

Appendix 5. ARTHROPOD GENOMICS RESEARCH IN ARS

Participants and Individual Research Summaries

Margaret Allen

***Biological Control of Pests Research Unit
Stoneville, MS***

I am rearing inbred, wild type, and phenotypically unique strains of the lady beetle *Coleomegilla maculata*. I sequenced partial transcriptomes (adult female) from two individuals of the highly inbred strain, differing in their adult diet. I also am examining transcriptome sequences from the invasive kudzu bug. The studies I presently am engaged in focus on insect feeding, diet and nutrition. For *C. maculata*, the question is “what makes an omnivore?” We want to elucidate biochemical/genetic mechanisms in insects of key importance to mankind and to improve our ability to live sustainably with the insects on our planet.

Dr. Katherine Aronstein

***Honey Bee Breeding, Genetics and Physiology Research Unit
Baton Rouge, LA***

My research is currently focusing on the development of genetic markers for the Marker Assisted Honey Bee Breeding Program. One of the future efforts will involve sequencing genomes of several important honey bee stocks (e.g., exhibited VSH traits, Russians, Minnesota Hygienic, common sources of Italians) to: 1) identify molecular markers, allele frequencies, and genomic patterns in diverse stocks of honey bees and 2) develop SNP panels characteristic for specific bee stocks as well as evaluate SNPs for linkage to the traits of interest. These new genomic tools will guide the honey bee breeding program in the future.

Anna Bennett, Bill Kemp and George Yocum

***Insect Genetics and Biochemistry Research Unit
Fargo, ND***

We are investigating insects’ transcriptome response to diapause development and environmental stresses. Diapause is a major component of insect life cycles and can have a substantial impact on rearing protocols and the availability of mass-reared insects. As well, mass reared insects are subjected to a wide range of stresses that can decrease their survival and post-storage quality. The goal of our research is the development of biomarkers that can be used for the optimization of storage protocols, thereby increasing the quality and quantity of beneficial insects.

Matthew Buffington
Systematic Entomology Laboratory
c/o National Museum of Natural History

Several SY's within SEL are working in collaboration with the Global Genome Initiative (GGI) of the National Museum of Natural History to collect 'genome quality' tissues, representing all genera of life on earth. This is a unique opportunity for partnership: USDA scientists bring six orders of insect and mite research to the GGI, providing authoritatively identified species to the project; the GGI provides the infrastructure for the collection and curation of tissues for genomic research, as well as state of the art means for generating and analyzing genomes. Members of SEL also are invited to sit on GGI steering committees.

Chiou Ling (Stella) Chang
USDA-ARS-DKI-USPBARC
Hilo, Hawaii

Fruit flies are very important pests worldwide, especially in Hawaii, Pacific area, and California. We are working on understanding fruit fly biology and related mechanisms with associated with their impact (including nutrition, aging, stage development, radiation, parasitism, attractant, chemosterilant, or strain development, etc.) using a proteomics approach. We are identifying/profiling differentially expressed factor-specific proteins/biomarkers. Any promising target genes or proteins will be further studied as candidates for gene interference, such as silenced (RNAi) or activated. Silenced or activated genes will be incorporated into control techniques to achieve or improve fruit fly control.

Brad Coates
Corn Insects & Crop Genetics Research Unit
Ames, IA

I use genomic data to correlate gene expression differences and/or segregation of structural mutations with insect traits. Adaptations in field populations of some insect pests of corn allow individuals to survive exposures to chemical insecticides and transgenic plants, or reside in alternative biological niches that circumvent current management practices and threaten sustainable production. Genetics approaches have identified genome regions and expression differences associated with population adaptations. Whole genome approaches are being conducted to identify the functional genomic basis of pesticide resistance and define population structure in order to enhance the efficacy of insect resistance management strategies used in corn production.

Christopher Dunlap
Crop Bioprotection Research Unit
Peoria, IL

My laboratory focuses on developing entomopathogens to control insect pests. Our primary insect pests are redbay ambrosia beetles and Asian citrus psyllids. We currently use several genomic approaches in our research, such as *de novo* microbial genome sequencing, amplicon-based population metagenomics and transcriptomics. In the future, we hope to study multitrophic interactions using metatranscriptomics and virus discovery based on microRNA.

Jay Evans
Bee Research Laboratory
Beltsville, MD

I am interested in genetic traits of honey bees that help them resist disease, and in genetic traits of their parasites and pathogens that can be used to develop novel controls. I have been involved with genome projects for the honey bee and two arthropod parasites of bees, and more tangentially with other insect genome projects, as well as genome efforts for viruses, bacteria, and fungi of bees. Current efforts are focused on *Varroa* mite genomics and RNA-Seq for disease management and parasite control. We will use results to improve bee health and decrease parasites via RNAi and targeted chemicals.

John Gaskin
Pest Management Research Unit
Sidney, MT

I am currently studying origins, taxonomy, phylogenetics, hybridization and population genetic structure of invasive weeds and weed biological control agents. I do this to assist classical biological control practitioners in developing arthropod and fungal agents with high host-specificity, low risk of non-target effect and high efficacy. I plan to determine when it would be efficient to move my research from sequencing and fragment analysis or relatively neutral markers to more genome-wide analyses that may include functional genes, with the same goal of improving classical biological control of weeds.

Felix Guerrero
Tick and Biting Fly Research Unit, Knipling-Bushland U.S. Livestock Insects Research
Laboratory, Kerrville, TX

I lead the international consortium that sequenced and is assembling the 7.1 Gbp genome of the cattle tick and the 1.4 Gbp genome of the horn fly. The horn fly was identified as the number one pest of cattle in the USA (estimated annual producer losses of \$1 billion). The cattle tick is threatening to reinfest the USA after being eradicated in the 20th century (estimated \$3 billion annual savings to the cattle industry). I believe the genome holds the key to development of novel control technologies for arthropods and we will mine these genomes to develop anti-arthropod vaccines.

Dawn Gundersen-Rindal
Invasive Insect Biocontrol and Behavior Lab
Beltsville, MD

A current research focus is comparative transcriptomics (across sexes, life stages, tissues) and genomics of agriculturally significant US invasive insect pests including brown marmorated stink bug (*Halyomorpha halys*), gypsy moth (*Lymantria dispar*), and others. Another research focus is analysis of transcriptome-level responses of pest insects to associated microbial pathogens that may help determine which microbes would be effective biocontrol agents. One goal underlying this research is the need to identify suitable and highly insect-specific pest gene targets for RNA interference strategies. The longer-term goal is to develop and deliver effective RNAi-based tools and effective biocontrol pathogens for management of agriculturally important invasive insect pests.

Robert Harrison
Invasive Insect Biocontrol and Behavior Laboratory
Beltsville, MD

I study insect-specific viruses, and also am attempting to identify genes encoding enzymes that catalyze biosynthesis of insect semiochemicals. Insect viruses have potential as safe, environmentally friendly tools to control damaging populations of insect pests. Also, it may be possible to use semiochemical biosynthesis enzymes for production of difficult-to-synthesize semiochemicals useful for monitoring and controlling insect pest populations. I would like to adapt a transcriptomic/genomic approach to characterize the response of insect pests to viral infection, to identify novel viruses in the transcriptomes of insect pests of interest, and to identify semiochemical biosynthesis genes.

Keith Hopper
Beneficial Insect Introductions Research Unit
Newark, DE

We work on the genetics of host specificity in parasitoids. The goal is to determine the likelihood of post-introduction evolutionary shifts in host specificity. We have measured host specificity of ten *Aphelinus* species. We are assembling their genomes and transcriptomes, annotating them with concentration on genes likely to affect specificity, and analyzing for genes highly divergent in sequence or expression. We are crossing species/lines differing in specificity and mapping underlying QTL. Using chromosomal FISH, we will test whether candidate genes occur in QTL. We are using the assemblies for an *Aphelinus* phylogeny, and mapping host use on this phylogeny.

J. Joe Hull

***Pest Management and Biocontrol Research Unit
Maricopa, AZ***

We currently are using RNA-Seq to profile the transcriptional response of western tarnished plant bug to thermal stress. A better understanding of the molecular basis underlying adaptation to extreme environments may facilitate development of new control tactics, such as RNAi, and can provide baseline information on how arthropods adapt to a changing climate. We want to continue utilizing RNA-Seq to profile other abiotic/biotic stressors and generate tissue specific transcriptomes. These molecular resources will provide valuable information on the molecular mechanisms driving plant bug biology, and as before, may facilitate the identification of genes for targeted disruption.

Kai-Shu Ling

***U.S. Vegetable Laboratory
Charleston, SC***

We are conducting a genome sequencing project on whitefly (*Bemisia tabaci*). Using DNA prepared from male whiteflies (haploid) selected from an isogenic colony, through Illumina sequencing and *de novo* assembly, we obtained a draft genome of 641.6 Mb with N50 length of 2.79 Mb. Additional PacBio data were generated and their incorporation is underway. Whitefly is a globally distributed vector that is capable of efficiently transmitting numerous plant viruses infecting many economically important crops. Through USAID, we are extending our successful genomics to a related whitefly species in Africa that spreads viruses causing serious brown streak disease on cassava.

Paul Madeira

***Invasive Plant Research Lab
Fort Lauderdale, FL***

- 1) What I am doing: Provide molecular support for 5 scientists, mostly molecular taxonomy and some genotyping. Examples: (1) molecular taxonomy- D2 analysis of *Neostromboceros albicomus*, biocontrol agent. Are 3 colonies imported in 2005, 2008, and 2014 the same subspecies? (2) genotyping (using GBS, NGS)- genotype foreign and domestic *Rhodomirtus tomentosa*, an invasive weed in Florida.
- 2) Why I am doing it? Support of classical biological control. Examples: (1) molecular taxonomy- Analysis showed all colonies were the same subspecies. Reduced need to repeat "Host specificity" testing. (2) genotyping- inform search for insect biocontrol agents by determining where the weed originated.
- 3) Where do I want to go? I want to be able to use reduced representational (and barcoded) NGS in order to genotype organisms leading to the ability to determine phylogeography (weed origins), for genotype comparison, and population analysis.

Dana Nayduch
Arthropod Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research
Manhattan, KS

We use RNA-seq to compare gene expression profiles in biting midges across diet and virus infection to reveal genes and pathways involved in hematophagy, reproduction, gut microbial-ecology, and vector competence. Subsequent studies employ qRT-PCR or RNAi to determine the temporospatial expression of these genes and knockout phenotypes, respectively. Our new NP104 project will use similar approaches with house flies, where transcription profiling is paired with microbiological techniques to understand fly-bacteria interactions and the impact on vector competence for pathogens. Such studies will update the molecular toolset for these species and can reveal targets for novel mitigation or transmission-blocking strategies.

Brenda Oppert
Stored Product Insect Research Unit
Manhattan, KS

The sequencing of the red flour beetle genome provided new tools to develop control products based on vulnerabilities in the biology of the beetle. Tissue-specific high throughput sequencing has identified gut transcripts as potential new targets for biopesticides (RNA interference and protease inhibitors) that are now being evaluated. Comparative gene expression technologies, such as RNA-Seq, are being used to probe the beetle's response to dietary inhibitors, toxins, and current control products, such as phosphine. We've found that beetle larvae fed dietary toxins and inhibitors mount a complex genetic response to survive. This information has provided us with details about genes that are transcribed by the beetle to survive toxin and inhibitor damage, and also identify genes that encode enzymes critical to digestion of protein in the diet. We apply these techniques to study insects lacking a sequenced genome (i.e., most of the stored product pests), but we also are sequencing the genome of other important storage pests, such as the lesser grain borer. As we identify potential new targets for biopesticides, we are using high throughput bioassays to evaluate purified proteins, peptides, or double-stranded RNA. Biopesticides have applications in the integrated pest management of storage pests in sprays, formulations, or transgenic cereals. These new insect control products for storage pests are urgently needed to replace those that are undergoing deregulation or to circumvent resistant pest populations.

O.P. Perera
Southern Insect Management Research Unit
Stoneville, MS

The focus of my current research program is the transcriptomics and genomics of heliothine moths and *Lygus lineolaris*. I use transcriptomes to profile gene expression in response to intoxication with Bt toxins and chemical insecticides. The goal is to characterize gene networks physiological processes associated with tolerance to insecticides. Genome analysis is carried out primarily to develop genetic markers for population genomics and QTL analysis. I expect to use the results to understand the genetic basis of resistance to Bt toxins and insecticides and to use the knowledge to help develop optimal resistance management approaches

Monica Poelchau and Christopher Childers
National Agricultural Library
Beltsville, MD

We are developing web-based resources to help access, curate, and visualize arthropod genomic data. Our rationale is to facilitate agricultural research on genomic 'big data', which will translate to faster and better agricultural findings for ARS scientists, and the scientific research community in general. Ultimately, we aim to provide comprehensive genomic database resources for any arthropod genome.

Gary Puterka and Scott Nicholson
Wheat, Peanut and Other Field Crops Research
Stillwater, OK

The *Diuraphis noxia* genome sequencing project, led by Dr. Gary Puterka, has sequenced, assembled, and annotated the entire RWA genome and transcriptome. The project was initiated to capitalize on earlier work that identified the salivary proteomes of RWA and Greenbug and their roles in aphid feeding, and has already identified several effective sequences for RNAi-based aphid control. The RWA genome and transcriptome are essential in analyzing the genetic basis of aphid feeding, evasion of plant defenses, and the plant damage that results, and will ultimately lead to novel genetically-based aphid control methods.

Brian Rector
Great Basin Rangelands Research Unit
Reno, NV

Currently, we mainly use genomic tools to generate markers for taxonomic and ecological studies in the context of weed biocontrol. Where we want to go is toward RNA-Seq studies to dissect the genetic factors associated with host specificity in arthropods. Host specificity is the most important criterion for evaluating classical biocontrol agents. We have two model systems, a mite and a beetle, each comprising multiple cryptic species that appear to differ only by host range; either highly specific or polyphagous. We hope to produce genetic screens for this trait, ideally with utility across many different taxa.

Thomas W. Sappington
Corn Insects & Crop Genetics Research Unit
Ames, IA

As co-leader of the *Diabrotica* Genetics Consortium, I have been co-organizing multi-institution, international efforts to obtain the genome sequence and transcriptome for western corn rootworm (WCR). Sequencing is completed, and assembly and annotation are beginning. WCR is the worst pest of corn in N. America and recently invaded Europe. It is exceptionally difficult to manage because it has evolved resistance to many management tools, including crop rotation and some transgenic Bt-corn varieties. Genomic/transcriptomic tools will facilitate characterization of resistance genetics. Although my research focuses on gene flow and adult dispersal, I would like to participate in annotating the genome.

Glen Scoles***Animal Disease Research Unit******Pullman, WA***

We are identifying vaccine targets to control disease or block transmission of tick-borne livestock pathogens. We use a combination of classical immunological methods, proteomics, transcriptomics, targeted gene silencing, pathogen attenuation/transfection, etc. We have genome sequences for the pathogens but need better genomic information for the vectors. Tick-borne pathogens have global economic and food security impacts and methods for protecting U.S. livestock currently rely on acaricides for tick control. Acaricide resistance and negative environmental impacts necessitate the development of alternative methods for controlling transmission. Effective transmission blocking vaccines would provide a more efficient and economical method for protecting livestock.

DeWayne Shoemaker***Imported Fire Ant Research Unit******Gainesville, FL***

We are using a comparative population genomics approach to reconstruct the evolutionary history of the *Gp-9* supergene, which is involved in regulation of fire ant colony social form. Results from the proposed study will provide detailed knowledge of the identity and potential functional roles of genes within the supergene, information that in turn is highly relevant to our efforts to develop novel fire ant control methods. More specifically, previous studies have linked variation within the supergene to traits that represent excellent targets for gene disruption (e.g., RNAi). These traits include colony queen number, queen viability, queen fecundity, and male fertility.

Steve Skoda***Screwworm Research Unit******Kerrville, TX***

The Screwworm Research Unit, in collaboration with Dr. Max Scott of North Carolina State University, is working to develop a transgenic, genetic sexing (male-only) strain of screwworm to be used in the International Program to Eradicate and Prevent Screwworms. Releasing only males will improve Program efficiency, protecting livestock in North America from this insidious pest and the >\$1.5 billion in annual losses. Moving forward, targeted insertion of genetic material (compared to current, random insertions) would improve the efficiency of developing genetic sexing strains of screwworms, therefore, better information on the screwworm genome is necessary.

David Stanley
Biological Control of Insects Research Laboratory
Columbia, MO

We are identifying genes that encode proteins (enzymes and receptors) responsible for the production of prostaglandins and their signaling of insect innate immune reactions to infections and invasions. We have reported that inhibiting prostaglandin biosynthesis can be lethal in infected insects. We developed a transcriptome and are now developing gene silencing constructs to seriously impair insect immunity, working with squash bugs, *Anasa tristis*. This work will contribute to development of novel, molecular-based pest insect management technologies. Future work will establish new, tissue-specific insect cell lines for research with gene silencing.

Lucy Stewart and Peg Redinbaugh
Corn, Soybean, and Wheat Quality Research Unit
Wooster, OH

The Maize Virology group in our research unit works on arthropod-transmitted maize viruses and phytoplasmas. These include pathogens vectored by leafhoppers, for which few genomics resources have been available, and which have not been model systems for studies of vector transmission. In collaboration with Ohio State University colleagues, we have developed transcriptome data for *Graminella nigrifrons*, the leafhopper vector of *Maize fine streak virus* and *Maize chlorotic dwarf virus*, and compared the transcriptome response of the soybean aphid *Aphis glycines* to vectored and non-vectored viruses. Our goal is to understand plant-virus-arthropod interactions important for vectoring.

Jamie Strange and Theresa Pitts-Singer
Pollinating Insect Research Unit
Logan, UT

I am currently working on transcriptome data from Hunt's bumble bee doing a comparative study to immune response genes in social Hymenoptera. I have two economically important species out for sequencing now to develop markers (microsatellites and SNPs) to look at the effects of bee movement on genetic introgression. I plan to expand into population genomics more in the future and continue exploring the genetic factors underlying disease susceptibility in bumble bees.

Kevin Temeyer
Tick and Biting Fly Research Unit, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX

What: I identify and express candidate antigens for anti-biting fly and anti-tick vaccines and conduct animal vaccination trials. I characterize function of chemosensory receptors; identify new attractants, repellents, toxicants and behavior-modifying chemicals; and identify, evaluate and develop tools for target assessment and novel approaches for biting fly and tick control.

Why: We seek to reduce the economic and health impacts of biting flies and ticks that increase costs of animal production systems and transmit pathogens among animals and humans.

Where going: Develop safer (health & environment) methods and tools for effective pest control.

Bill Wintermantel

USDA-ARS Crop Improvement and Protection Unit

Salinas, CA

Our group is examining the genome and transcriptome of *Bemisia tabaci* biotype B. Kai Ling (Charleston) will discuss the genome aspects. My program (Salinas) is analyzing the transcriptome of *B. tabaci* biotype B, and expression changes associated with whitefly feeding on plants infected with persistent and semi-persistent viruses, as well as other biological factors. Our goal is to determine how whitefly gene expression is influenced by feeding on different types of virus-infected plants and other conditions. Long term goals are to develop RNAi strategies to reduce populations of whitefly and associated viruses affecting agriculture in the USA and Africa.