

Texas A&M University Department of Entomology
Fifth Annual Graduate Student Forum



Christine E. Gray

Major Professor: Dr. Craig Coates

Ph.D. Candidate

Identification of an endogenous insulator for *Aedes aegypti* Transgenesis

Aedes aegypti Lin. is the primary vector of both the Yellow Fever and Dengue Fever viruses throughout much of the world. Introduction of transgenes targeted to viral products and/or receptors represent one means of reducing mosquito competence to harbor and transmit these viruses. Currently, transgene expression is highly variable both within and between transgenic families of mosquitoes. An endogenous boundary would be useful in reducing this variability of expression by insulating the transgene from the effects of neighboring genetic regulatory elements and unfavorable chromatin structure. A genomic library will be constructed and screened in a functional cell culture assay to identify specific sequences with boundary element activity. These sequences will be PCR-amplified, cloned and sequenced. The elements displaying the most consistent insulating properties in the transient assay will be used to produce transgenic mosquito lines. Transgene expression levels from these lines will be compared to identical transgenic lines constructed without boundary elements.



Jeremiah Dye

Major Professor: Dr. Kevin Heniz

M.S. Candidate

Effects of temperature, host plant and weevil strain on biological control of aquatic weeds (*Salvinia* spp.) using *Cyrtobagous salviniae*

Giant salvinia, *Salvinia molesta* Mitchell, and common salvinia, *Salvinia minima* Baker, are free floating aquatic ferns native to South America that can grow rapidly to cover the surface of lakes and streams. Giant salvinia has caused severe problems in the waterways of 13 countries (including the U.S.) on three continents, while common salvinia can be problematic in areas of the southeastern United States. The weevil *Cyrtobagous salviniae* Calder and Sands was first used successfully to control *S. molesta* infestations in Australia. Weevils collected in Australia have subsequently been redistributed to many other countries. When present in Florida, a genetically distinct strain of *C. salviniae* appears to reduce the severity of *S. minima* infestations. However, there has been no published comparison of the relative efficacy of each strain in controlling each salvinia species, and the effect of temperature has only been studied for the strain used in Australia. This study will seek to quantify differences between the two weevil strains by assigning experimental units to combinations of weevil strain, salvinia species and temperature profile in a factorial design. One daily temperature profile will be based on real world data for a water body in northern Texas and another for a water body in southern Texas. This will allow an assessment of the two strains of *C. salviniae* in terms of plant biomass reduction and weevil reproductive rate, allowing recommendations to be made regarding conditions under which each strain is most likely to be effective in reducing salvinia infestations.



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Andrea Julian

Major Professor: Dr. Craig Coates

M.S. Candidate

Using bioinformatics to investigate and analyze hot spots for insertion of transposable elements in the genomes of *Caenorhabditis elegans*, *Drosophila melanogaster* and *Anopheles gambiae*

It has become apparent that most transposons do not integrate completely randomly into their host genome. Target choice is dependent on the primary DNA sequence in that most transposable elements utilize a 2-8bp consensus nucleotide sequence, or target site, to insert into and duplicate. Appropriate target sites are selected by unknown means and are termed **hot spots** or preferred sites, as opposed to many other available target sites, which are **cold spots**. The hypothesis to be tested is that secondary and tertiary DNA structures, such as supercoiled DNA, bending of DNA and curved flanking DNA can also have a significant influence on target site preference. Bioinformatics will be used as a tool to predict and analyze the structure of the flanking DNA around hot spots for various genomes. More specifically, Hidden Markov Models are modeled and trained to analyze large datasets of transposable element insertion points in *Caenorhabditis elegans*, *Drosophila melanogaster* and *Anopheles gambiae*. Various measures are being investigated to predict local DNA structure, most of which are based on simple lookup tables of dinucleotide or trinucleotide values. The DNA profile parameters being considering for this analysis include DNA bendability, signed and unsigned nucleosome positioning or position preference, Intrinsic curvature, propeller twist, stacking energy and A+T content. Initial analysis reveals relatively high bendability, minimal preference for specific positions in the nucleosome, low propeller twist angles and low base stacking energies in and around the insertion points of transposable elements. These results suggest a relatively high degree of flexibility of the DNA flanking hot spots for insertion.



Jarrad Prasifka

Major Professor: Dr. Kevin Heinz

Ph.D. Candidate

Effects of landscape variables and cultural practices on generalist predators in cotton

Crop diversity has been repeatedly linked to increases in the abundance and diversity of natural enemies in agricultural systems, but how to use this knowledge requires additional information. For example, evidence suggests grain sorghum production contributes large numbers of generalist predators to cotton fields. However, studies of possible benefits have been confined to adjacent fields of these crops. To maximize the benefits to cotton production, the scale of predator enhancement and specific factors influencing predator abundance in cotton should be determined.

To examine the effect of grain sorghum on cotton predator abundance at the landscape level, direct manipulation is logistically and financially prohibitive. Therefore, we elected to use natural variation in agronomic and landscape variables in cotton production as a "natural experiment." Maps of agricultural fields across three counties in Texas' Southern Rolling Plains were generated using GPS technology, while data on abundance of predators and pests were collected from 70 cotton fields throughout the area. Additional information on cultural practices was gathered from cooperating cotton growers in all fields studied.

Separate regressions were conducted on data from three dates corresponding to stages of crop phenology. Data on pest abundance, cultural practices, and landscape variables were treated as independent variables, while predator abundance was used as the dependent variable. Results were examined for evidence of large-scale benefits of grain sorghum on cotton predator abundance and possible methods to increase this effect.



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Steven P. Holmes

Major Professor: Dr. Patricia Pietrantonio
Ph.D. Candidate

Functional analysis of a G protein-coupled receptor from the southern cattle tick *Boophilus microplus* (Acari: Ixodidae) identifies it as the first arthropod myokinin receptor

The myokinins are invertebrate neuropeptides with myotropic and diuretic activity. The lymnokinin receptor from the pond snail, *Lymnaea stagnalis* (Mollusca), was the only previously identified myokinin receptor. A G protein-coupled receptor (GenBank #AF228521) was cloned from the southern cattle tick, *Boophilus microplus* (canestrini) (Acari: Ixodidae), that is 40% identical to the lymnokinin receptor. This receptor has now been expressed in CHO-K1 cells. Exposure of transfected cells to myokinins at nanomolar concentrations induced intracellular calcium release as measured by fluorescent cytometry. It was determined that the receptor couples to a pertussis toxin-insensitive G protein. Absence of extracellular calcium did not inhibit the fluorescence response, indicating that intracellular stores were sufficient for the initial response. Control cells transfected with the expression vector only did not respond. These results identify the tick receptor as the first myokinin receptor cloned from an arthropod.



Maria Alice Pinto

Major Professor: Dr. Robert Coulson
Ph.D. Candidate

Mitochondrial DNA as a tool for assessing Africanization

In the present study the distribution of mitochondrial DNA (mtDNA) of feral honey bees present in the more temperate climate of the southern United States was investigated. Surveys of mtDNA of Neotropical Africanized honey bees have shown high frequencies (above 0.95) of African-type mitochondria in feral populations. Even before Africanized bees reached the United States, researchers predicted that the frequency of European mtDNA would be higher in this country. The temperate climate and larger size of the resident European population were the main arguments provided to support this view. Surveys of approximately 450 colonies, collected across the southern U. S. before Africanization, and an additional 316 colonies from the Welder Wildlife Refuge (Sinton, Texas), encompassing the pre- and post Africanization period, are reported. A single gene assay was used to distinguish bees carrying African-type mitochondria from bees carrying European-type mitochondria. The test consists of the polymerase chain reaction (PCR) amplification of a 485 bp section of the cytochrome *b* gene, followed by *Bgl II* digestion of the PCR products. Results from the survey of U. S. colonies are compared with those from 192 colonies of Old World bees representing all the races that were introduced into the United States.



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Ahmed Mohammed

Major Professor: Dr. Craig Coates

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Midgut gene expression in the potato tuber moth, *Phthorimaea operculella*

The potato tuber moth (PTM), *Phthorimaea operculella* Zeller, is widely distributed and causes serious damage to potatoes in storage, as well as to plants in the field. The larval stages mine the foliage and infest the tubers. The number of PTM generations per year ranges from 2-12, depending on the environmental conditions. PTM is the most important potato pest in the Middle East, North and Central Africa, Mexico and South America. Chemical insecticides are the most common control strategy used to protect the potato crop. In an attempt to reduce dependence on chemical insecticides, alternative control strategies have been successfully used to help potato producers lower their insecticide usage. Insect-tolerant potato strains were produced by genetic breeding programs. Biopesticides such as *Bacillus thuringiensis* (Bt) and granulosis virus (GV) have been used in different regions of the world to control PTM. The PTM sex pheromone has also been used as a tool for controlling PTM populations, under field and storage conditions, reducing the amount of insecticide usage per season. Most of the research previously performed on PTM has focused on the evaluation of these control strategies. Only a limited number of studies have concentrated on biological aspects of PTM to gain greater understanding of the insect's biology and to develop alternative control strategies. The insect midgut provides a rich target tissue for the identification of molecular targets that could be used to design a new control strategy. The main goal of this research is the identification of genes that are differentially-expressed within the PTM midgut. The suppression subtractive hybridization (SSH) method was used to amplify differentially expressed cDNA fragments and simultaneously suppress highly abundant messages. The midgut cDNA population is subtracted from the carcass (minus the midgut) cDNA population through two rounds of hybridization. Results from these SSH procedures on the PTM midgut will be presented.



Kristen Baum

Major Professor: Dr. Robert Coulson

Ph.D. Candidate

Patterns of cavity use by feral honey bees

The honey bee, *Apis mellifera* Lin., plays an important role in many ecosystems, pollinating a wide variety of native, agricultural, and exotic plants. The recent decline in the number of feral and managed honey bee colonies in North America has caused concern regarding adequate pollination for agricultural crops and natural plant communities. The arrival of Africanized honey bees has added additional concerns about the safety of humans and domesticated animals. This study investigates a population of feral honey bees on the Welder Wildlife Refuge in southern Texas. Changes in the distribution and abundance of these colonies over time were evaluated by examining nest site characteristics, population trends, and spatial and temporal patterns of colonies with European-type and African-type mitochondrial DNA. To evaluate cavity suitability, an index of cavity quality based on colony use was developed. The index ranged from 0 to 100, with higher numbers indicating greater use by the feral colonies. Cavities did not vary in suitability based on measured structural attributes, with the exception of a negative correlation with basal area. A negative correlation also existed with patch area (the size of the patches where the cavity trees were located), as well as patch shape. No differences existed between the structural attributes of cavities occupied by colonies with European-type or African-type mitochondrial DNA. Active cavities were spatially aggregated from 1992 through 2000. Africanized honey bees were first recorded on the refuge in 1994. Colonies with European-type or African-type mitochondrial DNA were aggregated during 1995 and 1996. The distribution of both colony types was random in 1997. From 1998 to 2000, colonies with European-type mitochondrial DNA were randomly distributed, while colonies with African-type mitochondrial DNA were aggregated in distribution. These spatial patterns of cavity occupancy represent the fragmentation of the European population as Africanized bees invaded the area and became established on the refuge.

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Paul D. Barron

Major Professor: Dr. Craig Coates

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Use of a yeast genetic system to select for randomly-primed arthropod cDNAs that contain a putative signal sequence

The Yeast Signal Sequence Trap (ySST) system utilizes an episomal plasmid with selectable markers and origins of replication allowing growth and selection in yeast and bacteria. The plasmid contains a mutated invertase gene that lacks both an initiation codon and a functional signal sequence. Yeast containing only mutated invertase genes are unable to grow on media in which sucrose is the only carbon source. Randomly primed cDNA molecules are cloned into the ySST plasmid, upstream of the mutated invertase gene. Those cDNA molecules that contain an initiation codon and a functional signal sequence will enable a functional, secreted invertase protein to be produced and allow growth on the sucrose containing media. The conservation of signal sequence structures across species allows the system to be utilized with diverse input molecules. It is likely that the rescued gene fragments will be from genes encoding secreted or membrane-bound proteins, which are of particular interest and biological significance.



Lara Lewey

Major Professor: Dr. Craig Coates

M.S. Candidate

Identification of a genetic basis for Varroa mite resistance in the honey bee, *Apis mellifera*

The Varroa mite, *Varroa destructor* Anderson and Truman, is a harmful parasite of the honeybee, *Apis mellifera* Lin. If unchecked, these mites can wipe out a bee colony within a few years. There are only limited methods of control, which must be repeated throughout the year to be effective. A strain of *A. mellifera* has been developed through selective breeding, which appears to display some level of resistance to Varroa mites. This particular resistance trait in *A. mellifera* has been termed SMR. In this study, susceptible and SMR queens are caged and encouraged to lay drone offspring. The drone pre-pupae are removed from the hive and placed into gelatin capsules, in a parasitized or non-parasitized situation. RNA is isolated from the drone pupae, cDNAs are synthesized, and a Suppression Subtractive Hybridization (SSH) procedure is performed. Genes that are differentially expressed between the two strains will be characterized to determine if they are involved in conferring resistance to Varroa mites.



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Veronica Manrique

Major Professor: Dr. Julio Bernal

M.S Candidate

Host location and discrimination mediated through olfactory stimuli in *Anaphes iole* (Hymenoptera: Mymaridae), an egg parasitoid of *Lygus hesperus* (Hemiptera: Miridae)

Lygus hesperus Knight is an important pest on different crops including cotton and alfalfa in the western U.S. It is polyphagous, feeding on 117 non-crop plants and at least 25 cultivated plants. In early spring, *Lygus* populations are mainly found on flowering weeds and also on volunteer alfalfa. As plants senesce or are harvested, *Lygus* migrate into nearby susceptible crops such as cotton. *Anaphes iole* Girault is the most commonly encountered parasitoid of *Lygus* eggs in the U.S. This parasitoid attacks *Lygus* eggs that are partially embedded in plant tissue. Chemicals derived from both host adults or eggs, and physical properties of protruding eggs are used as host recognition and acceptance cues by this parasitoid. However, no studies have addressed the influence of plant volatiles in long-distance foraging by *A. iole*. Therefore, a four-arm olfactometer will be used to examine the behavior of *A. iole* females in response to the odors of infested plants compared with uninfested ones. Common groundsel, shepherd's purse, annual ragweed, pigweed, cotton and alfalfa will be infested with *Lygus* females, and will be allowed to lay eggs for 48hs. The olfactometer bioassays will be conducted between 8:00 to 12:00 h, and four odors will be tested simultaneously in each trial, 1) an infested plant (with *Lygus* eggs), 2) an uninfested plant 3) a blank control (no odor), and 4) a second blank control. Female responses will be observed for 5 min on a computer monitor, and the time spent in selection or visit regions will be recorded for each arm. The importance of olfactory cues exploited by biological control agents will be discussed.



Darren Hagen

Major Professor: Dr. Craig Coates

M.S. Candidate

Identification of *vasa*-like genes in *Aedes aegypti*, *Anopheles gambiae*, and *Apis mellifera*

Aedes aegypti Lin. and *Anopheles gambiae* Giles are both vectors for pathogens that have a dramatic effect on world health. *Apis mellifera* Lin. is an organism that is important economically to the agricultural industry. Currently, methods of transformation are being developed for these species in order to create transgenic lines incapable of transmitting, or in the case of *A. mellifera*, being infected by disease agents. The creation of genetic transformants relies on the expression of a transposase protein in the germline cells to promote the integration of transgenes into the insect chromosomes. The use of a germline-specific promoter would enable transposase expression in the gonadal tissues, without affecting somatic tissues. The *Vasa* gene from *Drosophila melanogaster* Meigen has been determined to be essential to the formation of germline precursor cells. The research presented here focuses on the identification of a *vasa* homolog in all three species and the subsequent identification of the promoter region and any other regulatory sequences involved in conferring germline specificity. Identification of such regions would allow the use of promoters native to the organism and potentially increase the levels of transposase expression and thus the frequency of transgenic individuals produced.



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Mei-Er Chen

Major Professor: Dr. Larry Keeley and Dr. Patricia Pietrantonio
Ph.D. Candidate

Cloning of the fire ant, *Solenopsis invicta*, vitellogenin receptor cDNA and its transcriptional expression profile in reproductive females during development

Insect vitellogenin is a phosphoglycolipoprotein that is a precursor of the major yolk protein, vitellin. It is synthesized in fat bodies and internalized by growing oocytes via receptor-mediated endocytosis. To date, vitellogenin receptors (VgRs) are sequenced in only two insects, the mosquito and fruit fly, *Aedes aegypti* Lin. and *Drosophila melanogaster* Meigen, respectively. Both VgRs are members of the low-density lipoprotein receptor (LDLR) superfamily. I have cloned and sequenced four cDNA fragments encompassing the entire coding region of a putative fire ant, *Solenopsis invicta* Buren VgR (SiVgR). The complete cDNA has a length of 5220 bp coding for a 1740-residue protein with a predicted molecular mass of ~196 kDa. The deduced amino acid sequence of the SiVgR reveals that it encodes a protein belonging to the LDLR superfamily and contains two putative ligand binding domains as observed in the mosquito and fruit fly VgRs. The deduced amino acid sequence of the SiVgR exhibits 38% and 32% identity to those of mosquito and fruit fly VgRs respectively. The 7.5-kb SiVgR mRNA is present only in reproductive female ovaries. The current data show that SiVgR transcript is present at higher levels in alate females compared to dealate females. The profile of developmental transcriptional expression will be determined by relative quantitative reverse transcription polymerase chain reaction (RT-PCR). The cellular distribution will be determined by *in situ* hybridization.



Rodrigo Diaz

Major Professor: Co-Chairs Dr. Julio Bernal and Dr. Allen Knutson
M.S. Candidate

What eats beet armyworm eggs in cotton fields?

Insect predators likely play important roles in cotton pest management maintaining pest levels below economic thresholds. Different techniques have been used to quantify predation, including exclusion with field-cages, recovery of labeled prey items, fecal analysis, post-mortem methods and direct observations. Red imported fire ant *Solenopsis invicta* Buren (RIFA hereafter) is a keystone species in southern agricultural landscapes. Observations of beet armyworm egg masses were carried out in two cropping seasons and locations. Egg masses were placed on plants that were either protected or not from RIFA. Each egg mass was observed for a period of 5 seconds every 15 minutes. Predators feeding on the eggs were identified in the field, and numbers of predatory events were scored during the period between 8:00 pm and 1:00 am. *Abrolophus* sp. (Acari: Erythraeidae), spiders (several genera), cotton fleahopper [*Pseudatomoscelis seriatus* (Reuter)], minute pirate bug (*Orius* sp.) and RIFA were among the most frequent predators observed feeding on egg masses and more egg masses disappeared due to active predation by RIFA. Observations suggest that most predators feed *in situ*, whereas RIFA removes completely beet armyworm eggs from the plant, which may lead to under appreciation of RIFA's importance as a predator. Mismanagement of RIFA populations could lead to serious outbreaks of species previously suppressed in part by this exotic invasive species.

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Fifth Annual Graduate Student Forum Committee Members

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Mark Johnsen

Chair Assistant: Teresa Gold



Graduate Student Forum Award Recipients

<u>2001</u>	<u>2000</u>	<u>1999</u>	<u>1998</u>
1-Christine E. Gray	1-Jarrad Prasifka	1-Carlos Bogran	1-Carlos Bogran
1-Ronald D. Weeks	2-Robert Kula	2-Jarrad Prasifka	2-Richard Houseman
1-Steven P. Holmes	3-Ahmed Mohammed	3-Karol Burns	3-Jim Martin
4-Mei-Er Chen	4-Ronald Weeks	4-Steve Holmes	4-Steve Holmes
5-Marcia Trostle	5-F. Mariana Tenorio	5-Andrea Jensen	5-Henrique Serra
6-Matt J. Yoder	6-Jason Mottern	6-Matt Buffington	6-Ron Vogtsberger
7-Ahmed Mohammed	7-James Martin	7-Ronald Weeks	7-Cordelia Rasa



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