State University

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Schedule of Events

- 8:00 – 8:45 AM** **REGISTRATION & CONTINENTAL BREAKFAST**
Science Lab Building First-floor Atrium
- 8:45 – 9:00 AM** **OPENING REMARKS**
Science Lab Building 121
C. Gatto, Director, School of Biological Sciences
C. Thompson, Oral Session Moderator
- 9:00 – 10:00 AM** **ORAL SESSION 1**
Science Lab Building 121
- 9:00 Rachael Van Essen: Deuterium, tree bats, and wind farms
9:20 Christine Hodges: What limits clutch size in house wrens
9:40 Deborah Petrik: PMT in the lignin biosynthetic pathway in
Brachypodium distachyon
- 10:00A – 12:00 PM** **POSTER SESSION**
Science Lab Building Atrium
- 12:00 – 1:30 PM** **LUNCH**
Science Lab Building 121
- 1:30 – 2:30 PM** **ORAL SESSION 2**
Science Lab Building 121
- 1:30 Keith Bowers: Brood size and parental provisioning
1:50 Chris Loebach: Epizoochory in garlic mustard
2:10 Kristin Duffield: Spermatophylax composition following mortality cue
- 3:00-3:45 PM** **PRE-SEMINAR REFRESHMENTS**
Felmley Science Annex, outside room 133
- 3:45 – 4:00 PM** **TRAVEL AWARDS & RAFFLE DRAWING**
Moulton Hall 208
- 4:00 – 5:00 PM** **KEYNOTE ADDRESS**
Dr. Fred H. Smith, Illinois State University
Moulton Hall 208

Keynote Address

Fred H. Smith
Professor
Illinois State University



**“Something Old, Something New.
Neandertals Revisited.”**

Moulton Hall 208
4:00-5:00 PM

ORAL PRESENTATION ABSTRACTS

*** participating in travel award competition**

Oral presentations should be 20 minutes in total
(15 minutes for presentation & 5 minutes for questions)

Using deuterium and GIS to estimate geographic extents of source populations of tree bats killed at a central Illinois wind farm

Rachael Van Essen and Angelo Capparella

Bats are killed at an astonishing rate by wind facilities—an estimated 600,000 nationwide in 2012. Approximately 75% of these bats are of three species, the Eastern Red (*Lasiurus borealis*), Hoary (*Lasiurus cinereus*) and Silver-haired (*Lasionycteris noctivagans*). While the number seems large, we have little understanding of the impact of this high mortality on these species' population persistence, in part because we have poor knowledge of their breeding sites and migration pathways.

My research focuses on the Eastern Red and Hoary bats using deuterium ratio (δD) analysis in a novel way through combining (GARP: Genetic Algorithm for Rule-set Prediction) with (Isomap: Isoscapes Modeling, Analysis, and Prediction) technologies. My goal is to determine whether the bats being killed at an Illinois wind facility are coming from a large or small portion of their summer geographic range. This will help researchers understand autumn tree bat migration patterns through central Illinois. It will also be an important stepping stone towards understanding population and long-term impacts that high wind facility mortality will have on these two species.

**What limits clutch size in female house wrens
(*Troglodytes aedon*)**

Christine J. Hodges, Charles F. Thompson, and Scott K. Sakaluk

To maximize fitness, an organism must optimize the allocation of resources among competing physiological functions, which often results in trade-offs. A well-studied trade-off is that between current and future reproduction and survival. It is hypothesized that the amount of energy invested in a current breeding attempt may reduce the amount available to invest in future breeding attempts, or oneself. To test this hypothesis, we used a clutch-size manipulation to increase reproductive effort in the first brood by inducing females to produce and incubate extra eggs and to raise additional young than they normally would attempt. We then observed the reproductive success of their second broods and future breeding attempts. Females producing enlarged first-clutches laid the same number of eggs in the second brood as unmanipulated females, but suffered reduced hatchling survival, subsequently fledging fewer offspring from second brood nests. However, the manipulation did not affect maternal or offspring return rates. Despite suffering decreased second-brood success, experimental females had higher fitness, producing more first brood, high-quality offspring which are most likely to recruit into the breeding population, suggesting that females are poorly-adapted to their environment, producing smaller-than-capable clutch sizes.

p*-Coumaroyl-CoA:Monolignol Transferase (PMT) acts specifically in the lignin biosynthetic pathway in *Brachypodium distachyon

Deborah L. Petrik, Steven D. Karlen, Cynthia L. Cass, Dharshana Padmakshan, Fachuang Lu, Sarah Liu, Philippe Le Bris, Sébastien Antelme, Nicholas Santoro, Curtis G. Wilkerson, Richard Sibout, Catherine Lapierre, John Ralph, and John C. Sedbrook

Grass lignins contain substantial amounts of *p*-coumarate (*p*CA) acylating sidechains of the phenylpropanoid polymer backbone. An acyltransferase, named *p*-coumaroyl- CoA:monolignol transferase (OsPMT), that could acylate monolignols with *p*CA *in vitro* was recently identified from rice. *In planta*, such monolignol-*p*CA conjugates become incorporated into lignin via oxidative radical coupling, generating the observed *p*CA appendages. However, *p*-coumarates also acylate arabinoxylans in grasses. To test the authenticity of PMT as a lignin biosynthetic pathway enzyme, we examined *Brachypodium distachyon* plants with altered *BdPMT* gene function. Using newly developed analytical methods, we determined that the transferase was specifically involved in monolignol acylation. A sodium azide-generated *Bdpmt-1* missense mutant had no (<0.5%) residual *p*CA on lignin, and *BdPMT* RNAi plants had levels as low as 10% of wild-type. The amounts of *p*CA acylating arabinosyl units on arabinoxylans in these *PMT* mutant plants remained unchanged. *p*CA acylation of lignin from *BdPMT* overexpressing plants was found to be over three-fold higher than that of wild-type, while the level on arabinosyl units remained unchanged. Together, these data are consistent with a defined role for grass *PMT* genes in encoding BAHD acyltransferases that specifically acylate monolignols with *p*CA, producing monolignol *p*-coumarate conjugates that are used for lignification *in planta*.

Brood size and parental provisioning in house wrens: who cares?

E. Keith Bowers, Daniela Nietz, Charles F. Thompson, and Scott K. Sakaluk

In biparental species, parents are often expected to ‘negotiate’ over how much care to provide their offspring, where the investment put forth by one parent influences that of its mate. We studied parental provisioning in house wrens (*Troglodytes aedon*), predicting that male provisioning rates would be more strongly affected than female provisioning rates by differences in offspring number because this would influence the marginal return on a male’s investment. Parents delivered more prey to experimentally enlarged broods, but provisioning rates did not vary sex-specifically with brood size. Parents also did not respond to changes in their mate’s provisioning as predicted from current models of sexual conflict. We also assessed consequences of parental food-provisioning rates for offspring, and found that increased provisioning early in the nestling period, particularly of medium-sized prey, enhanced offspring pre-fledging mass, survival to fledging, and recruitment into the breeding population. Although the potential for sexual conflict in our study species remains, our data suggest that its resolution does not likely involve negotiation over provisioning, possibly because the effects of provisioning on offspring favor heavy investment by both parents, thus reducing the extent to which parents should negotiate over care.

Epizoochory as a seed dispersal mechanism for garlic mustard (*Alliaria petiolata*)

Chris Loebach and Roger Anderson

Garlic mustard, an herbaceous Eurasian plant, has aggressively invaded northeastern North American deciduous forests. It is a major threat to native groundlayer species. Garlic mustard's impact on groundlayer species has been extensively studied, but seed dispersal mechanisms of this wide-spread species have not been investigated. Epizoochory (external animal transport) is often cited as a dispersal mechanism, but this has never been tested. In this study, we tested for epizoochory under field conditions using a randomized block design. The study was conducted in second-growth forests. Each block contained a mammal inclusion treatment (MIT), designed to increase small mammal activity over a germination tray filled with potting soil, and a control, which excluded mammal activity over its tray. Four treatment blocks were placed around three garlic mustard patches on 3 July 2013. After most seeds were dispersed, germination trays were transported to ISU on 7 August 2013 and placed outdoors to receive cold-moist stratification. On 20 February 2014, trays were placed in a heated greenhouse, and emerging garlic mustard seedlings in each tray were counted daily. Preliminary results indicate epizoochory is a dispersal mechanism as there were significantly more seedlings ($F_{1,11}=48.89, P<0.001$) in the MIT trays (42.5 ± 7.7) compared to control trays (6.0 ± 1.5).

**Spermatophylax composition in male decorated crickets
(*Gryllodes sigillatus*) following mortality cue**

Kristin Duffield, James Rapkin, John Hunt, and Scott Sakaluk

The trade-off between key life history traits (survival, age-specific growth, and reproduction) is central to life-history theory; the expression of these traits is physiologically constrained by the amount of resources available to an organism whereby increased allotment of resources to one trait is necessarily traded off against a reduced allotment to other traits. A prominent trade-off includes one between current and future reproduction, which is likely dependent on life expectancy. The terminal investment hypothesis posits that as residual reproductive value (the age-specific expectation of future offspring) for an individual decreases, investment in current reproduction should increase.

I tested the hypothesis that male decorated crickets (*Gryllodes sigillatus*), when cued to their impending mortality, would alter investment in current reproduction by shifting the composition of their nuptial gifts, or spermatophylaxes, in a way that increases their gustatory appeal to females (i.e. increasing the ratio of free amino acids to water). I analyzed the contents of spermatophylaxes derived from males before and after a treatment of either a saline control or a solution of heat-killed gram-negative bacteria. Testing these trade-offs are important because our understanding of evolutionary and ecological processes relies on our knowledge of the mechanisms that drive variation in reproductive tactics.

POSTER PRESENTATION ABSTRACTS

*** participating in travel award competition**

The role of microtubule branching nucleation in cortical microtubule array reorientation in plant cells responding to growth hormones

Samantha Atkinson, Angela Kirik, and Viktor Kirik

Plant cortical microtubules orient perpendicularly to the growing cell axis in response to different environmental cues. The hormones auxin and gibberellic acid induce reorientation of longitudinal arrays into transverse in hypocotyl cells of light grown plants. The mechanism driving this reorientation is unknown.

We investigated the mechanism of microtubule reorientation and the role nucleation plays in that mechanism. The rates and types of microtubule nucleation were determined for wild type and *ton2* hypocotyl cells during hormone-induced microtubule reorientation. The *ton2* mutant, which is deficient in branching nucleation, provides a tool to assess the importance of branching nucleation in reorientation. Our data showed that cortical microtubules of the *ton2* mutant are able to reorient despite their deficiency in branching nucleation; though at a slower and less efficient rate. We also found that hormones did not cause a significant increase in nucleation for either wild type or the *ton2* mutant. Hormones caused an increase in the amount of microtubules that entered from the cell side for both wild type and the *ton2* mutant.

The effects of invasive macrophytes on nitrate removal and wetland sediment attributes in high nitrate, low carbon systems

Lauren Beal and William Perry

Constructed wetlands are quickly becoming inundated with invasive species like *Phragmites australis* and *Typha angustifolia* which are capable of changing whole ecosystem function and community composition. We wanted to assess how plants affect dissolved oxygen (DO) and dissolved organic carbon concentrations (DOC), and in turn if they increase nitrate ($\text{NO}_3\text{-N}$) removal rates relative to barren sediments. Using a $\text{NO}_3\text{-N/Br}$ tracer test, we tested $\text{NO}_3\text{-N}$ removal rates, DO, and DOC concentrations. After the tracer test, we measured above- and below-ground plant biomass and sediment characteristics. $\text{NO}_3\text{-N}$ removal did not differ across the treatments. *Phragmites australis* microcosms had significantly higher sediment bulk density and organic matter concentrations compared to *T. angustifolia* ($P=0.0049$, $df=5,23$) and barren sediment ($P=0.0033$, $df=5,23$) microcosms, however *T. angustifolia* and barren sediment treatments were not significantly different ($P=0.1021$, $df=5,23$). In $\text{NO}_3\text{-N}$ saturated environments, carbon outputs from roots may be insufficient to sustain microbial demands for denitrification. With DOC and DO concentrations near zero at all times in the rhizosphere, it seems likely that detritus and organic matter build up drive nitrate removal.

How do yolk corticosterone and fluctuating incubation temperatures affect hatchling behavior and physiology?

Amanda W. Carter, Laura M. Zimmerman, Ryan T. Paitz, and Rachel M. Bowden

In reptiles, the thermal and endocrine environments are influential in determining offspring phenotype. We conducted two studies examining the effects of thermal fluctuations during incubation and exogenous corticosterone on red-eared slider (*Trachemys scripta*) hatchling phenotype including physiology, morphology, and behavior. In the first study, we incubated eggs under three thermal flux regimes, that only differed in their fluctuation frequency with a normal frequency (24 hr cycle), a hypo-flux (48 hr cycle), and a hyper-flux (12 hr cycle) to disentangle the effects of temperature and fluctuations on hatchling phenotype. Fluctuation frequency did not affect any of the measured aspects of phenotype including morphology, behavior, and sex ratios. In the second study we dosed freshly laid eggs with corticosterone (0, 0.05, 0.15, 0.5 ng/ 5 μ l) and incubated eggs under a constant temperature. Corticosterone dose increased malformation frequency and decreased size at hatch, but did not affect behavior. Both studies demonstrated consistent individual behavior in a juvenile reptile, and we are currently working to determine if individual behavior influences dispersal ability and survival in the field.

Female choice in sagebrush crickets: are risks rewarded?**Charles Collis, Oscar Fernandez, Daniel Greenblatt, Zoe Marquis-Kelly, and Carly Osherow**

During their breeding season, male sagebrush crickets (*Cyphoderris strepitans*) climb into sagebrush to secure a singing perch at dusk. Sexually receptive females use auditory cues from the males to find prospective mates. Field experience suggests that individual males have characteristic calling behaviors. Some males call nearly continuously and are quick to recover from interrupting stimuli they interpret as a risk of predation. Other males call only sporadically and are reluctant to call after interruption. Previous studies have shown that acoustic signaling by males is an expensive behavior that requires a large expenditure of energy and increases the risk of predation. Presumably female choice in mating has balanced these expenses by rewarding males who recover quickly from interruptions and expend more energy calling than their rivals. To explore the role of female choice, we first scored calling behaviors and recovery from interruption behaviors of individual males in the field. These data were then used to separate the males into two groups: bold and timid. We hypothesized that the males' behaviors would remain consistent when a bold male, a timid male, and a female were placed in an outdoor pen together. We also hypothesized that females would mate with bold males more often than timid males. While the calling and recovery behaviors of the bold males at normal field density were not significantly different during mating trials, timid males called significantly more often in the artificially dense conditions in the pen than they did at normal field density. Females mated with bold males more often than timid males, but the differences were not statistically significant.

Metamodulation: Dopamine causes long-lasting modifications of neuromodulator-induced motor patterns

Marissa Cruz and Wolfgang Stein

Neuromodulators are chemical messengers that by modifying neuronal and synaptic properties alter the activity of whole networks of neurons. Despite the abundance of neuromodulators, only the actions of individual neuromodulators are well characterized. Their interactions, release-dynamics and long-term effects are less well understood.

We are studying the interactions of the Proctolin and Dopamine, which are both released from modulatory neurons in the stomatogastric nervous system of the crab, *Cancer borealis*. Without modulatory neurons, the pyloric motor pattern slows down or stops. Application of Proctolin restores the rhythm, but Dopamine has no immediate effect although Dopamine receptors are present in this system. **We hypothesize that Dopamine has long-lasting effects on pyloric neurons that affect *subsequent* neuromodulator actions.**

We recorded pyloric motor neuron activity and measured the pyloric cycle frequency. Preliminary data (N=7) indicates that Proctolin consistently elicited a pyloric rhythm, while Dopamine did not. However, Dopamine displayed long-term effects: 45 minutes after Dopamine wash-out, a second Proctolin application elicited a slower pyloric rhythm than before Dopamine. Phase relationships, burst and spike frequencies were different. Thus, even without apparent effects on the neural activity, neuromodulators cause long-term influences on the actions of other neuromodulators.

Supported: ISU Undergraduate Research Fellowship to Marissa Cruz.

Incubation Behavior Prior to Clutch Completion in European Starlings (*Sturnus vulgaris*)

Jason T. Hanser and Joseph M. Casto

In general, birds lay eggs over a series of consecutive days and begin incubation after the clutch is completed. However, in many species, females will often begin incubation prior to the completion of the clutch. Incubation prior to clutch completion causes eggs to hatch asynchronously and, consequently, later hatched individuals often experience greater mortality and slower growth rates. Despite hatching asynchrony being a widespread phenomenon across avian taxa and the subject of a considerable amount of research, we know surprisingly little about the patterns of incubation prior to clutch completion. Here, we present a comprehensive analysis of early incubation behavior in European Starlings.

Searching for Spore killer: A meiotic drive element in *Neurospora* fungi

**Austin Harvey, Dilini Samarajeewa, Kevin Sharp, David Rehard,
Patrick Shiu, and Thomas Hammond**

Mendel's laws of inheritance ubiquitously describe the mechanisms of chromosomal inheritance of genes. One such Mendelian law states that during meiosis, two homologous alleles have an equal chance of being passed to the progeny. However, meiotic drive has the ability to suspend Mendelian inheritance. Meiotic drive describes the phenomenon in which an allele is able to increase its transmission to offspring well beyond the standard Mendelian 50/50 chance of inheritance. In this study we are investigating a meiotic drive element called Spore killer discovered in *Neurospora* fungi. Spore killer in *Neurospora* works via a 'killer and resistance' model. In this system there are two tightly linked loci, one for killing and one for resistance. Recently, the resistance locus was identified. In this work, we are using molecular genetic techniques to identify the location of the killer gene. Identification of the Spore killer could potentially lead to a novel control technology for agriculturally detrimental fungi.

Expression, localization, and characterization of CTP: Phosphocholine cytidyltransferase from *Leishmania*

Justin D.T. Lange and Jon A. Friesen

The eukaryotic parasite *Leishmania* is the causative agent of visceral leishmaniasis. A common phospholipid found in the cell membranes of *Leishmania* is phosphatidylcholine. The enzyme CTP: phosphocholine cytidyltransferase, or CCT, catalyzes the addition of cytidine triphosphate to phosphocholine, a critical step in the formation of phosphatidylcholine. Amino acid sequence analysis of CCT from *Leishmania infantum*, *Leishmania major*, *Caenorhabditis elegans*, *Plasmodium falciparum*, and rat shows a high level of homology in the CCT catalytic region but a lack of a known carboxy-terminal membrane-binding region in *L. infantum* and *L. major*. However, this also shows an exclusive amino-terminal region in *L. infantum* and *L. major* which has a high level of homology with the sequence for choline phosphotransferase, the enzyme which catalyzes the next and final step of phosphatidylcholine synthesis, in other organisms. Here we present progress toward our goal of cloning the gene encoding CCT with a red fluorescent protein marker, expressing the recombinant protein in non-pathogenic *Leishmania tarentolae*, characterizing possible multi-step catalysis by the enzyme in vitro, and observing cellular localization of the enzyme using confocal microscopy.

Developing a transformation protocol for pennycress, a high potential oilseed crop and biofuel feedstock.

Michaela M. Lottes, Cynthia Cass, and John Sedbrook

Thlaspi arvense (field pennycress) of the family Brassicaceae is an annual cover crop that has the potential to increase and expand the plant oils profile of the nation without displacing food crops or requiring any additional tilled acreage. Pennycress seeds are roughly 36% oil, which amounts to potentially 840 liters of oil being produced per hectare, an amount double the oil production of soybean. After extracting the oil from crushed pennycress seeds, the remaining 1,470 kg/hectare of seed meal can be utilized as a nutrient filled, high protein animal feed.

Agrobacterium-mediated transformation in pennycress will allow for quick and effective genetic alteration of this potential oilseed crop. The Sedbrook lab is in the process of developing an *Agrobacterium* transformation protocol for pennycress, as one has yet to be established. At present, a mixed protocol utilizing steps from reputable *Arabidopsis* and *Camelina* protocols is being tested. This new protocol includes the standard steps of bacterial growth, sucrose/silwet resuspension and vacuum infiltration. Pennycress specific preferences such as bacterial strain, vacuum pressure and length of exposure are being tested.

Impact of light limitation on a hemiparasite

Brandon Mardoian and Victoria Borowicz

Root hemiparasites are autotrophic plants that parasitize the vascular systems of neighboring plants' roots for water and nutrients. Obligate hemiparasites require connections to hosts. It is hypothesized that a hemiparasite's ability to establish these connections depends on early growth, which may be limited by shade from taller neighbors. We tested this hypothesis with young *Pedicularis canadensis* plants (low-lying perennial obligate root hemiparasites) transplanted from a prairie to pots containing hosts in a greenhouse. We predicted that hemiparasites shaded early in development would experience higher mortality than plants either shaded later in their lives or never light-limited. We found mortality of these early light-limited plants was significantly higher than in plants that were not light-limited, but not significantly different from plants that were shaded later in life. Furthermore, we are analyzing biomass differences between the two light-limited treatments and control to determine if shading the hemiparasite indirectly affected the rest of the plant community. A current model describing the role of the hemiparasite in a plant community is based on annual hemiparasites whereas *P. canadensis* is a perennial hemiparasite. Therefore data gathered here make an important contribution to understanding hemiparasite interactions.

Engineering vegetative oils accumulation in the model grasses *Brachypodium distachyon* and *Zea mays*

Jack Munz, Cynthia Cass, Yang Yang, Rajandeep Sekhon, Shawn Kaeppler, Sanjaya, Cristoph Benning, and John Sedbrook

Increasing demand for energy has led to concerns as to whether finite fossil fuel reserves can meet the requirements of a growing world. Alongside fuel supply doubts are worries of continued climate change due to increasing atmospheric carbon dioxide levels from burning fossil fuels. At present biodiesel is one of the only viable liquid fuel alternatives. The hydrocarbon chains combusted in biodiesel are made from photo-assimilated sugars synthesized from atmospheric CO₂ and internal H₂O meaning biodiesel is essentially carbon neutral. Biodiesel production is limited most by lack of arable farm land and biofuel feedstock yield. Significant increases in oil yield per plant are necessary to equal global demand for plant based fuel and nutrition.

WRINKLED1 (WRI1), first identified in the model plant *Arabidopsis thaliana* (*Arabidopsis*), encodes a transcription factor responsible for stimulating production of enzymes involved in fatty acid (FA) biosynthesis for lipid droplet production in seeds and seedlings. *WRI1* constitutive overexpression mutants have been found to ectopically produce lipid droplets in leaves and stems in *Arabidopsis*. This phenomenon can be further enhanced to five-fold that of wild type plants when combined with a knockdown of the committal starch synthesis step catalyzed by ADP-glucose pyrophosphorylase (AGPase) (Sanjaya *et al.*, 2010).

Soil characteristics effects on female frequency in *Lobelia spicata*

Josef Oremus, Robert Philips, Tyler Rippel, Matt Trygg, and Diane Byers

Today, remaining prairie habitat is fragmented into patches with most being isolated from other prairies and surrounded by agriculture. This fragmentation can negatively impact the reproductive success of species when random effects determine the dynamics of the breeding system. We have been examining consequences of prairie loss and fragmentation on the variation in female frequency of a gynodioecious native prairie plant, *Lobelia spicata*. Gynodioecy is a breeding system that consists of either hermaphrodite or female plants, which is genetically determined. Initially we proposed the variation in female frequency in prairies was determined by genetic drift (variation in allele frequencies due to random sampling). While we found support for genetic drift, the type of prairie also appears to influence female frequency. We propose differences in soil characteristics found in the different types of prairies imposed sex-specific selection. Our current study will determine which soil traits are associated with the increase in female frequency. Assessment of how prairie habitat characteristics and *L. spicata* population size impact the reproductive success and female frequency will be presented.

**Geometric morphometric measurement of sexual selection
on the song-producing tegmina of the sagebrush cricket,
Cyphoderris strepitans (Orthoptera: Haglidae)**

Geoffrey D. Ower and Scott K. Sakaluk

Male sagebrush crickets attract females with song produced through stridulation of their tegmina. Linear and nonlinear sexual selection were measured on the shape, size and symmetry of tegmina using geometric morphometric techniques. Both top and bottom tegmina were found to be under directional selection for increased shape symmetry. However, all other significant selection gradients were found solely on the bottom tegmen, conceivably because it interacts more freely with the subtegmenal airspace that is likely used for song amplification. The anterior (lateral) edge of the bottom tegmen, which likely acts as a baffle against noise cancellation, was found to be under selection for enlargement. Despite the strong stabilizing selection found previously on the dominant frequency of the song of sagebrush crickets, significant stabilizing selection was not found on the resonator that regulates dominant frequency according to the clockwork cricket model. However, the shape principal components showed little variation in the landmark placement around the resonator, and this reduction in variance suggests that strong stabilizing selection has occurred in the past.

Aged vs young B1 cell immune response in a SCID mouse model

Hazel Ozuna and Laura A. Vogel

Different factors contribute to age-related immune dysfunction such as changes in white blood cell numbers and function. In particular, humoral responses are negatively impacted by age, due to both direct and indirect effects on antibody-producing B cells. However, controversy exists as to whether mucosal B cell responses may be preserved during aging. The objective of this project was to study if B1 cells, which mainly participate in T cell-independent responses but retain numbers and function in aged individuals, are capable of producing T cell-dependent (TD) responses characteristic of B2 cells. Our hypothesis was that under optimal conditions, B1 cells from aged individuals can produce antibodies to TD antigens. We predicted that aged B1 cells transferred to SCID recipients, where competition with B2 is removed, could produce antigen-specific IgA in the intestine. To test the prediction, antigen-specific B1 cells from young or aged donors were adoptively transferred along with antigen-specific T cells to recipient SCID mice. After homeostatic expansion, recipients were immunized by oral gavage with cognate antigen (NP-OVA). Flow cytometry was used to examine cell numbers and distribution and ELISA was used to examine levels of antibody secretion (quantity and quality) between young and old donors. Results demonstrated a significant difference in expanded cell populations between the animals that received old donor B1 cells compared to those that received young B1 cells. A difference was also observed between naïve and immunized groups among the animals that received old donor cells. Analysis of fecal and serum antibody production showed background levels of NP-binding IgA in naïve samples of both age groups, however only mice receiving young B cells were able to produce antigen-specific IgA upon immunization. These results provide important insight to the effectiveness of mucosal B cell responses in the elderly.

p*-Coumaroyl-CoA:Monolignol Transferase (PMT) acts specifically in the lignin biosynthetic pathway in *Brachypodium distachyon

Deborah L. Petrik, Steven D. Karlen, Cynthia L. Cass, Dharshana Padmakshan, Fachuang Lu, Sarah Liu, Philippe Le Bris, Sébastien Antelme, Nicholas Santoro, Curtis G. Wilkerson, Richard Sibout, Catherine Lapierre, John Ralph, and John C. Sedbrook

Grass lignins contain substantial amounts of *p*-coumarate (*p*CA) acylating sidechains of the phenylpropanoid polymer backbone. An acyltransferase, named *p*-coumaroyl- CoA:monolignol transferase (OsPMT), that could acylate monolignols with *p*CA *in vitro* was recently identified from rice. *In planta*, such monolignol-*p*CA conjugates become incorporated into lignin via oxidative radical coupling, generating the observed *p*CA appendages. However, *p*-coumarates also acylate arabinoxylans in grasses. To test the authenticity of PMT as a lignin biosynthetic pathway enzyme, we examined *Brachypodium distachyon* plants with altered *BdPMT* gene function. Using newly developed analytical methods, we determined that the transferase was specifically involved in monolignol acylation. A sodium azide-generated *Bdpmt-1* missense mutant had no (<0.5%) residual *p*CA on lignin, and *BdPMT* RNAi plants had levels as low as 10% of wild-type. The amounts of *p*CA acylating arabinosyl units on arabinoxylans in these *PMT* mutant plants remained unchanged. *p*CA acylation of lignin from *BdPMT* overexpressing plants was found to be over three-fold higher than that of wild-type, while the level on arabinosyl units remained unchanged. Together, these data are consistent with a defined role for grass *PMT* genes in encoding BAHD acyltransferases that specifically acylate monolignols with *p*CA, producing monolignol *p*-coumarate conjugates that are used for lignification *in planta*.

Probing the limits of unpaired DNA detection during meiosis**Jay Pyle, Morgan McCall, and Thomas Hammond**

Homologous chromosome pairing is an essential process during meiosis in the life cycle of the filamentous fungus *Neurospora crassa*. During this phase the fungus, through a process known as Meiotic Silencing of Unpaired DNA (MSUD), is able to scan its genome and utilizes an RNAi pathway to effectively silence genes which appear to be unpaired between the homologous chromosomes. MSUD directs transcripts from unpaired genes along with all homologous copies of that gene, including paired copies, through RNAi mediated by MSUD-associated small interfering RNAs (masiRNAs). While the mechanism behind RNA processing is well defined in the cytosol the system for identification of unpaired DNA within the nucleus has remained obscure. This study aims to characterize the basic capabilities for MSUD to detect and silence unpaired DNA. Specifically, analysis of phenotypic outcomes will be analyzed after sexual reproduction with crosses containing ectopic transformations of gene fragments known to cause distinct phenotypic modifications in the progeny. Our analysis will attempt to better define *Neurospora crassa*'s ability to recognize repetitive DNA sequences and inverted transgenes as either paired or unpaired DNA segments.

SAD-6: an SNF2-family protein involved in meiotic silencing by unpaired DNA

Dilini A. Samarajeewa, Nick A. Rhoades, Kevin A. Edwards, and Thomas M. Hammond

Meiotic silencing by unpaired DNA (MSUD) is a process that detects and silences unpaired DNA between homologous chromosomes for the duration of meiosis. It is believed that gene silencing works through an RNA-interference-related pathway via the production of a theoretical aberrant RNA molecule. However, the nature of the unpaired DNA scanning mechanism and the proteins involved in the nuclear aspects of MSUD are completely unknown. Rad54 is a protein involved in repairing double stranded DNA breaks by homologous recombination in yeast. SAD-6 is a putative SNF2-family protein closely related to Rad54 in *Neurospora crassa*. We found that MSUD is significantly suppressed by the deletion of *sad-6* locus, suggesting that it is required for the process. Moreover, confocal microscopy studies have confirmed that GFP tagged SAD-6 protein is localized in the nucleus during meiosis suggesting that SAD-6 could be a protein involved in the unpaired DNA detection process of MSUD. Further studies will seek to identify the theoretical aberrant RNA and determine if SAD-6 is required for its production.

**Determining the parameters of a gene silencing mechanism
in the model organism *Neurospora crassa***

Pegan Sauls, Zach Smith, Kevin Sharp, and Thomas Hammond

When model organism *Neurospora crassa*'s cells undergo meiosis, its genome undergoes a gene silencing mechanism referred to as MSUD (Meiotic Silencing of Unpaired DNA). MSUD can be triggered during homologous chromosome pairing if any gene "looks" unpaired. This has been shown to occur when a gene is deleted or when an extra ectopic copy is added to one of the chromosomes. In our research we have taken a gene marker and integrated it at different spots into the *N.crassa* genome. After performing multiple crosses between strains containing the differently located gene marker, the progeny of those crosses were examined to determine spore phenotypes and the degree of silencing. We have discovered that the amount of gene silencing is dependent upon the distance between the gene markers on each homologous chromosome.

The reproductive cost of fighting an infection: a test of Life History Theory using the mosquito

Molly Schumacher and Steve Juliano

Investment in life history traits such as immune function, reproduction, and maintenance is determined by limited resources, resulting in trade-offs within these traits to maximize lifetime fitness of an individual. This may occur when an upregulated immune response to combat an infection further restricts investment into reproductive effort. Conversely, the Terminal Investment Hypothesis suggests that an infected individual may display dishonest signals of their condition, and enhance energy investment in reproductive efforts to maximize terminal reproductive success. To determine whether immunocompromised males will display honest or dishonest reproductive signaling, binary-choice mating trials were conducted with an *Aedes aegypti* female and 2 males: a control and an immunocompromised male (either inoculated with *Escherichia coli*, heat killed *E. coli*, or saline). Male and female reproductive success and survivorship were monitored to determine fitness differences between treatments. Immunocompetence was measured 48 hours post-infection to compare immune responses between treatments. The outcome of this study may be important in understanding current population control efforts (especially those that rely on male mating behavior). Mosquito mate-choice behavior is poorly investigated but important for success of those control efforts.

Characterization of Butyrate Kinase from *Listeria monocytogenes*

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Listeria monocytogenes, the causative agent of listeriosis, can build up to dangerous levels in refrigerated foods. The bacterium's growth at low temperatures is aided by its increase in the branched-chain fatty acid (BCFA) anteiso C15:0 content of its membrane which imparts greater membrane fluidity. Mutants in the branched-chain α -keto dehydrogenase (*bkd*) complex are deficient in BCFAs but can be restored by feeding C4 and C5 branched-chain carboxylic acids (BCCAs) thus modifying the membrane fatty acid profile. This suggests an alternate pathway for production of acyl CoA precursors for fatty acid biosynthesis which we believe involves two enzymes, butyrate kinase (Buk) and phosphotransbutyrylase (Ptb).

The *buk* gene of *L. monocytogenes* 10403S was cloned into the expression vector pRSETa and the recombinant protein was purified using affinity chromatography. Steady state kinetic data showed that Buk had high affinity for BCCAs although it had the highest activity with pentanoate. A dramatic increase in the K_m for hexanoate (C6) when compared to pentanoate (C5) illustrates that long chain carboxylic acids are not suitable substrates for this enzyme. Our data also indicates that Buk catalysis occurs through a ternary complex intermediate and that Buk from *L. monocytogenes* phosphorylates a remarkable number of straight and BCCAs.

Sub-second measurement of electrically-evoked dopamine release in the European starling

Amanda R. Smith and Joseph M. Casto

In the striatum and nucleus accumbens, dopamine is released in response to novel or otherwise salient sensory stimuli. To date, dopamine release in response to drug or food presentation has been fairly well characterized, but the role of dopamine in processing complex natural stimuli, such as social or sexual signals, remains largely unexplored. Due to the clarity and distinct ecological context of their social signaling, songbirds, such as the European starling, present a desirable model for investigating the relationship between neural signaling and social signal perception. To address this, we have recently implemented fast-scan cyclic voltammetry (FSCV), a powerful technique able to measure sub-second changes in electroactive chemicals, to quantify dopamine overflow in the striatum of the European starling. This represents, to our knowledge, the first *in vivo* use of this technique in a non-mammalian vertebrate. This work is an important first step in establishing FSCV, a technique allowing specific, real-time quantification of dopamine, in a songbird system, thereby laying the groundwork for future studies investigating dopamine release during complex social stimuli.

Effects of pre- and post-natal corticosterone on fitness-related traits in nestling house wrens (*Troglodytes aedon*)

Meghan Strange, Scott K. Sakaluk, and Charles F. Thompson

Corticosterone is a glucocorticoid hormone present in birds and is involved in modulating behavioral and physiological responses to environmental challenges. Corticosterone secretion in response to environmental challenges is beneficial to an individual, but prolonged exposure to elevated corticosterone levels can be detrimental. Corticosterone is included in the hormonal environment during both the pre-natal (i.e., embryonic) and post-natal (i.e., nestling and fledgling) stages of avian development. Although corticosterone is essential for development, exposure to elevated concentrations can have significant organizational phenotypic effects on offspring and can therefore affect Darwinian fitness.

This study tested the hypothesis that elevated pre- and post-natal corticosterone levels within the physiological range modify offspring development and phenotype in house wren (*Troglodytes aedon*) nestlings. Corticosterone levels were elevated pre-natally through egg injections and elevated post-natally through diet. I measured the fitness-related traits mass, hematocrit, tarsus length, and immune response of nestlings 11 days after the first egg of the clutch hatched. Results reveal that fitness-related traits in nestling house wrens are not affected by pre- and post-natal corticosterone elevation.

Characterization of yolk glucocorticoids and their metabolism during the embryonic development of the red-eared slider (*Trachemys scripta*)

Lisa A. Treidel, Ryan T. Paitz, and Rachel M. Bowden

Oviparous vertebrate eggs contain a number of steroids, including glucocorticoids, at the time of laying. During embryonic development, maternally derived glucocorticoids can act to modify the offspring's phenotype, while embryonically produced glucocorticoids are important for hatching. The multiple roles of glucocorticoids make it likely that regulating the timing of embryonic exposure throughout development is necessary for proper offspring development and hatching. Yet, little is currently known about the mechanisms by which this occurs, especially in oviparous reptiles such as the red-eared slider (*Trachemys scripta*). In our first study, we characterized the changing concentrations of yolk corticosterone occurring during embryonic development. Eggs from ten clutches were sampled throughout incubation and yolk corticosterone was quantified via a radioimmunoassay. We found that while prior to the start incubation only trace amounts of corticosterone are present, late in development, yolk corticosterone levels spike. Next, to investigate the metabolism and movement of corticosterone during embryonic development, we topically applied 150,000 cpm of tritiated corticosterone to eggs. Using eggs sampled at different points in development, an ether extraction was used to separate and quantify ether soluble and water soluble metabolites in the yolk, extraembryonic fluid, and embryo. From this study we found that applied corticosterone is rapidly metabolized during development and remains as metabolites mostly in the yolk and extraembryonic fluid. Together, these two studies suggest that the glucocorticoid environment is subject to modulation prior to the embryonic production of glucocorticoids.

Neuromodulator-induced stability of neuronal activity patterns

Abigail Tubia and Wolfgang Stein

Central Pattern Generators (CPG) are networks of nerve cells that govern essential rhythmic behaviors such as breathing, walking, and chewing. The open question remains, as to how the nervous system can be stable while maintaining its flexibility to execute an appropriate response for changing environmental conditions. The pyloric CPG in the stomatogastric nervous system (STNS) of the crab, *Cancer Borealis*, is a well-characterized model system for studying the effects of neuromodulators on neural flexibility and stability. The neuropeptide Proctolin for instance, initially speeds up the pyloric rhythm, but fails to accelerate the cycle frequency beyond approximately 1 Hz. *We hypothesize that, while the rhythm appears unaltered by higher concentrations of Proctolin, the robustness of the motor pattern will increase instead.*

We performed *in vitro* intracellular recordings from the pyloric pacemaker neuron PD and perturbed it with randomly timed current injections. The response to perturbation was measured as a phase response curve to determine changes in pyloric cycle frequency. Our preliminary data indicate that higher concentrations of Proctolin increased pyloric motor pattern stability, independently of cycle frequency. This suggests that neuromodulators like Proctolin, can increase the robustness of motor patterns without affecting the speed and structure of the pattern.

Circadian and light driven modulation of rhythmic motor activity in the intact crab, *Cancer borealis*

Alexandra M. Yarger and Wolfgang Stein

Virtually all physiological processes vary rhythmically over time and it has been well established that such variations can occur in response to changing endocrine and neuromodulatory conditions. Circadian rhythms are controlled by internal biological clocks that regulate the timing of hormonal release and neuronal activity (Hastings *et al.* 2007, J Endocrinol, 195). However, the processes by which biological clocks affect the motor circuits driving these behaviors are not well described. We hypothesize that modulatory pathways cause long-term changes in neurons of motor circuits that mediate circadian fluctuations in activity. To test this, we are using the well-characterized gastric mill and pyloric motor circuits in the crab stomatogastric nervous system (Stein, 2009, J Comp Physiol A, 11). These rhythms drive processing of food in the crab foregut and are controlled by an identified neuromodulatory system.

We performed *in vivo* long-term extracellular recordings in three different light conditions (12h dark/12h light, 24h dark, or 24h light) to determine the frequency of the pyloric rhythm, the occurrence of the gastric mill rhythm and activity patterns of the motor neurons. Preliminary results suggest an absence of light and circadian influences on the pyloric rhythm, but revealed considerable interactions between pyloric and gastric mill rhythms.

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