

Biology (and Biology related collaborations) student abstracts 2014
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The Effect of Methoctramine on Acetylcholine Release in Toad Gastrocnemius Muscles

Amanda Babbin and Andy Gannon

Myasthenia Gravis, a skeletal neuromuscular junction disorder, can lead to limb and respiratory muscle weakness due to the production of antibodies that can degrade receptors for the neurotransmitter acetylcholine (ACh). There are five muscarinic receptor subtypes, M₁-M₅, for ACh on the presynaptic (nerve) membrane. The M₂ muscarinic receptor regulates the release of the neurotransmitter, ACh through negative feedback. Methoctramine is an antagonist of the M₂ muscarinic receptor. Therefore, we hypothesized that the blocking effect of methoctramine on presynaptic muscarinic receptors would enhance the release of ACh into the neuromuscular synapse. An enhanced amount of ACh in the synapse should increase the likelihood of ACh binding to receptors. Because Myasthenia Gravis victims have smaller numbers of ACh receptors, methoctramine could have therapeutic value. We treated the gastrocnemius muscle of Cane Toads, *Rhinella marina*, with increasing concentrations of methoctramine and observed the force of contraction and latent period of multiple nerve-stimulated contractions, since enhanced ACh release should affect these parameters. Our results showed no significant difference between the control muscle contractions and the contractions of the muscle treated with methoctramine, even though doses were up to two orders of magnitude greater than those used in previous experiments. The Cane Toad is apparently not an appropriate model organism for Myasthenia Gravis. It has also shown a lack of response to acetylcholinesterase inhibitors.

The Effects of Intraguild Predation on Growth and Metamorphosis of Salamander Larvae

Hank Ballard and Megan Gibbons

Intraguild predation occurs when individuals of different species engage in both competitive and predator/prey interactions. The intraguild predator benefits in two ways from this interaction: eliminating competition and gaining a meal. Understanding what types of impacts intraguild predation has on the species that are involved can help formulate different conservation models and shape preservation efforts. This study was designed to further explore the effects of intraguild predation on the growth rates and metamorphosis of intraguild predator and prey using two salamander species, *Ambystoma opacum* (intraguild predator) and *Ambystoma maculatum* (intraguild prey). We hypothesize that when these congeners occur together, they affect one another's growth rates and development. To test this hypothesis, I sampled individuals in three vernal pools containing either one or both species of salamanders. Snout-vent lengths of several individuals of both species were measured periodically throughout development to determine growth rates of the populations. Metamorphosis of *A. opacum* was determined based on diminishing larval abundance during sampling and presence of metamorphs under cover objects near the pools. The snout-vent lengths of the individuals from the different pools were compared to determine if there was a significant difference between the population's growth rates. The conclusion of this experiment will further the knowledge of the effects of intraguild predation on community species and help to structure community conservation efforts and eliminate local extinction of species.

Investigating the effectiveness of dental treatment provided by Cahaba Valley Healthcare

Arpan Bosmia, Megan Gibbons, and Edwina Taylor

Oral health is an integral component of one's well-being. According to the CDC, 2 out of 5 Americans ages 6-64 have untreated dental caries (tooth cavities), and poor oral health has been linked to coronary heart disease. We investigate the effectiveness of dental treatment provided by the non-profit organization Cahaba Valley Healthcare (CVHC) for underserved patients (patients not receiving sufficient dental care) in Alabama. CVHC wanted to determine whether their patients' oral health improved in the following areas after being treated: pain, sleep, embarrassment level, eating ability, and ability to work/find employment. When all patients were analyzed collectively, they improved in all areas except sleep. The same results were found when Jefferson County patients were analyzed separately. Shelby County patients improved for only pain and eating ability. CVHC appears to be providing effective treatment for its patients; however, further issues need to be investigated, which include: repeating this analysis with a larger sample size for Shelby County, examining CVHC patients' oral health in the long term, identifying potential benefits of fluoridating more of Shelby County water, the effect of using clinical assessments in addition to self-assessments, and investigating barriers to dental treatment.

Effects of Riparian Forest Buffers on Urban Stream Macroinvertebrate Biodiversity

Cameron Budzius and Andy Gannon

Urban development is needed to support growing populations; however, urbanization destroys natural habitats, leading to reductions in environmental quality and losses in biodiversity. It is well-documented that urban land use can negatively affect stream biodiversity. Urban streams are associated with impervious surface cover and inorganic pollutants and therefore these pollutants from surface water run-off are less filtered by the environment before it makes its way into the stream. In the present paper we study the effects of riparian forest buffers as a management tool to maintain stream macroinvertebrate biodiversity and water quality in urban streams of North Central Alabama. We hypothesized that streams with greater amounts of riparian forest buffer would exhibit higher levels of biodiversity and water quality than streams without buffer zones in urban areas. Sampling sites were selected on six streams in the Shades Creek Watershed using ArcGIS maps. With the use of a Surber sampler to collect macroinvertebrate samples, we measured taxa richness, taxa density, species evenness, and the Shannon diversity index of the benthic macroinvertebrates of the streams. At each stream, we also evaluated water quality by measuring stream depth, width, flow rate, turbidity, temperature, pH, and dissolved oxygen. Our results show that there is some correlation between macroinvertebrate population size and amounts of riparian forest buffers of urban streams. The results from water quality measurements suggest there is not one particular water quality measurement that determines macroinvertebrate biodiversity, but when looking at all water quality measurements together, there is some influence on biodiversity.

Analysis of the Effects of Continuous Intensive Trapping on the Catch Rates and Body Size of an Invasive Crayfish Population at Roebuck Springs

Corbin Burns and Dr. Megan Gibbons

Invasive species have a negative impact on local ecosystems. These impacts can arise from competition, predation, or hybridization, leading to the possible elimination of a native species from a habitat. An example of an invasive species is the crayfish *Orconectes virilis*, which may be a potential threat to both the endangered watercress darter (*Etheostoma nuchale*), and the native crayfish populations (*Cambarus striatus* and *Procambarus acutus*) at Roebuck Springs. We observed the impacts of intensive trapping of *O. virilis* individuals at Roebuck Springs in Jefferson County, Alabama. In order to eliminate the risks that the *O. virilis* population imposes upon this habitat, we continued an intensive trapping effort, which began in 2008, of this invasive species. With the use of continued intensive trapping efforts, we proposed that there should be observed decreases in both catch rates and body size of *O. virilis* individuals. The perceived effects of intensive trapping of *O. virilis* in Roebuck Springs can potentially provide a model for eliminating or reducing populations of invasive crayfish in other local ecosystems.

Constitutive and Induced Alkaloid levels of *Baptisia australis* and *Baptisia leucantha* Grown With and Without Rhizobia

Campbell Crocker and Peter Van Zandt

Plants have the capacity to defend themselves through constitutive or induced means relying on chemical or physical barriers to deter herbivores. Constitutive defenses are always present in the plant regardless of herbivory, and induced defenses are usually formed as a plants response to herbivory. These different types of defenses are all composed of a secondary compound known as an alkaloid. Alkaloids are developed in plants when excess nitrogen is available. Plants gain access to increased levels of nitrogen through the cooperation of nitrogen fixing bacteria. Nitrogen fixing bacteria such as rhizobia situate themselves in the root nodules of plants and then act as nitrogen factories to the plants. I chose to grow two different species of legumes known to produce alkaloids, *Baptisia australis* and *Baptisia leucantha*. To evaluate if there is a difference in alkaloid production in plants with nitrogen fixing bacteria in relation to without, I grew *Baptisia australis* and *Baptisia leucantha* with and without rhizobia and measured their levels of defense. I grew 78 of each species of plant, half of which were exposed to rhizobia. To measure constitutive levels of defense, half the plants were left unharmed. To measure induced levels of defense, *Anticarsia gemmatilis* damaged half the plants I grew 10%, 20%, 30%, 40%, and 50% of total leaf area. The alkaloids were then extracted using an acid-base protocol and assessed with the use of a gas chromatograph. This data will offer future experiments a proper protocol when the necessity for higher alkaloid containing plants must be grown.

Effects of the *geal* E643K Mutant on Septation and Drug Sensitivity in Fission Yeast

Ben Dickey and Melanie Styers

Golgi specific Brefeldin A resistance factor 1 (GBF1) is a guanine nucleotide exchange factor (GEF) responsible for protein trafficking between the endoplasmic reticulum (ER) and the Golgi, and is also required for replication of a subset of viruses, including hepatitis C and poliovirus. GBF1 acts by mediating the activation of ADP ribosylation factors (Arfs), which promote the formation of COP1 vesicle coat proteins and vesicle budding. However, there is still little known about the regulation of ArfGEF function, which is vital to understanding how viruses exploit the GBF1-mediated trafficking process for replication. Previous research found that cells expressing the haploinsufficient *geal* yeast ortholog of GBF1 exhibited sensitivity to GBF1 inhibitor Brefeldin A (BFA), and defects in the formation of septa. In order to establish a model system to further characterize ArfGEF regulation, we observed the effects of expression of *geal* E643K, a dominant negative mutant of the fission yeast ortholog of GBF1, on *Schizosaccharomyces pombe* sensitivity to BFA and septation. Spot assays suggested that both *geal* and *geal* E643K-expressing cells display resistance to BFA, compared to wild type cells. In addition, staining of cell septa with calcofluor revealed that *geal* E643K-expressing cells have a higher rate of septal defects than *geal* or wild type cells. These defects in cell septa suggest that ER-Golgi trafficking in these cells is impaired, where proteins responsible for the assembly, localization, and breakdown of the septum are unable to exit the rough ER.

Determining if the Hydroperiod of the Vernal Pool at Oak Mountain State Park is Sufficient for Breeding Amphibians

Hillary Dreyfus and Megan Gibbons

Vernal pools are seasonal wetlands that develop in permanent depressions. After a dry period, they begin to become inundated with water in the winter and inundation usually lasts through the spring. Amphibians use vernal pools during the breeding season to lay their eggs. Vernal pools are often the preferred habitat for amphibian breeding because they provide an environment for the eggs to hatch and larvae to metamorphose without the threat of fish predation. Vernal pools are important for biodiversity and the destruction of these habitats could cause local extinction of species. The hydroperiod, the time period for which the basin is inundated with water, can affect the success of reproduction and metamorphosis of amphibian larvae. This study investigates the hydroperiod of a vernal pool at Oak Mountain State Park in Pelham, AL to determine if it is sufficient to support the community of amphibians that breed there. The timing of inundation and drying of the pool were recorded and the water level, pool width, and pool depth were periodically monitored and recorded throughout the hydroperiod. The area in and around the pool was sampled periodically for amphibian species throughout the hydroperiod in order to determine the arrival of breeding species to the pool if they were able to successfully complete metamorphosis before the pool dried. Understanding the hydroperiod of a specific vernal pool and the breeding seasons of its inhabitants can provide insight into conservation and management strategies for temporary breeding habitats.

Regulation of vacuolar fission and morphology by *lvs1* and *ypt4* in *Schizosaccharomyces pombe*

Kaitly A. Dorsett and Melanie L. Styers

The pathways that regulate vacuolar homeostasis in fission yeast have yet to be fully characterized. However, vacuole size is known to depend on proper regulation of fission and fusion. We have identified two proteins involved in regulation of vacuole size: *ypt4*, a Rab4 homolog, and *lvs1*, the ortholog of the human *LYST* gene. When the vacuoles were stained with fluorescent dye MDY-64, both *ypt4* Δ and *lvs1* Δ cells were observed to have an increase in vacuole size. *LYST* has been identified as the mutated gene in the human disease Chediak-Higashi Syndrome (CHS). This disease has been characterized as a lysosomal disorder, in which cells exhibit enlarged lysosomes, the human equivalent of the yeast vacuole. Thus, these proteins may regulate a pathway of clinical significance. Here, we propose that *ypt4* and *lvs1* coordinately regulate vacuole size in fission yeast. After osmotic shock, *ypt4* Δ and *lvs1* Δ cells exhibited vacuolar fusion, similar to the control cells. However, when the cells were allowed to recover, both deletion strains exhibited a delay in vacuole fission. These results implicate impaired vacuole fission as a cause of the increased vacuole size in *ypt4* Δ and *lvs1* Δ strains. Most importantly, when *lvs1* is overexpressed in *ypt4* Δ cells, the vacuolar size defect is rescued. Based on these results, we suggest that *lvs1* and *ypt4* work together in the same pathway to regulate vacuolar morphology in fission yeast cells. To further test this hypothesis, we plan to assess the effects of expression of GFP-tagged *ypt4* in *lvs1* Δ cells on vacuolar size. These studies are currently in progress.

Threshold of salinity change and location of osmoreceptors for physiological response in the Blue Crab, *Callinectes sapidus*

Stephanie Esman and Andrew Gannon

Callinectes sapidus, the Blue Crab is an efficient osmoregulator that can survive in freshwater and hypersaline conditions. Blue crabs osmoregulate at hyposaline conditions and osmoconform at hypersaline conditions. When exposed to hyposaline conditions the blue crab has an initial increase of heart and ventilation rates. Blue crabs are able to adapt to various levels of salinity shown by their ability to migrate from saltwater to freshwater and back. Crustaceans respond to changes in salinity, ranges of 25% seawater (SW) to 100% SW but it is not known if a smaller degree of change of salinity can trigger physiological response. Osmoreceptors detect changes in salinity, locations of these vary among species and appendages of crustaceans. This study will determine the threshold of salinity change that triggers physiological responses in the Blue Crab. This study will also find the location of osmoreceptors by looking at heart and ventilation rate of the crabs when they are exposed to concentrations of salinities (1100mosm, 900mosm, 700mosm, and 500mosm) at those sites. The crabs were acclimated to 100% SW (1100mosm) for two weeks. Heart rate and ventilation rates were collected for 30minutes using an impedance converter. The threshold for physiological response was found to be 700mosm with $p=0.03$ for heart rate and $p=0.02$ for ventilation rate. The location of the osmoreceptors was found to be in the gill chamber with significance for heart rate at 1 minute $p=0.03$ and at 1.5minutes $p=0.05$.

Assessing the Efficacy of the Deworming Protocol of Birmingham Jefferson County Animal Control for Dogs

Jeremy Evans, Andy Gannon, and Nicole Metcalf

To maintain the health of pets and reduce the spread of infectious parasites, the efficacy of veterinary treatments must be assessed. Gastrointestinal parasites are common among canines, with tapeworms, hookworms, whipworms and roundworms being among the most prevalent. These parasites are frequently passed to humans via zoonotic transference, and this danger makes the assessment of deworming procedures a high priority for not only animal health, but also human health. The canine deworming treatment of the Birmingham-Jefferson County Animal Control (BJCAC) was evaluated by analyzing fecal samples from before deworming treatment, 5 days after, and 10 days after with the McMaster technique to calculate eggs per gram. Hookworm eggs were the most common, found in approximately 80 percent of the animals examined. The least common were tapeworm eggs, with none found. By ten days after deworming, the parasite burden was almost completely eliminated by the treatment in nearly every dog. Typically, all whipworm eggs were eliminated after treatment, and almost all hookworm eggs were removed. A mean of approximately 2/3 of roundworm eggs were eliminated by the treatment after 10 days. In two different animals, coccidian protozoa were found in the fecal samples. On multiple occasions, parasites not seen in the pre-deworming sample were identified in fecal samples after treatment occurred, indicating a possible infestation within the clinic. While on average, worm burden removal was nearly complete 10 days after treatment, slightly more than half of the examined animals were left without any worm burden.

The potential impact of artificial bias on taxonomy and biodiversity at a fossil human site, Sterkfontein, South Africa

Ahmer Festok and Jason Heaton

Cave Taphonomy presents a unique set of difficulties for identifying how bones and other fossils were interred, either as a result of carnivore or hominid activity, or from the complex nature of the cave's structure. Taxonomists must recognize when discussing faunal biodiversity the necessity of taking into account the formation of the assemblage. They must observe and identify any potential bias present in the fossil collection. The purpose of the study is to analyze the Sterkfontein fossil primate sample for clues about its deposition and collection. It is hypothesized that there will be a significant difference in the pattern of bones collected at Sterkfontein to the pattern of known bone collectors. In the present paper we observed the differences between the pre-1966 and post-1966 Sterkfontein collections, with a total estimate of 3000 skeletal elements. We then categorized the skeletal elements into discrete categories and computed the MNI and NISP. The Sterkfontein collection was compared to the assemblages accumulated by non-human bone collectors, such as leopards, porcupines, and hyenas. There appears to be a significant difference between the pre-1966 sample and post-1966 sample of the fossil assemblage. In addition, there appears to be a significant difference between the fossil assemblage that we observed to that of hyenas, leopards, and porcupines.

Identifying morphometric variation of the hand phalanges of anthropoids

Muhamad Festok and Jason Heaton

The hand and foot phalanges of primates (including gorillas, chimpanzees and baboons) show strong similarities in morphology. Hand and foot phalanges are often recovered during digs in sites like Swartkrans located in South Africa. Due to morphological similarities, identifying the species of primate based on a recovered phalange can be challenging. Our goal is to identify any morphometric variance in the phalanges of the hand and foot of primates. We hypothesize that there will be a morphological difference between the phalanges of the various primates. Modern primate phalanges from the Smithsonian National Museum of Natural History were photographed in three views for analysis. The Various measurements such as distal width, proximal height, mid-shaft width, etc. of the proximal and intermediate phalanges were measured and collected using a computer program called MorphoJ. A Fourier analysis was used to identify the variation in the primate phalanges. This analysis identifies features that are useful in discriminating the various primates in a mixed sample. There appears to be a common trend in the variation phalange morphology of the various primates.

Size of foramen lacerum in relation to cranial measurements in Primates

Shay Gibson and Jason Heaton

The foramen lacerum is the only opening in the human skull through which nothing passes and in the living person, is filled with cartilage. However, little research has been completed on its origin, and its purpose is not known. Because little is known about the foramen lacerum, putting it in an evolutionary context could be helpful in understanding its function. This project focuses on researching the foramen lacerum within an evolutionary context. The cranial length, breadth, and height were measured for a primate sample and correlated with the size of the foramen lacerum. The primate sample researched included humans, chimps, baboons, gorillas, mangabeys, and howler monkeys. The results showed a significant correlation in the size of the foramen lacerum compared to cranial length and breadth.

Longleaf pine restoration: establishing and verifying control plots at Oak Mountain State Park

Sarah Gilkerson and Scot Duncan

This research focused on establishing and evaluating control plots for montane longleaf pine (*Pinus palustris*) restoration at Oak Mountain State Park. Relationships between slope position and plot treatment were also evaluated. Eight control plots were established and data was taken regarding forest structure and composition. In order to determine equivalency between control and experimental plots, two-one-sided t-tests were performed (alpha level 0.1). For the number of trees the set of plots were equivalent for the overall longleaf community, the upland community, and six tree species. For diameter at breast height, Blackjack Oak (*Quercus marilandica*), Northern Red Oak (*Quercus rubra*), and Red Maple (*Acer rubrum*) were found to be equivalent between treatments. Neither a statistically significant equivalence nor difference was found for canopy or midstory coverage. Interactions between slope and treatment were detected using a two-way ANOVA with an alpha level of 0.05. The only instances of treatment/slope interactions was for the count of loblolly pine (*Pinus taeda*) and Pignut Hickory (*Carya glabra*) and the diameter of Pignut Hickory. Overall, it was determined that the plots will be good controls as they have high levels of equivalency and low treatment/slope interactions.

Effects of inhibition of the guanine nucleotide exchange factor *gea1p* on membrane trafficking in fission yeast

Cory Gonzalez and Melanie L. Styers

Golgi specific Brefeldin A Resistance factor 1 (GBF1) is a human guanine nucleotide exchange factor (GEF) that has been linked to the replication of many RNA viruses, including Ebola. It is also a member of the Sec7 family of GEFs. GBF1 has been shown to operate by driving ADP-Ribosylation Factor 1 (ARF1) GDP/GTP exchange, which allows ARFs to recruit COPI complexes required for membrane trafficking. COPI is a coat protein complex known to operate between the Endoplasmic Reticulum (ER) and the Golgi. COPI drives vesicle formation by regulating the incorporation of cargo proteins into the developing vesicle. Fission yeast is a model organism that has a single GBF1 ortholog known as *gea1*, and deletion of *gea1* is lethal in yeast cells. *Gea1* has been suggested to regulate COPI-dependent trafficking in fission yeast, just as GBF1 does in humans. In this study, I created a yeast strain that expresses a dominant negative allele of *gea1*, known as *E643K*. We hypothesized that this *gea1* mutant would inhibit ARF activation, which would in turn block recruitment of COPI and secretory traffic. To test this hypothesis, secretion of acid phosphatase, a marker for the general secretory pathway, was measured for control cells, cells expressing wild-type *gea1*, and cells expressing *E643K*. Results from this analysis showed that strains overexpressing *E643K* and *gea1* inhibit acid phosphatase secretion equally relative to the control strain. These observations suggest that overexpression of wild-type or mutant *gea1* inhibits secretory traffic.

The Role of Heat Shock Proteins in Acquired KP1019 Resistance in Yeast

Evan Grovenstein and Pamela Hanson

Heat shock proteins, also known as HSPs, are some of the most abundant intracellular proteins found in cells. They are present during normal cellular function, but are expressed in abundance when cells are exposed to sudden increases in temperature or other cellular stresses. Elevated HSP expression has been correlated with acquired resistance to various chemotherapies. KP1019 is an anticancer drug that has been shown to cause a decrease in number of colorectal tumor cells. A mild heat shock was administered to yeast cells to increase HSP expression and was then given KP1019 to test for resistance to KP1019. Also, yeast cells were pre-exposed to mild doses of KP1019 and then heat shocked for various lengths of time to test for resistance to increased temperatures. It was discovered that a mild heat shock does not cause any significant resistance to KP1019, but a mild KP1019 pre-treatment does cause significant resistance to increased temperatures.

KP1019 derepresses HSF1 transcriptional activity in *Saccharomyces cerevisiae*

Alex Hunsucker and Pamela Hanson

The heat shock response (HSR) is a highly conserved cellular reaction to proteotoxic stress that results in the rapid induction of molecular chaperones known as heat shock proteins (HSPs). In the budding yeast *Saccharomyces cerevisiae*, thiol-reactive molecules modify the cysteine residues of the Hsp70 chaperone Ssa1, resulting in derepression of the heat shock transcription factor HSF1, which mediates the HSR by binding to heat shock elements (HSEs) within promoter regions of heat shock genes. Previously unpublished research showed that indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)] (KP1019), a ruthenium-based anticancer agent, also induces transcription of HSPs. Given that ruthenium (II) complexes, such as the activated form of KP1019, have a thermodynamic preference for binding to sulfur-donating ligands, it was hypothesized that KP1019 modifies Ssa1 cysteines, activating the HSR. Thus, the objectives of this study were to determine if KP1019 activates the HSR by examining HSE-*lacZ* induction by KP1019 and to determine if KP1019 is a thiol-reactive compound by using UV-vis spectrophotometry to examine the reaction of KP1019 with cysteine. The observed derepression of HSF1 transcriptional activity by KP1019 corroborates the preliminary finding that KP1019 induces transcription of HSPs; however, the results regarding the mechanism by which KP1019 activates the HSF1-mediated HSR were inconclusive. Moreover, the findings suggest that KP1019 is not a thiol-reactive compound under *in vitro* conditions; despite this, the drug could exhibit thiol-reactivity under *in vivo* conditions.

Identification of Single Nucleotide Polymorphisms in Genes Related to Imatinib Mesylate Therapy in Patients with Neurofibromatosis Type 1

Katie Hutchins, Andrew Gannon, and Michael Ferguson

Imatinib mesylate, a tyrosine kinase inhibitor, is currently being tested in clinical trial as a treatment for patients with Neurofibromatosis Type 1 (NF1). In other diseases employing imatinib for treatment, certain single nucleotide polymorphism (SNP) mutations have been linked with resistance to imatinib. Our goal was to determine if these SNPs are present in NF1 patients and if so, reveal any correlations with imatinib resistance. We hypothesized that the presence of one or more variant alleles in any SNP studied would correlate with one or more of the disease outcomes evaluated. Using NF1 patient frozen serum samples from the Imatinib Clinical trial, DNA was extracted, quantified, and concentrated. SNP genotyping was run using primers for 20 SNPs in drug transporter, receptor, and metabolism genes associated with imatinib function. The genotypes were then correlated with four clinical responses to imatinib, or disease outcomes. All patients with the variant allele of *CYP2C8**3 underwent dose reduction during clinical trial, $p = 0.01$. The variant allele presumably caused reduced metabolism of imatinib, leading to toxicity that necessitated dose reduction. All patients with wildtype *ABCB1* genotypes were able to receive full dose imatinib, $p = 0.04$. More toxicity was associated with *3 variants of *CYP3A5*, although this was not statistically significant. The long-term goal of this project is to run multivariable analyses on the genotyping results and create risk prediction scores for imatinib failure. These scores would enable treatment personalization to determine the likelihood of successful imatinib therapy for the treatment of plexiform neurofibromas.

Habitat preference of the invasive crayfish (*Orconectes virilis*) in relation to habitats used by the endangered watercress darter (*Etheostoma nuchale*)

Kayla King and Scot Duncan

The Southeast is home to the greatest freshwater biodiversity in the nation. There are many threats to these species, one being the introduction of invasive species. The crayfish, *Orconectes virilis*, may be negatively impacting the watercress darter, *Etheostoma nuchale*, an endangered Alabama fish species, found in only five springs within Jefferson County. Both live in habitats of dense vegetation. Research conducted in the spring season found no differences in habitat preferences by crayfish. This autumn study analyzed the relationships between crayfish capture frequencies, sizes, and sex ratios between different habitats to determine if there is a relationship between darter and crayfish densities across habitats. To better understand habitat preference, crayfish were trapped during autumn, in five habitats of Roebuck Spring. This spring contains both *E. nuchale* and *O. virilis*, and is home to the largest known watercress darter population. Based upon previous results, we hypothesized there would be no frequency differences among the darter habitats, and no correspondence of habitat preferences between *E. nuchale* and *O. virilis*. *A. philoxeroides*, had significantly more crayfish in autumn than spring but no other seasonal differences were detected. *C. demersum* had a significantly smaller crayfish population when compared to *M. aquaticum*, *S. americanum*, and the silt habitat. Sex ratios were similar in all but the silt habitat, where there were significantly more females than males. These findings may lead to a better understanding of the relationship between the invasive crayfish and darters, which may inform attempts to protect and expand the darter's population.

Restoration of the Montane Longleaf Pine (*Pinus palustris*) Woodlands: The Effects of Fire Intensity on Composition and Structure.

Ryan Laughlin and Scot Duncan

The longleaf pine (LLP) ecosystem once occupied 37 million hectares across the Southeast; due to commercial logging, lack of replanting, and interruption of natural fire cycles, only 1 million hectares remains. LLP ecosystems, including montane LLP (MLLP) ecosystems, depend upon frequent, low-intensity fires for structure, function, and composition. Managers of Oak Mountain State Park in Pelham, Alabama have recently reintroduced fire into portions of the MLLP ecosystem as part of a restoration study. Two prescribed fires burned different units of the park with unintentional intensity differences; the Campground Unit (CGU) had a more intense burn in 2008 than the Lake Hills Unit (LHU) in 2009. This study investigates whether these fires differentially affected forest composition and structure, if scorch mark heights (SMHs) on the bark of trees can be used to predict changes in forest structure and composition, and whether slope affects these changes. SMHs and vegetation cover (before/after the burns) were measured for every tree within three 50-m transects, or subplots on the shoulder, mid, and toe slopes of the ridges in three plots, one in LHU and two in CGU. SMHs for LLP trees were higher in CGU than LHU. SMH in CGU increased with increasing slope for all trees, and SMHs were significantly higher on the mid-slope than the toe or shoulder slopes. A strong, positive relationship between SMHs and mortality of invasive hardwood species was observed. These results could provide information concerning optimal fire intensities that could help restoration managers eradicate invading trees in LLP woodlands.

Determining the distinct functions of *sec71p* and *sec72p* in membrane trafficking in fission yeast

M'Kayl Lewis and Melanie Styers

Autosomal recessive periventricular heterotopia with microcephaly (ARPHM) is a rare inherited developmental brain abnormality resulting from mutations found in the ADP-ribosylation factor guanine nucleotide-exchange factor-2 (ARFGEF2) gene, which encodes the BIG2 protein. This ARFGEF2 mutation affects the production and migration of neural stem cells during development as a result of a disruption in membrane trafficking. BIG2, as well as its homolog BIG1, activates ADP-ribosylation factors, or ARFs, by catalyzing the release of GDP and binding of GTP, which drives vesicle formation in membrane transport. Although previous studies suggest BIG1 and BIG2 have distinct functions, their specific roles remain unclear. Thus, the goal of this study was to establish those distinct functions using *sec71p* and *sec72p*, proteins in *Schizosaccharomyces pombe* homologous to BIG1 and BIG2. Strains heterozygous for *sec71* or *sec72* were analyzed for potential blocks in endocytosis. Cells were incubated with the fluorescent dye FM4-64 in order to trace the endocytic pathway. Results from confocal fluorescence microscopy of FM4-64 showed that endocytosis was slowed, with the dye reaching the vacuole by 15 minutes in wildtype cells, compared to 30-60 minutes for the *sec71(+/-)* and *sec72(+/-)* strains. Flow cytometry also revealed a delay in uptake of FM4-64 in *sec71(+/-)* and *sec72(+/-)* cells. Together, these results suggest that both *sec71* and *sec72* play an important role in endocytosis, which may provide insight into the functions of BIG1 and BIG2 and the specific membrane trafficking disruptions associated with ARPHM.

Second to Fourth digit (2D:4D) ratio in relation to age at onset of puberty

Cody Mann and. Jason Heaton

The ratio between the second and fourth digits (2D:4D) is negatively correlated to prenatal and adult levels of testosterone. Current research has also shown that among a single ethnic group that 2D:4D ratio is a sexually dimorphic trait. Dissimilarities between male and female 2D:4D digit ratios in humans have been found in recent studies and others that date back to the late 1800's. Lower digit ratios suggest higher levels of testosterone and lower levels of estrogen, confirming that on average males have a lower digit ratio than females. The study of 2D:4D ratios are linked with many different types of diseases such as diabetes, autism, depression, and many others. With this it is also found that over time, individuals are starting puberty earlier than previous generations. By looking at the digit ratios and age at onset of puberty, we will confirm a lower digit ratio is correlated with earlier onset of puberty in both males and females.

Determination of the effect of enhanced green fluorescent protein on enzyme activity and tetramer formation of *D. viviparus* acetylcholinesterase and human butyrylcholinesterase

Claire McIlwraith and Leo Pezzementi

Butyrylcholinesterase can act as a bioscavenger which protects against organophosphate (OP) agents like sarin gas. When OP toxins are present, acetylcholinesterase is inhibited leading to toxic symptoms; intravenous BChE functions to sequester and neutralize these toxins preventing AChE inhibition. However, BChE would have to be synthesized *in vitro* because large amounts of purified human BChE required for this technique limits its commercial availability. Synthesis of the enzyme in its soluble tetrameric form would be most beneficial due to an increased residence time. The structure of these soluble tetramers *in vivo* is promoted by a poly-proline sequence of lamellipodin (Lpd). When the mechanism for this assembly was studied *in vitro*, we found that the expression of the full length Lpd sequence via the Lpd-pEGFP-N1 vector caused a decrease in enzyme (nematode AChE and huBChE) activity as well as tetrameric formation. Further testing and research directed us to propose that EGFP, enhanced green fluorescent protein, is responsible for the decrease in enzyme activity. We performed site directed mutagenesis to insert a stop codon into the Lpd-pEGFP-N1 vector between the sequences coding for lamellipodin and EGFP. After confirming the mutations with DNA sequencing, we will co-transfect COS-7 cells with the mutated vector and the cDNA of ChE, harvest the enzyme, and perform an Ellman's esterase assay to determine the enzyme level. Finally, we will use velocity sedimentation on a sucrose gradient to separate the molecular forms of the enzyme present in order to determine the proportion of tetramers formed.

The effects of pond desiccation on growth and development of *Ambystoma opacum* (marbled salamander)

Moya Moore and Megan Gibbons

Concerns about the negative impacts of humans on the natural world are steadily growing, and detecting local degeneration of the environment is paramount. Animals with complex life cycles frequently serve as indicator species, due to their susceptibility to environmental conditions. An amphibian's larval habitat may dictate whether it survives to metamorphosis as well as, the length of time takes to metamorphose, its body size at metamorphosis, and its morphology after metamorphosis. For example, a pond that dries quickly may lead to early metamorphosis, small body size at metamorphosis, and small post-metamorphic body size, which has been linked to reduced fitness and increased mortality. We tested these predictions in the laboratory with larvae of the marbled salamander, (*Ambystoma opacum*) under rapid and slow desiccation rate treatments. We hypothesized that the larvae in the rapid desiccation rate treatment would reach metamorphosis earlier and at a smaller body size than the larvae in the slow desiccation rate treatment group. We compared the effects of desiccation rate on growth rate, time to metamorphosis, and body size at metamorphosis with t-tests and a MANOVA. We expected to see that salamander larvae living habitats with a slow desiccation rate would reach metamorphosis later, have a larger body size at metamorphosis, and have a longer SVL (mm) at metamorphosis than larvae in habitats with rapid desiccation rates. Studying the effects of these factors can give us insight into management and conservation strategies for protecting amphibians and their habitats.

The establishment and analysis of spatial control plots at Oak Mountain State Park to determine the effects of restoration burns on the montane longleaf ecosystem

Abigail Morrison and Scot Duncan

The longleaf pine (*Pinus palustris*) ecosystem of the Southeast is endangered. It is dependent upon low-intensity fire to keep the understory relatively clear, the organic soil layers minimal, and for some understory plants reproduction. Wildfire suppression has allowed hardwood trees to invade and outcompete many species native to the longleaf systems, shading the understory and deepening and wetting the organic soil layers. Many attempts to restore the longleaf system occurred through prescribed and controlled restoration burns,. Most studies of restoration burns are from the coastal plain; little is known about the montane longleaf ecosystem. A study of montane longleaf pine restoration at Oak Mountain State Park is investigating how prescribed fire affects fuel, soil structure, and forest composition and structure. While data are collected from before and after burns, until now the study has lacked spatial control plots. The 25-meter long spatial controls were established in adjacent locations that have not been burned in many decades and were described using the variables. The resulting data was examined using statistical analysis, primarily a two-tailed t-test to check for equivalence (TOST) between the control plots and the treatment plots. Preliminary results indicate that the spatial control plots identified are not equivalent to the treatment sites, and cannot be used for comparison for all variables analyzed in the study. Another plot should be identified as a potential spatial control to be used to study the affects of the controlled burns at Oak Mountain State Park.

Effects of KP1019 on *PDR5* and *TKL2* Expression in *Saccharomyces cerevisiae*

Nikki Patel and Pamela Hanson

Indazolium *trans*-[tetrachlorobis(1*H*-indazole) ruthenate(III)], also known as KP1019 or FFC14A, is a ruthenium-based anticancer agent capable specifically targeting and killing tumor cells while being less toxic to normal, healthy cells than platinum-based anticancer agents. Additionally, KP1019 is able to overcome cellular drug resistance encountered with the use of platinum-based drugs for treatment of certain cancers. With promising activity against some carcinomas, it is imperative to understand the mechanism by which KP1019 functions. Since many basic biological pathways are evolutionarily conserved between yeast and mammals, the budding yeast, *Saccharomyces cerevisiae*, was used as our model organism. We examined KP1019's effect on the induction of yeast genes involved in the cellular stress response, such as *PDR5* and *TKL2*. Using a permeabilized cell assay and a *PDR5-lacZ* reporter construct, we confirmed *PDR5* was induced in wild-type yeast in response to KP1019 treatment. Then by subjecting the wild-type and *stb5* Δ strains to a KP1019 dose response treatment, we concluded *STB5* does contribute to the regulation of *PDR5* expression. We next hypothesized *STB5* contributed to the regulation of *TKL2* in *S. cerevisiae* in response to KP1019 treatment. Marking *TKL2* with green fluorescent protein (GFP), we used flow cytometry to measure the expression of this gene in response to KP1019 treatment. Our data suggests that the deletion of *STB5* does not completely inhibit the induction of *TKL2* in yeast treated with KP1019. With a better understanding of cellular response to DNA-damaging agents, such as KP1019, researchers may develop improved drug treatments for various forms of cancer.

Do Pik1p and Gea1p Form a Positive Feedback Loop in Fission Yeast?

Ross Pearlman and Melanie L. Styers

From intracellular and intercellular signaling to disposal of waste products, membrane trafficking plays many critical roles in the function of a cell. Fundamentally, proteins working together to package cargo into transport units called vesicles drive membrane trafficking. Vesicle formation at the Golgi is initiated by PI4K, which produces phosphatidylinositol 4-phosphate (PI4P). PI4P then recruits GBF1, which activates ARFs to recruit vesicle coat proteins to the membrane. Coat proteins recruit cargo into the forming vesicles, preparing the newly formed vesicle to transport its contents. To characterize the process of rapid protein recruitment for vesicle formation, we have used *Schizosaccharomyces pombe* as a model system to study the yeast protein orthologs of PI4K (*pik1p*) and GBF1 (*gea1p*). We hypothesized that *pik1p* and *gea1p* form a positive feedback loop that drives increased recruitment of *gea1p* to the Golgi membrane and increases activity of *pik1p*. We used spot assays to determine whether cells expressing the kinase-dead *pik1-D709A* mutation inhibited the function of *gea1p*, resulting in brefeldin A (BFA) sensitivity. Spot assays indicated that cells expressing *pik1-D709A* were not consistently sensitive to BFA. Next, we tested PI4P levels in cells expressing a *gea1* dominant negative mutant (*gea1-E643K*) to assess whether *pik1p* activity had been affected. Our results showed no significant difference between the amounts of PI4P produced by cells expressing *gea1-E643K* versus the wild-type control. Based on these results, it remains unclear whether a *gea1p-pik1p* feedback loop is present in fission yeast.

Melatonin Effects on Heart Rate and Ventilation in *Procambarus clarkii*

Eric Pinheiro and Andrew Gannon

The hormone melatonin signals circadian rhythm, physiological responses to the differences between light and dark periods, but its exact function varies greatly between organisms. In animals, melatonin secretion typically occurs at night, and is often associated with decreased physiological activity during sleep. Tachycardia, however, is an acute side effect of supplemental melatonin in humans. Prior research has revealed the function of melatonin in crustaceans is varied, such as antioxidation and the regulation of limb regeneration rate. Previous papers on crustacean physiology have yet to make a connection between melatonin concentration and heart rate and ventilation. Impedance conversion may be used to detect the heart beat and scaphognathite movement in crustaceans. This paper demonstrates this technique used to monitor the heart rate and ventilation in *Procambarus clarkii*, with varying concentrations of melatonin in the hemolymph. Acclimation of crayfish to darkness causes them to enter a free-running endogenous circadian rhythm. After recording for 24 hours under these conditions, injection of melatonin during times when endogenous levels are low reveals a potential increase in mean heart and respiratory rates over a 6 hour period, contrary to melatonin studies on other species that show a decrease in these rates.

Investigation of Selected Moth Species for Presence of Pollen on Mouth Parts and Proboscides as a Measure of their Potential Role in Pollination

William Pitts, Wayne Shew, and Peter Van Zandt

The interaction between flowering plants and animal pollinators has long been an area of keen interest for biologists. The mutually beneficial relationship between diurnal insect pollinators and plants is well documented, but the role of nocturnal insect pollinators is not as well studied or understood. Previous studies have shown that some species of moths regularly visit and pollinate flowers, but the full extent of the involvement of nocturnal moths in pollination is not known. We examined six species of moths housed in the BSC moth collection, 14-20 specimens of each species, to determine if they had pollen grains on their proboscides and mouthparts. The species examined were *Mythimna unipuncta*, *Darapsa myron*, *Catocala amica*, *Spodoptera ornithogalli*, *Anicla infecta*, and *Marimatha nigrofimbria*. Each specimen examined had its mouth parts and proboscis swabbed with a cube of glycerin jelly containing basic fuchsin stain. The glycerin jelly cube was placed on a microscope slide, a coverslip was added, and the glycerin jelly was then melted. The slide was examined at 400x magnification and any pollen grains present were counted and identified to the lowest taxon possible. Previous studies proposed that moths carrying five or more pollen grains on their mouthparts should be considered plant visitors, and if 20% of the specimens of a given species have five or more pollen grains on their mouthparts, then the species should be considered a regular plant visitor and potential pollinator. The species *Catocala amica*, *Spodoptera ornithogalli*, and *Anicla infecta* were found to be plant visitors and potential pollinators.

Outcome of Aggressive Interactions between One Invasive and One Native Species of Crayfish at Roebuck Springs, Alabama.

Morgan Pullen and Megan Gibbons

Invasive species are not native to and have a negative effect on an ecosystem. Invasive species are often successful because of competitive advantages that they have over native species in the new ecosystem. *Orconectes virilis*, a crayfish species invasive to the Southeastern United States, is extremely common in a stream in Roebuck Springs in Birmingham, Alabama. Two native species of crayfish, *Procambarus acutus* and *Cambarus striatus*, also occupy the spring, but in relatively low numbers compared to *O. virilis*. The purpose of this project was to determine if differences in aggression could explain the displacement of the indigenous species of crayfish at Roebuck Springs. Specifically, I ran competition trials between the invasive species *O. virilis* and the native species *C. striatus*, to determine if *O. virilis* was more aggressive. All crayfish were collected from Roebuck Springs. The competition trials were performed between same size/sex pairs of *O. virilis* and *C. striatus*. Pairs were placed into a testing arena and the time that each crayfish spent in various aggressive behaviors was recorded. If invasive species are typically more aggressive than native species of crayfish, then I expected that *O. virilis* would show more aggressive behavior than the native species in competition trials. Preliminary results do not support this expectation. The results of this project could be used to better understand the dynamics between invasive and native species in Roebuck Springs.

Content of polyphenols in leaves of select species of *Camellia* and cultivars of *C. japonica*

Lauren E. Smith and Wayne Shew

Determination of total polyphenol content and antioxidant activity of the tea plant, *C. sinensis*, has been investigated by numerous researchers. Why is *C. sinensis*, but not other species in this genus, used for making tea? One possible reason is that *Camellia* species differ in levels of production of secondary metabolites that make them less flavorful and aromatic, or cause them to differ in their physiological effects. This study seeks to determine if the content of polyphenols differs between species of *Camellia* and between varieties of one particular species, *C. japonica*, flowering *Camellia*. A positive correlation between the antioxidant properties of extracts from *C. sinensis* leaves and their total polyphenol content have been shown in previous studies. However, minimal studies have examined the total polyphenol content and antioxidant activity of species of *Camellia* other than *C. sinensis* or cultivars of the species *C. japonica*. We hypothesize that there will be significant differences in the content of total polyphenols present in the leaves of *C. sinensis* compared to other species of *Camellia*. We also predict that there will be no significant differences in the content of polyphenols in leaves from different cultivars of *C. japonica*. A convenient, rapid, and simple in vitro colorimetric procedure, the Folin-Ciocalteu (F-C) assay, was used to estimate the total phenolic content in fresh leaf samples obtained from *Camellia* plants growing at the Birmingham Botanical Garden. Preliminary results indicate significantly higher polyphenol content in leaves of *C. sinensis* compared to leaves from other species of *Camellia*.

The effect of fire intensity longleaf pine (*Pinus palustris*) woodlands at Oak Mountain State Park, Pelham, AL

Grant Talbert and Scot Duncan

Longleaf pine (*Pinus palustris*) woodlands once covered over 37 million ha of the Southeast. Longleaf pines depend on fire for preventing competitive, fire-intolerant trees from establishing and out-competing them. Fire suppression has eliminated the natural fire regime that once allowed longleaf to flourish. Oak Mountain State Park (OMSP), Alabama harbors a remnant of the mountain longleaf pine woodlands. Prescribed fires are being used in a portion of OMSP to restore the longleaf woodland. Part of the restoration includes replacing the nonnative fire-intolerant species with the native fire-tolerant species. An ongoing project at the park uses data from before and after each of the fires to improve restoration efforts. The project evaluates the vegetation cover, soil layers, tree composition, and fuel levels in the campground and lake hills units of the park. The initial campground fire seemed to be very intense while the lake hills fire was of low intensity. We expected corresponding differences in scorch mark heights on trees, tree mortality, and forest composition between the two sites. Scorch mark height were higher in the campground unit. However, between the two units there was no significant difference in mortality between the native and nonnative tree species. Regressions between growth after the fire and scorch mark height show that fire intensity has a negative effect on growth after the fire. In summary, fire intensity had a large effect on the longleaf woodland and a better understanding of this can lead to more successful restoration efforts in the future.

Reduction Sequences and Skill in Naïve Flintknappers

Lillie Teague and Jason Heaton

Many animals use different techniques for survival. Apes, such as modern chimps, have been known to use many different types of tools for survival including stone tools. With this information, we can infer that their ancestors used tools as well, although very little record exists. This mode of adaptation opens up new food resources that were not previously available to apes. Stone tool technique relies heavily on the steps used by the flintknappers, or tool-makers, because this determines how useful the tools are and also their potential functions. In this experiment, we aim to determine how naïve knappers, or participants with no previous knowledge, vary in their approach to construct a stone handaxe. As a tool type, handaxes first appear approximately 1.8 million years ago (1.8Ma, or mega annum) and were constructed by our ancestors with brains over 25% smaller than the modern human. However, they require technical skill to produce. Based upon previous research, we expect to find that naïve knappers will work one end of the tool, preferentially the end farthest from the toolmaker. If this hypothesis is supported, then this information may be used to further understand the origins of the shape, or morphology, of early stone tools found in the early Paleolithic, such as Gona, Ethiopia (ca. 2.6Ma).

Prevalence of Feline Gastrointestinal Parasites in the Birmingham-Jefferson County, Alabama Area

Caroline Wilder and Andrew Gannon

Gastrointestinal (GI) parasites in stray cats pose health risks for domestic cats as well as humans. Parasites can be transmitted through fecal exposure from infected hosts to other healthy animals and humans. Stray cats are a considerable threat for spreading GI parasites because their infections do not get treated. Veterinarians should know which feline parasites in their locality are the highest GI parasite risk factors for domestic cats in order better treat local animals. However, veterinary availability is not uniform and the number of veterinarians per capita varies between municipalities due to different socioeconomic conditions. We hypothesized that parasite prevalence in stray cats would inversely correlate to both median household income and property values of the municipality in which they were collected. In order to test this, fecal samples were taken from 189 stray felines collected by Birmingham-Jefferson County Animal Control Services from multiple municipalities in the Birmingham-Jefferson County Area and analyzed for parasite prevalence. The most prevalent helminthic parasites were Ascarids and Hookworms (Nematoda), while the most prevalent protozoan parasites were *Giardia* and *Isospora*. Very few cats were free of GI parasites, while more than half of cats were infected with three or more parasite, making feline parasites in roaming cat populations a public health risk. However, there seems to be no correlation between parasite prevalence and median household income and property values because feline GI parasites are ubiquitous throughout all municipalities of the Birmingham-Jefferson County Area.

Acorn predation and its effect on Oak seedling recruitment: An integrative approach to acorn predation by weevil

Bethany Winslett and Peter Van Zandt

Acorns are vital food sources for deer, rodents, and a variety of insects. Acorn predation has the potential to influence ecosystem structure, by limiting seedling recruitment and forest regeneration. Acorn weevils, which grow inside of the acorn and eat the cotyledon, can result in substantial mortality rates for acorn seeds. This study hypothesizes that the percentage of predation by weevils greatly influences acorn recruitment and could be used as predictors for rates of infestation in oak species. Acorns were collected from Oak Mountain State Park in Alabama from five species of oak from September through November. Rates of infestation determined by the use of two methods, looking for weevil emergence holes and the dissecting of acorns. Dissection is useful in cases where the weevil died before emergence but also killed off the seed. The percentage of acorn predation at Oak Mountain were compared to percentages of seedling recruitment for plots at both Oak Mountain and an alternate site. It is expected that oak species with larger seeds, faster developmental time periods, and lesser levels of tannins will experience the greatest rate of weevil infestation. It is also expected that species of oak with high predation rates will have lower numbers of saplings growing in the area. Acorn predation has the potential to affect the structural elements of an ecosystem by restraining seedling recruitment and in turn, forest regeneration. If a forest is not regenerating, the likelihood of its continuation is limited.