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Arthropod Genomics Symposium

Platform/Oral Presentations

i5k

1 - Assembly Validation and Scaffold Extension for Complex Genomes Using Extremely Long Single-Molecule Imaging

Brown, Sue; VanSteenhouse, Harper

De novo genome assemblies using only short read data are generally incomplete and highly fragmented due to the intractable complexity found in most genomes. Consisting mainly of large duplications and repetitive regions, this complexity hinders sequence assembly and subsequent comparative analyses. We present a single molecule genome analysis system (Irys) based on NanoChannel Array technology that linearizes extremely long DNA molecules for observation. This high-throughput platform automates the imaging of single molecules of genomic DNA hundreds of kilobases in size to measure sufficient sequence uniqueness for unambiguous assembly of complex genomes. High-resolution genome maps, based on fluorescently labeled single-strand nicks (produced by modified restriction enzymes), retain the original context and architecture of the genome. Comparison of the genome maps produced by the Irys system with *in silico* maps based on the genome assembly improves contiguity and accuracy of whole genome assemblies, permitting a more comprehensive investigation of functional genome biology, comparative genomics and other downstream analyses.

2 - Progress and lessons learned from the BCM-HGSC i5K pilot

Richards, Stephen; Qu, Jiaxin; Dugan, Shannon; Lee, Sandy; Liu, Yue; Doddapaneni, HarshaVardhan; Muzny, Donna; Worley, Kim; Gibbs, Richard

The i5K is an initiative to sequence the genomes of 5,000 arthropods of medical, agricultural and scientific importance. As a pilot project to identify potential problems and challenges in species selection, identification and acquisition, DNA isolation, sequencing strategies and assembly, automated and manual annotation, analysis and publication, the BCM-HGSC is sequencing 30 arthropods, selected by the i5K species selection committee.

Our extensive experience in arthropod genomics, has allowed us to quickly identify new challenges in scaling the number of de-novo genome projects. For example, many arthropod species have much larger genome sizes than initially expected (e.g. the American Cockroach's is 3Gb, the Velvet worm - a living fossil out-group to the arthropods ~ 5Gb, most Crustacea are 3Gb+). For a number of arthropod orders, small body sizes yield tiny amounts of DNA, for example sand flies, thrips and protura – an out-group to the insects with no wings. Other challenges include the inability to inbreed, a common situation for most arthropods (extreme examples are the cicadas with life cycles of 13 and 17 years) or inability to sex individuals – for example arthropods where only larvae can be easily collected. An additional challenge is to identify ongoing genome projects to avoid replication of work.

To date, we have collected samples and isolated DNA for all 30 species, produced genome sequence for over 20, and assembled 10. We present genome assembly successes, problems and statistics, initial results from extremely low quantities of DNA, and our automated analysis plans. Also we present an outline of the i5K community annotation and analysis process.

Perhaps the biggest contribution of this project will be bringing the genomics revolution to the field of arthropod biology, establishing a molecular baseline to the study of animals that affect our society so greatly.

In addition to providing an introduction to this newly available technology, we will describe the production of genome maps for the red flour beetle *Tribolium castaneum* and their use to independently validate the genome assembly, estimate gap-lengths, extend scaffolds and improve chromosome builds.

3 - WebApollo: A Web-based Sequence Annotation Editor for Distributed Community Annotation. *Lee, Ed; Helt, Gregg; Reese, Justin; Munoz-Torres, Monica; Childers, Christopher; Buels, Robert; Stein, Lincoln; Holmes, Ian; Elsik, Christine; Lewis, Suzanna*

Better sequencing technologies increase our access to whole genome sequences; annotation efforts must keep pace converting these sequence data into knowledge. The growing number of genome sequencing projects will then largely rely on contributions from domain specialists. This is indicative of a curation environment shifting from a traditional, centralized model to a geographically dispersed community annotation one, which requires new tools to support collaborative annotation. WebApollo provides a web-based environment that allows multiple, distributed users to conduct and share manual annotations. The WebApollo client is an extension to JBrowse, a genome browser with a fast, highly interactive interface for visualization of genomic data. WebApollo allows users to create and modify transcript and exon structures through intuitive gestures, and flags potential problems within manual annotations. A server handles the edit logic and deals with the complexities of modifications in a biological context, where a single change can have multiple cascading effects (e.g., splitting/merging transcripts). Edits are persistently stored on the server, ensuring reliability and allowing the client to review histories and providing undo and redo functions. The server provides synchronized updates over multiple browser instances, so annotation edits are immediately visible to all users who are working on the same region. And WebApollo has been optimized for displaying and editing results from the MAKER annotation pipeline. WebApollo has enabled annotation efforts for diverse research communities working on population genomics of stickleback fish, evolution of morphology and eusociality with organisms such as pigeons and ants, and agriculturally relevant species such as honey bee, cow and fruit rust fungus. WebApollo is available at <http://icebox.lbl.gov/webapollo/releases> Public demo & More information at <http://icebox.lbl.gov:8080/WebApolloDemo> and <http://gmod.org/wiki/WebApollo>.

4 - Structural Variation Detection and De Novo Assembly in Complex Genomes Using Extremely Long Single-Molecule Imaging

VanSteenhouse, Harper

De novo genome assemblies using only short read data are generally incomplete and highly fragmented due to the intractable complexity found in most genomes. This complexity, consisting mainly of large duplications and repetitive regions, hinders sequence assembly and subsequent comparative analyses. We present a single molecule genome analysis system (Irys) based on NanoChannel Array technology that linearizes extremely long DNA molecules for observation. This high-throughput platform automates the imaging of single molecules of genomic DNA hundreds of kilobases in size to measure sufficient sequence uniqueness for unambiguous assembly of complex genomes. High-resolution genome maps assembled *de novo* from the extremely long single molecules retain the original context and architecture of the genome, making them extremely useful for structural variation and assembly applications.

Genome map-based scaffolding in shotgun sequencing experiments performed in parallel with second or third generation sequence production offers an integrated pipeline for whole genome *de novo* assembly solving many of the ambiguities inherent when using sequencing alone. Additionally, genome maps serve as a much-needed orthogonal validation method to NGS assemblies. As a result, genome maps improve contiguity and accuracy of whole genome assemblies, permitting a more comprehensive analysis of functional genome biology and structural variation.

In addition to providing an introduction to this newly available technology, we will demonstrate a number of examples of its utility in a variety of organisms, including arthropods.

Emerging Genomes

5 - The genome of the primitively social wasp *Polistes dominula*: insights and opportunities for understanding the genomic basis of eusociality

Toth, Amy

Eusocial insects are excellent models for understanding the evolution of complexity. The evolution of eusocial life marks one of the major transitions in evolution due to the shift from individual selection to colony level selection. There has been great interest in understanding the genomic changes that accompany the evolution of eusociality. To date, published genomes are available for eight eusocial insects—one bee and seven ants. But, there have been no genomes sequenced for one of the three major eusocial lineages, the paper wasp family Vespidae, nor genomes for primitively eusocial species. These deficits have hampered comprehensive comparative analyses of eusocial evolution. In this talk, I will describe progress on the *de novo* sequencing of the genome, transcriptome, and methylome of the primitively social paper wasp *Polistes dominula*. These results will be discussed in light of three non-mutually exclusive ideas about genomic changes influencing the evolution of sociality: 1) the role of deeply conserved genetic toolkits, 2) the influence of epigenetic modifications such as DNA methylation, and 3) the importance of novel genes.

6 - Sequencing the Antarctic midge, *Belgica antarctica*: the smallest insect genome

Denlinger, David

The Antarctic midge *Belgica antarctica*, the only insect endemic to Antarctica, inhabits a cold, desert environment inhospitable to most forms of terrestrial life. The genome assembly we present for the midge represents the first dipteran in the family Chironomidae and the first Antarctic eukaryote to be sequenced. At 98 Mb, this is the smallest insect genome sequenced thus far. This unusually small genome has low repeat content and few transposable elements. The transposable elements that are present are mainly retro-elements. RNA-seq was used to quantify genome-wide mRNA changes in response to dehydration, one of the most significant environmental challenges confronted by organisms living in a polar environment, where water is frozen and therefore unavailable for much of the year. Midge larvae tolerate a loss of up to 70% of their body water, a feature that enables them to survive in a frozen state. In response to dehydration, larvae up-regulate cellular recycling pathways including the ubiquitin-mediated proteasome and autophagy, while concurrently down-regulating general metabolism and ATP production. Metabolomics results reveal shifts in metabolite pools that correlate closely with changes in gene expression, indicating that coordinated changes in gene expression and metabolism are critical components of the dehydration response. Another unique feature of the polar environment is the dramatic seasonal change in daylength, ranging from nearly constant light to nearly constant darkness. Clock genes fail to cycle under these conditions, and the midges remain constantly active during the austral summer.

7 - Genomic adaptation of Glanville fritillary butterfly in living in fragmented habitats

Ahola, Virpi; Lehtonen, Rainer; Somervuo, Panu; Kvist, Jouni; Koskinen, Patrik; Salmela, Leena; Välimäki, Niko; Mäkinen, Veli; Holm, Liisa; Paulin, Lars; Auvinen, Petri; Frilander, Mikko; Hanski, Ilkka

The Glanville fritillary butterfly (*Melitaea cinxia*) is emerging as an ecological model organism to study genetic consequences of habitat loss and landscape fragmentation. To facilitate large-scale genetics studies in this model system and investigate the genetic basis of local adaptation to fragmented landscapes, we have sequenced the whole genome of the Glanville fritillary. The first version of the genome consists of 8,262 scaffolds (N50=119Kbp) with total length of 390 Mbp and 16,667 predicted protein-coding genes. In spite of high level of polymorphism in terms of SNP and indel polymorphism, low DNA quality, and relatively high (34%) fraction of transposable elements, we were able to construct the reference genome with overall coverage of 70X. The genome has low number of chimeric scaffolds (9%) compared to linkage map which covers 75% of the total genome length.

To study genomic adaptation, we conducted two RNA-Seq experiments using thorax samples. One experiment involved a flight treatment with a control, the other experiment compared gene expression of individuals from fragmented versus continuous landscapes. Our results show that 755 genes were induced in active flight including genes belonging to hypoxia and downstream tricarboxylic acid cycle and glycolysis pathways. Many genes in these functional groups show similar gene expression patterns across individuals living in continuous and fragmented landscapes and across individuals with poor and good flight performance. Since previous studies have shown that dispersal capacity is higher in individuals living in fragmented populations, expression differences in these genes may be pertinent for adaptation to living in fragmented landscapes.

8 - Water-walking insects: marrying evo-devo with ecology for a better understanding of morphological evolution

Khila, Abderrahman

Three decades after the birth of evo-devo, there is an increasing recognition that a strong integration of evolutionary ecology with developmental genetics is required for a more comprehensive and thorough understanding of the origin of animal diversity. Three major challenges have been hindering such integration efforts. First is the paucity of suitable natural systems where state of the art tools for functional studies of developmental genetic processes can link the genotype to phenotypes within the context of their ecological relevance. Second is the longstanding focus of the field on single-species analyses. With this deeply reductionist approach, although helpful in elucidating mechanisms that are specific to particular taxa, it is hard to pinpoint which of these principles can be generalizable for a better understanding of the evolutionary process as a whole. Finally, the third challenge has been, until recently, the difficulty of generating genomic resources.

Here we propose a set of original model organisms, water-walking insects, to overcome the challenges facing an efficient integration of developmental and evolutionary genetics with ecology. About 200 million years ago, the common ancestor of water-walking insects (Heteroptera, Gerrhormorpha) invaded water surfaces and radiated into a diverse array of niches, from shorelines to open oceans. This ecological transition and specialization is associated with an array of adaptive changes that enabled these insects to support their body weight and generate efficient propulsion on the water surface. Our recent effort in developing state of the art tools of gene function analyses across multiple species, along with the on going sequencing of genomes and transcriptomes, establishes this group as a sustainable model for integrative studies. This approach on water walking insects will provide a fresh and unbiased insight into the developmental genetic mechanisms underlying species diversification within the context of their ecological environment. This will constitute a step forward in evo-devo studies by providing the link between molecular function, phenotypes, and a concrete measure of fitness associated with morphological evolution.

Epigenomics Session

9 - Expression of Alternative Splice Variants in the Wing Polyphenism of the Pea Aphid

Chaffee, Mary; Brisson, Jennifer

Many insect species express phenotypic plasticity, in which the same genotype produces multiple phenotypes in response to environmental conditions. An extreme form of plasticity is known as polyphenism, where distinct morphs are produced. The pea aphid (*Acyrtosiphon pisum*) expresses a wing polyphenism where migratory (winged) and reproductive (wingless) morphs exist during the summer months when the pea aphid reproduces asexually. When an asexual, wingless, female is subjected to stressful conditions such as crowding, deteriorating plant quality, and predator presence, she begins producing offspring that will be winged as adults. The winged offspring, although phenotypically different from their wingless mother, are genetically identical to her. In addition to the different phenotypes, the morphs are also transcriptionally distinct. One method of creating diverse transcriptomes is alternative splicing, which produces different isoforms of a gene. Multiple gene isoforms at different expression levels may be important for the maintenance of morph-specific traits. I hypothesize that differentially expressed gene isoforms from alternative splicing are important for morph differences and are the result of an epigenetic mechanism, differential methylation of DNA. In this study I have begun characterizing alternatively spliced genes in the pea aphid. I will present data on 12 genes (six non-differentially expressed and six differentially expressed genes between morphs) that are alternatively spliced. Two alternative splice variants in two genes (Chico and a serine protease) show significant differential expression between the morphs. In future, I will look for a causative agent, which I hypothesize to be DNA methylation. This study begins to characterize the molecular basis of the wing polyphenism in the pea aphid. How DNA methylation can cause alternative splicing is just beginning to be studied, but what that alternative splicing can do to an organism is unknown. My work is novel because it starts analyzing the direct cause and effect of epigenetic control in insect polyphenisms. This is done by studying both the expression of alternative splicing between morphs and in future what causes the alternative splicing.

10 - The function of DNA methylation in insects

Hunt, Brendan; Glastad, Karl; Yi, Soojin; Goodisman, Michael

Epigenetic inheritance plays a fundamentally important role in developmental plasticity. One of the most important and widely conserved forms of epigenetic information is the methylation of genes. However, the function of intragenic DNA methylation remains poorly understood. In this talk, I present research aimed at understanding the function of DNA methylation in insects. Recent studies have demonstrated that DNA methylation is targeted to active, ubiquitously-expressed genes. Moreover, DNA methylation patterns in insects are highly conserved among taxa. DNA methylation has also been implicated in messenger RNA splicing variation within species. Interestingly, DNA methylation is frequently associated with other types of epigenetic information, such as specific modifications to histone proteins. Overall, current research suggests that DNA methylation is a single component of a conserved, integrated, multi-layered epigenetic and regulatory landscape in insect genomes.

11 - Parent of Origin Effects on the Gene Expression in the Honey Bee

Hunt, Greg; Kocher, Sarah; Tsuruda, Jennifer; Emore, Christine; Arechavaleta, Miguel; Robles-Rios, Carlos; Grozinger, Christina; Queller, David; Strassman, Joan

Genomic imprints silence one parental allele through intergenic CpG islands or histone modifications in mammals and plants. However the phenomenon is largely unstudied in invertebrates, which have primarily gene body methylation that is not associated with gene silencing. The discovery of a paternal effect on stinging behavior motivated us to look for parent-of-origin (PoO) effects on transcription. We sequenced RNA transcripts from reciprocal hybrid workers derived from crosses between one European and Africanized honey bees, and genomic DNA from the parents, as well as one of the two F1 families. We identified heterozygous single nucleotide polymorphisms (SNPs) that were identical in both families and used read counts to determine whether the maternal allele or paternal allele was overexpressed. About 1,250 to 2,400 transcripts could be analyzed in larvae, adults and brains. We identified 150, 120 and 95 transcripts showing parentally-biased transcription in larvae, adults and brains. Surprisingly, about 90% of transcripts showing PoO effects were maternally biased. Results were asymmetric between the two reciprocal F1 families. The family with European maternity (EA family) had many more transcripts that were highly maternal/European in expression than the AE family. The high maternal bias and asymmetric results suggest that mitonuclear interactions affect allele-specific expression in the honey bee and may have been a factor in the evolution of PoO effects. Cellular OXPHOS metabolism and associated gene networks may influence hybrid fitness and could help to explain the success of Africanized bees in tropical America. However, paternal expression of some transcripts, particularly in brains suggest that the kinship theory of genomic imprinting may also explain our results.

12 - DNA methylation in the primitively social wasp, *Polistes dominulus*

Weiner, Susan; Geffre, Amy; Temple, Jon; Toth, Amy

Recent work in many social animals, from bees to rats to humans, suggest a key role of DNA methylation in the epigenetic regulation of social phenotypes. In highly social honey bees, DNA methylation is important in nutritionally-dependent queen and worker caste differentiation in early development. We investigated the role of DNA methylation in *Polistes*, a primitively eusocial wasp with more flexible caste differences that has long been a model system for studying social evolution in insects. Through recent genome sequencing efforts in *Polistes dominulus*, we have found potentially unusual elements in the DNA methyltransferases. However, through AFLP analysis, we found that genome-wide that DNA methylation levels in *P. dominulus* are among the highest known for any social insect. In addition, we have confirmed from our AFLP data that there are significant differences between castes in methylation patterns in *P. dominulus*. We also found that inhibiting DNA methylation in early development by treating larvae with the methylation inhibitor zebularine can affect whether they become members of the worker or queen caste as adults; zebularine treated wasps showed more worker-like traits, and an increase in foraging and nest work behaviors. We will also present some early results from bisulfite sequencing and transcriptome sequencing looking at the patterns of genome-wide methylation and gene expression. These results suggest DNA methylation may play an important role in caste phenotypes even in primitively social species, and thus may have been important in the evolution of sociality in insects.

Comparative Genomics

13 - Orthology-based genome annotation and interpretation

Waterhouse, Robert

The rapidly increasing rate of sequencing of arthropod genomes means that comparative genomics approaches are becoming ever-more powerful as tools to improve and extend genome annotation and interpretation for newly-sequenced species. Orthology delineation is a cornerstone of comparative genomics, offering qualified hypotheses on gene function by identifying “equivalent” genes in different species. This provides the means to begin to interpret characteristic genome biology traits of a species or clade, highlighting genomic commonalities and differences that underlie their diversity. The success of such interpretative analyses relies on the comprehensiveness and accuracy of the input data - genome assemblies and their gene annotations. Here too, orthology delineation provides a rich source of data to assess the quality and completeness of genome assemblies and gene annotations, to prioritize improvements, and to ensure that initial “drafts” develop into high-quality resources that benefit the entire research community. With more than 55 species, OrthoDB (www.orthodb.org) offers the most comprehensive orthology resource for arthropod comparative genomics, helping researchers to make the most of their newly-sequenced arthropod genomes.

14 - The genome and germline of the emerging model crustacean *Parhyale hawaiiensis*

Winchell, Christopher; Patel, Nipam

The marine amphipod *Parhyale hawaiiensis* is becoming a recognized model for comparative embryology and the developmental-genetics of arthropod body plan evolution. Recently available genomic resources (a partially sequenced BAC library, two maternal/embryonic transcriptomes, and a high-density microarray) serve to advance *Parhyale* as a model system, but a complete genome sequence is necessary to better infer, for example, the cis-regulatory changes contributing to the diversification of arthropod morphology and developmental mechanisms. This talk will, first, briefly describe our approach and progress toward sequencing and assembling the *Parhyale* genome, as well as draw some basic comparisons between its genome and those of other animals. Second, the bulk of the talk will present our preliminary results and ongoing effort toward characterizing the remarkable phenomenon of germline replacement in *Parhyale*. We have found that ablation of *Parhyale*'s germline progenitor cell (specified autonomously in the early embryo), is compensated for by the formation of a *de novo* germline during mid to late juvenile stages. *Parhyale* thus possesses two mechanisms of germline specification: during normal development, the germline forms via topographic restriction of maternally provided determinants, and during “contingency-based” development, a lost germline is replaced post-embryonically via an inductive mechanism effecting soma-to-germline stem cell transdifferentiation. In no other animal has this particular flexibility been demonstrated. To gain insight into the replacement process, we are using transgenesis-mediated lineage tracing to identify the embryonic source of the replacement germline. We are also using transgenesis techniques to implement a conditional, cell type-specific ablation system, which will allow us to test whether germline replacement is possible in adults. Through transcriptome analysis of intact versus germline-ablated gonads, we hope to illuminate the signaling pathways and chromatin remodeling factors that drive germline replacement.

15 - Evolutionary transcriptomics associated with developmental color switching in an adaptive radiation of Hawaiian *Tetragnatha* spiders (Araneae: Tetragnathidae)

Brewer, Michael; Croucher, Peter; Gillespie, Rosemary

Hawaiian *Tetragnatha* spiders have undergone an adaptive radiation resulting in various life history strategies and associated color morphs. Within the most well studied lineage, the “spiny-leg” clade, all 17 species have abandoned building an orb web characteristic of most species in the family and adopted a wandering, active hunting strategy. Further niche partitioning has resulted four easily distinguishable ectomorphs readily identified on the basis of color (Green, Maroon, Small Brown, and Large Brown) which corresponds to diurnal refuge substrate type (green leaves, maroon mosses, brown twigs, and brown branches). Among the basal clades on Kauai and Oahu, two species (*T. kauaiensis* and *T. polychromata*) exhibit a maturity-associated shift in refuge substrate and corresponding body color: leaves/Green to mosses/Maroon. This developmental switching is lost in a sister species on Oahu, and in all the more derived clades on younger islands. To investigate the genomic underpinnings of this phenotypic shift, we have sequenced the transcriptomes of six specimens representing four species in the basal clades: two *T. kauaiensis* (Green & Maroon morphs), one *T. polychromata* (Green Morph), one *T. tantalus* (Green), and two *T. perreirai* (Maroon). Among the predicted ORFs at least 50 amino acids in length, 2,647 putative orthologs were found in all specimens via reciprocal best-hit BLAST searches (e-cutoff= 1e-20). Because *T. kauaiensis* and *T. polychromata* are not sister species, we could test whether genes show differing selective regimes among lineages and, if so, whether they are associated with phylogeny or the color-switching condition. Additionally, phylogenies were reconstructed for all loci using both amino acid residues and nucleotide data and compared to the species relationships. Gene tree versus species tree discordances have the potential to reveal loci under strong selection associated with the color-switching life history. Candidate loci from both the selection regime and phylogenetic analyses were identified representing targets for downstream analyses of the genetic mechanisms controlling color-switching and associated life history characteristics.

16 - Organization and evolution of piRNA clusters in *Anopheles gambiae*

George, Phillip; Vaury, Chantal; Jensen, Silke; Sharakhov, Igor

The Piwi-interacting RNA (piRNA) pathway is an important mechanism in the defense against transposable element (TE) mobilization in many species including *Drosophila melanogaster* (the fruit fly) [1], *Aedes aegypti* (the Yellow Fever mosquito) [2], and *Mus musculus* (the mouse) [3]. In *Drosophila*, it has been shown that clusters of piRNA produce transcripts that, when interacting with PIWI proteins, create a complex that can recognize and silence retrotransposable elements in the germ line. In *Aedes* mosquitoes and the mouse, the piRNA clusters appear to involve a higher number of protein coding genes. The aims of our study were 1) to see if the piRNA mechanism in *Anopheles gambiae* is more closely related to the *Drosophila* pathway or to the *Aedes* pathway and 2) to test if incipient species of *An. gambiae* differ in the structure of piRNA clusters. The recent emergence of two molecular forms, M and S, as incipient species of *An. gambiae* has provided a unique opportunity to explore how the piRNA mechanism has diverged in terms of piRNA cluster content and positioning. The M and S forms are morphologically indistinguishable, yet behaviorally and ecologically dissimilar. We hypothesize that the piRNA transcripts generated by these forms may differ to combat the form-specific TE mobilization. We sequenced small RNAs from M (Mali) and S (Zanu) forms of *An. gambiae*, and have created a database of uniquely mapping piRNAs using the NucBase software. To identify clusters of piRNAs, all unique piRNAs have been mapped to the PEST reference genome. The most abundant clusters of piRNAs were found primarily in high TE-content areas—the intercalary and pericentromeric heterochromatin. Our results demonstrate that piRNAs, much like in *Aedes* and *Drosophila*, are present and active in Anopheline mosquitoes. Cluster locations and content suggest that the piRNA pathway in *Anopheles* compares more favorably to the *Drosophila* pathway than to the *Aedes* pathway. The top 15 clusters identified in *Anopheles gambiae* potentially produce ~74% of the total number of piRNAs; all 15 of these clusters map primarily to TE vestiges. Our study has also detected divergence in piRNA sequences and cluster composition between the M and S forms. Our data indicate that although speciation between the two forms is recent, there are differences in TE vestiges that make up the clusters, as well as the piRNA sequences that can be present and absent when mapped to their respective genomes. We hypothesize that the defense mechanism in the two incipient species has begun to rapidly evolve to protect its respective genome against novel TE invaders that have not incorporated into both populations.

References:

1. Brennecke, J., et al., *Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila*. Cell, 2007. 128(6): p. 1089-103.
2. Arensburger, P., et al., *The mosquito Aedes aegypti has a large genome size and high transposable element load but contains a low proportion of transposon-specific piRNAs*. BMC Genomics, 2011. 12: p. 606.
3. Aravin, A.A., et al., *A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice*. Mol Cell, 2008. 31(6): p. 785-99.

Systems Biology/Population Genomics

17 - Population genomic inference from a global diversity reference panel of *Drosophila melanogaster* *Clark, Andrew*

The *Drosophila* Genetic Reference Panel has demonstrated the utility of a reference set of *Drosophila* lines for the analysis of genetics of complex traits. By constructing a set of lines from 5 globally distributed populations, and applying genomics methods to score sequence and expression variation, we have developed an expanded resource for asking questions about global differentiation and population history. After introducing the genetic variation among these lines, this talk will present applications of these lines in understanding differences in flight, energy metabolism and lipid composition.

18 - Global analysis of the dorsal-ventral patterning regulatory network in the wasp *Nasonia vitripennis* using quantitative transcriptomics. *Lynch, Jeremy; Oezueak, Orhan; Buchta, Thomas; Roth, Siegfried*

Gene regulatory networks underlie developmental patterning and morphogenetic processes, and changes in the interactions within the underlying GRNs are a major driver of evolutionary processes. One of the most thoroughly characterized GRNs is the dorsal-ventral patterning system of the *Drosophila* embryo. Using this as a starting point, we have endeavored to characterize the DV system of the wasp *Nasonia vitripennis*. This wasp has convergently evolved a mode of embryonic development similar to that of the fly, and it is of interest to know whether the similarity at the gross level also extends to the molecular level. Taking advantage of our ability to produce dorsalized and ventralized embryos, and combining this with quantitative next-generation sequencing, we have identified a set of over 200 genes that appear to be differentially regulated along the DV axis of the wasp embryo. This set includes many of the genes identified in a similar experiment performed in *Drosophila*. Crucially, we have also identified a set of *Nasonia* genes with distinct expression patterns that are not expressed in the fly embryo. We propose that at least some of these genes were recruited in the wasp to carry out the unique morphogenetic movements and patterning processes that occur in the wasp embryo at gastrulation. The general strategy we have employed is likely to be widely applicable both in terms of species, and in biological processes.

19 - Genetic pathways induced by mating have a key role in the reproductive biology of *Anopheles gambiae* *Mitchell, Sara; Kakani, Evdoxia; Mariezcurrena, Ainhoa; Shaw, W. Robert; Teodori, Eleonora; Gabrieli, Paolo; Baldini, Francesco; Catteruccia, Flaminia*

Female *Anopheles gambiae* mosquitoes exhibit major behavioural and physiological changes in the first twenty-four hours after mating, including increased egg development and laying, and refractoriness to further mating. To investigate the molecular basis of this response we performed a tissue specific transcriptional time-course of five female tissues including the female lower reproductive tract (LRT) using whole-genome microarrays. The LRT samples comprised the atrium, in which the male deposits seminal secretions in the form of a mating plug, the spermatheca, an organ for sperm storage, and the parovarium, a secretory gland of unknown function. Tissues were collected at three time-points after mating and compared to those from age-matched virgins, with the aim to identify not only single genes but also gene networks that respond to mating. After microarray analysis, performed in the R environment using linear modelling, a total of 1109 genes showed differential expression with mating in the LRT over the three time points. GO and enrichment analysis (DAVID) highlighted a number of functional groups and pathways significantly enriched in the dataset, including an early up-regulation of peptidases, potentially involved in male mating plug processing, and putative lipid transporters with implications in oocyte provisioning. Later responses were characterised by an induction of the proteasome pathway, indicative of high protein turnover, V-Type ATPases with a potential role in epithelial transport, and genes involved in energy metabolism suggestive of high energy demands in mated females. Functional RNA interference analyses of a number of mating-induced transcripts are revealing a role for these genes in key aspects of female physiology, including egg development and sperm viability. All together these results identify previously unknown genetic pathways important for the reproductive biology of *An. gambiae* mosquitoes, and provide novel targets for future vector population control strategies in the field.

Ecological Genomics

20 - Dissecting mosquito host-seeking behavior through loss-of-function genetics

Vosshall, Leslie

Female mosquitoes of some species are generalists and will blood-feed on a variety of vertebrate hosts, whereas others display marked host preference. *Anopheles gambiae* and *Aedes aegypti* have evolved a strong preference for humans, making them dangerously efficient vectors of malaria and Dengue haemorrhagic fever. Specific host odours probably drive this strong preference because other attractive cues, including body heat and exhaled carbon dioxide (CO₂) are common to all warm-blooded hosts. Insects sense odours via several chemosensory receptor families, including the odorant receptors (ORs), membrane proteins that form heteromeric odour-gated ion channels comprised of a variable ligand-selective subunit and an obligate co-receptor called Orco. Here we use zinc-finger nucleases to generate targeted mutations in the orco gene of *A. aegypti* to examine the contribution of Orco and the odorant receptor pathway to mosquito host selection and sensitivity to the insect repellent DEET (N,N-diethyl-meta-toluamide). orco mutant olfactory sensory neurons have greatly reduced spontaneous activity and lack odour-evoked responses. Behaviourally, orco mutant mosquitoes have severely reduced attraction to honey, an odour cue related to floral nectar, and do not respond to human scent in the absence of CO₂. However, in the presence of CO₂, female orco mutant mosquitoes retain strong attraction to both human and animal hosts, but no longer strongly prefer humans. orco mutant females are attracted to human hosts even in the presence of DEET, but are repelled upon contact, indicating that olfactory- and contact-mediated effects of DEET are mechanistically distinct. We conclude that the odorant receptor pathway is crucial for an anthropophilic vector mosquito to discriminate human from non-human hosts and to be effectively repelled by volatile DEET.

21 - Complexity in the Function and Evolution of Insect Immunity

Lazzaro, Brian

Individual variation in resistance to infection is ubiquitous in human, plant and animal populations. This variation can have both genetic and non-genetic origins, or, critically, can stem from an interaction between genetic and environmental factors. Using genetic and environmental manipulations, we demonstrate that both dietary nutrition and reproductive status affect defense quality in *Drosophila*, even though neither of these is expected to act through the canonical immune system. Pleiotropy between seemingly unrelated components of physiology is important because such linkages constrain the evolution of all traits involved. Natural *D. melanogaster* populations harbor genetic variation for immunological sensitivity to reproductive status and dietary perturbation, indicating polymorphism in the genes linking nutritional assimilation and reproduction to defense against infection. Establishing the genetic basis for these linkages and the mechanisms underlying genotype-specific responses to environment is critical to understanding the physiological and evolutionary entirety of defense.

22 - Related insects show differing amounts of population differentiation and localization of transcribed genes in response to climate

Hellmann, Jessica; O'Neil, Shawn; Emrich, Scott; Dzurisin, Jason; Williams, Caroline

Population differences may determine geographic range shifts and adaptive evolution under climate change. Local adaptation in peripheral populations could preclude or slow range expansions, and populations with different genetic make-up could have distinct trajectories that produce complex spatial patterns of population change. To investigate the genetic extent of local responses to climate change, we exposed poleward-periphery and central populations of two Lepidoptera to reciprocal, common-garden climatic conditions and compared whole-transcriptome expression. We found significant expression differences between populations in both species. Several hundred genes, including genes involved in energy metabolism and oxidative stress, responded in a localized fashion in the species that exhibits greater population structure and local adaptation. Expression levels of these genes are most divergent in the same environment in which we previously detected phenotypic divergence in metabolism. By contrast, we found no localized genes in the species with higher gene flow, reflecting the lack of previously observed local adaptation. These results suggest that population differences do not generalize easily, even for related species living in the same climate, but some taxa deserve population-level consideration when predicting the effects of climate change.

23 - Genomic and transcriptomic regions conferring saltwater tolerance in anopheline mosquitoes
Smith, Hilary; White, Bradley; Kundert, Peter; Romero-Severson, Jeanne; Andolfatto, Peter; Besansky, Nora

Larval tolerance of saltwater is interspersed among genera and even members of species complexes in mosquitoes, including many vectors of disease. Here, we report QTL mapping and multiplexed shotgun genotyping to investigate the molecular basis of saltwater tolerance of malaria vectors within the *Anopheles gambiae* cryptic species complex. We genotyped 384 backcrossed progeny from crosses of the freshwater species *An. coluzzi* (formerly *An. gambiae* s.s. M form) and the saltwater-tolerant taxon *An. merus*. Analyses from Bayesian interval mapping and random forests yielded four genomic regions on autosomes 2 and 3 associated with saltwater tolerance. Homozygosity for the allelic form of the *An. merus* parental genotype is needed across at least two regions to confer tolerance, but itself does not guarantee survivorship in saltwater. The results are consistent with an additive or epistatic mode of inheritance on the autosomes. Our findings have ramifications for understanding the process of convergent evolution of saltwater tolerance in the *An. gambiae* complex, such as the existence of fungible genes or gene products allowing for multiple evolutionary paths. Our results are complemented with the investigation of differential gene expression via RNA-Seq analysis, comparing mRNA levels of *An. coluzzii* and *An. merus* exposed to freshwater (reverse osmosis water), or to artificial seawater.

Posters

i5k

24 - Progress on insect genome sequencing at Illinois

Robertson, Hugh

We are undertaking the sequencing of several insect genomes from Hymenoptera, Diptera, and Coleoptera using an all-ILLUMINA approach. While drafts for several genomes have been completed, these are relatively small (less than 500 Mbp), for example the wheat stem sawfly *Cephus cinctus*. Two large genomes, those of the apple maggot fly *Rhagoletis pomonella* (1.5 Gbp) and the corn rootworm beetle *Diabrotica virgifera* (2.6 Gbp) are proving difficult. I will report on our progress on these and other insect genomes.

25 - Hymenoptera Genome Database

Childers, Christopher; Reese, Justin; Bennett, Anna; Hagen, Darren; Elsik, Christine

The Hymenoptera Genome Database (HGD; <http://HymenopteraGenome.org>) provides informatics resources for hymenopteran species. HGD incorporates data from three *Apis* species (*A. mellifera*, *A. florea*, *A. dorsata*), two bumble bee species (*Bombus impatiens*, *B. terrestris*), the parasitoid jewel wasp (*Nasonia vitripennis*) and nine ant species (*Acromyrmex echinator*, *Atta cephalotes*, *Camponotus floridanus*, *Cardiocondyla obscurior*, *Harpegnathos saltator*, *Linepithema humile*, *Pogonomyrmex barbatus*, *Wasmannia auropunctata* (Roger) and *Solenopsis invicta*). This phylogenetic group spans a distance of 200 million years, and the continued incorporation of new species into HGD improves the power of comparative genomics, and facilitates research efforts toward increased understanding of biological and behavioral processes. Datasets include genome assemblies and mapped features including protein homologs, cDNA sequences, non-coding RNA sequences, RNA-Seq data and genetic markers. Gene features generated using computed prediction algorithms and manually-annotation methods are also available for each species. All way comparisons between the official gene sets (OGS) for each species at HGD and the FlyBase gene set for *Drosophila melanogaster* were used to compute ortholog relationships between species. Users may access the data via genome browsers (GBrowse), web-based BLAST searches and direct file downloads. The genome browsers leverage the pre-computed ortholog information to link between the species, allowing users to navigate across the genomes at HGD. Database cross-references directly connect features to external resources. BLAST queries to a reference assembly are linked to the genome browsers, allowing visualization of BLAST hits in the context of the genome. We have recently deployed WebApollo at HGD to support community annotation.

26 - Additional evidence of genome size diversity in Arthropods

Hanrahan, Shawn; Johnston, J. Spencer

Arthropod genome size diversity remains poorly sampled, with far less than one percent of species surveyed. Herein are presented over 300 estimates that allow a first glimpse into the extent of genome size variation. To date, the genome size for these species has ranged from 98 Mbp for a midge (Chironomidae) all the way up to 18,000 Mbp for a grasshopper (Acrididae). The upper range has been exclusively found within the Orthoptera, with many species uniquely surpassing 10,000 Mbp. Other orders contain a wide range of estimates, and while many estimates group around a modal value within each order, additional sampling finds outliers that strongly deviate from other known estimates. Examples of such deviation include a Tetrigidae with an estimate of 870 Mb, as compared with the range of 6,000 – 18,000 Mb in the other grasshoppers. Another is the *Euglossa spp.* orchid bees which include estimates as high as 4,000 Mb, as compared with most of Hymenoptera with estimated genome sizes under 600 Mb. The full range of genome size has yet to be discovered for any order. It is evident that this range will change as additional sampling is performed. Because genome size will directly correlate with the cost of any sequencing project, a genome size estimate is an important part of any complete sequencing project. Moreover, an accurate estimate is essential at the end of a complete sequencing effort as a measure of the completeness of that effort. Closely related species may differ very significantly in genome size; and knowledge of the genome size of a relative can be a poor predictor of the genome size of a given species. We recommend estimation of genome size for several individuals selected from the strain used for sequencing and suggest that sexes be scored separately. Our lab is pleased to offer genome size estimates for any fresh or frozen arthropod sent to us and correctly identified to the species level.

27 - The HGSC i5K Arthropod Genomes Pilot: Thirty Arthropod Whole Genome Assemblies

Qu, Jiaxin; Liu, Yue; Dugan, Shannon; Worley, Kim; Gibbs, Richard; Richards, Stephen

The i5K pilot is a project to sequence the genomes of 30 Arthropods to learn the procedures necessary to reach the i5K 5,000 species goal. The variety of genome sizes, DNA homozygosity, and other species specific features provide valuable information for the accomplishment of this task.

We describe here our sequencing plan, assembly strategy, assembly software pipeline, efforts to improve automation and our successes and failures. Our current pipeline includes sequence preparation steps, the ALLPaths-LG assembler (using the haploidify option), Atlas-Link, and one or multiple rounds of Atlas-GapFill followed by a gap fill consolidation step.

The process is susceptible to failure with large amounts of polymorphism in the input DNA sequence, but is generally robust, in most cases produces publishable assemblies. Contig N50 statistics range from as high as 60kb to a low of 3kb, but the majority of assemblies have achieved > 10kb needed for gene annotation and publication. Scaffolds generated with high quality 8kb mate pair data are generally in the Mb range.

Finally, we present our views on expanding this pipeline to 5,000 species and advice on using this assembly pipeline for your own species.

28 - A roadmap for a satellite i5k genome portal

Poelchau, Monica; Evans, Jay; Moore, Gary; Liu, Simon; McCarthy, Susan; Gourley, Donald; Hackett, Kevin

The i5k initiative is an umbrella project that aims to sequence 5,000 arthropod genomes over the next few years. Dedicated genome portals are necessary to display detailed information on genome features, allow for interactive exploration of the genome, and enable manual editing of genome annotations by the broader community. However, establishing and maintaining a genome portal is often beyond the expertise of individual labs, and continued, long-term maintenance of the portal can be prohibitively expensive. Here, we propose one such portal dedicated to a subset of the arthropod genome projects arising from the i5k initiative, which we have named the 'i5K Workspace'. A main entry page will provide access to a dedicated website for each genome project. The portal will implement open-source software to enable genome viewing via GBrowse and JBrowse, searchable gene pages, and BLAST searches. We plan to integrate WebApollo into the portal, allowing researchers to both visualize manually annotate gene models. Servers will be provided by the National Agricultural Library (NAL) and maintained by support staff. Further functionalities may be added to the portal depending on future funding sources. A centralized access point for genome information arising from the i5k initiative will greatly facilitate comparative studies of arthropod genomics. We solicit feedback from the i5k community on other desired features to create a useful resource for all.

Emerging Genomes

29 - Building a reference transcriptome for analysis of global gene expression in *Frankliniella occidentalis*, an agricultural crop pest and insect vector of tospoviruses

Schneweis, Derek; Whitfield, Anna; Rotenberg, Dorith

Frankliniella occidentalis, the western flower thrips, is a member of the family Thripidae within the order Thysanoptera, an insect order comprised of over 7,000 species of thrips. *Frankliniella occidentalis* causes extensive damage to numerous food and fiber crops worldwide by direct damage and through transmission of *Tospovirus* species, including the type member *Tomato spotted wilt virus* (TSWV). In recent years, our lab generated a collection of transcript sequence resources (Sanger ESTs and 454 contigs) and assembled a partial transcriptome of coding RNA to enable studies of gene function in *F. occidentalis*. In an attempt to assemble a more comprehensive transcriptome for global expression analysis of genes involved in metabolism, development, and vector competence, we are currently constructing a *de novo* assembly of RNAseq reads obtained from four biological replications of an experiment that includes larval, pre-pupal, and adult developmental stages of non-infected and TSWV-infected *F. occidentalis*. In total, 24 RNAseq libraries were generated with TruSeq chemistry and multiplex-sequenced (by bio-replication) using Illumina Hi-Seq 2000. The resulting data set includes 68,441 Mb single-end reads with average Phred quality scores ranging from 33.1 to 34.5. Our pipeline for assembly includes trimming and removal of low quality sequence reads, *de novo* transcriptome assembly using the Trinity pipeline, and generating a high quality hybrid assembly of the RNAseq-derived assembly, 454-contigs, and Sanger EST sequences using MIRA. The comprehensive coverage and assembly of the transcriptome of *F. occidentalis* will subsequently be used as a reference transcriptome for quantitative RNAseq analysis of global gene expression in response to tospovirus infection. It will also enable the pursuit of research questions pertaining to thrips biology, development, vector competence, and genetics of virus transmission as well as pave the way for genomic studies of other Thysanopterans.

30 - The proliferation of long terminal repeat retrotransposons within the *Diabrotica virgifera virgifera* genome

Coates, Brad; French, B. Wade; Sappington, Thomas

The Western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is a major pest of cultivate corn in the United States and Europe that has evolved resistance to chemical and transgenic insecticides. A project to sequence and assemble the 2.58 Gb (2.80 pg) *D. v. virgifera* genome has been initiated, but difficulties have been encountered due to the large size and frequency of repetitive DNAs with high sequence similarity. The proliferation of retrotransposons contributes to increased genome sizes, and can affect the structure and expression of eukaryotic genes. In attempts to understand the structure of this emerging genome, full-length long-terminal repeat (LTR) *gypsy*, *copia*, *Bell/Pao*, and DIR group transposons were annotated from *D. v. virgifera* bacterial artificial chromosome (BAC) sequence and a low coverage whole genome assembly. Sequence divergence within each *gypsy*, *copia*, *Bell/Pao*, and DIR lineage suggest that LTR retrotransposons are ancestral within the *D. v. virgifera* genome, but results from mapping reads back to LTR retrotransposon-containing contigs indicate differential rates and recency of proliferation. These data are useful for understanding the mechanism of genome size increases and will be important for the annotation of repetitive elements within the eventual genome assembly.

31 - Annotation and expression of glycolysis, TCA and nonessential amino acid biosynthesis pathway genes in a galling insect

Shreve, Jacob; Shukle, Richard; Subramanyam, Subhashree; Williams, Christie

The Hessian fly, *Mayetiola destructor* (Say) (Diptera:Cecidomyiidae), is a model species for gall forming insects and is also a major pest of wheat in the southeastern United States. Using the recently available genome sequence for this insect we have annotated the genes in the glycolysis, TCA and nonessential amino acid biosynthesis pathways. Additionally, we have documented the expression of the genes in these pathways during development. Hessian fly larvae infest seedling wheat between developing leaves at the base of the whorl and on susceptible wheat larvae induce major systemic changes that include irreversible stunting and development of a nutrient rich tissue layer that nourishes the larvae. The hypothesis tested in this study is that expression of genes in the metabolic pathways and biosynthesis of nonessential amino acids will fluctuate in larvae on susceptible and resistant wheat. Results are discussed in the context of Hessian fly's interactions with susceptible and resistant wheat.

32 - Draft sequencing and assembly of two large insect genomes: the New Zealand giant weta and common stick insect

Twort, Victoria; Wu, Chen; Newcomb, Richard; Ross, Howard; Buckley, Thomas

We are using Illumina technology to generate draft genomes of two New Zealand endemic insects: the giant weta and common stick insect. The Poor Knights giant weta (*Deinacrida fallai*, Orthoptera) is one of the largest extant insects and is restricted to a small offshore island. The common stick insect (*Clitarchus hookeri*, Phasmatodea) is widespread through much of New Zealand and exhibits geographic parthenogenesis. The genomes of both these species are large, the stick insect and weta being 10 and 20 Gbp respectively. Our current draft assemblies have been obtained from a mixture of paired end (200 bp, 500 bp, and 1 Kbp) and mate pair libraries using SOAPdenovo. RNAseq data are also being obtained from both species from a variety of tissues to aid in genome annotation and to generate candidate genes. For example, we are comparing expression patterns in male and female antennae to detect candidate genes in mate recognition. These draft genomes along with comparative SNP and RNAseq data will be used to investigate a range of evolutionary questions and conservation genetics issues in these and related species.

33 - A first genome draft of an endoparasitoid wasp, *Cotesia plutellae*, which encodes a symbiotic polydnavirus

Lee, Dae-Weon; Kim, Yonggyun

With various parasitic factors, an endoparasitoid wasp, *Cotesia plutellae*, parasites young larvae of the diamondback moth, *Plutella xylostella*, and alters immune and developmental processes. The parasitic factors are divided into maternal and embryonic origins. Maternal factors include ovarian proteins, venom, and polydnavirus, while embryonic factors include teratocytes and wasp larvae. We do not fully understand the genetic characteristics of these parasitic factors, though all these factors are encoded in the genome of *C. plutellae*. Our current deep sequencing analysis of *C. plutellae* genome shed light on the origin of these parasitic factors. An isofemale line was selected through at least 20 successive generations. The haploid males were collected from this isofemale line and used for genome sequencing with both an Illumina HiSeq2000 and 454 pyrosequencing. Both sequencings read more than 37Gb with 62x coverage of an expected genome size (=239,950,975 bp), of which 37% sequences are estimated to be repetitive. Assembly was performed by constructing scaffolds with 454 pyrosequencing reads and filling gaps with Hiseq reads. A total of 21,030 scaffolds were constructed with an average size of 14,458 bp. In addition, 474,530 contigs (> 100 bp) were obtained. Using these scaffolds and contigs, 46,854 protein-coding genes were predicted. Each predicted gene contains an average of three exons. Almost 50% genes were annotated into different GO categories. Interestingly, 66 genes are predicted into protein-tyrosine phosphatases, some of which are viral and can be classified into a largest gene family of the symbiotic polydnavirus.

34 - A molecular population genetic survey of the Z-chromosome of the cotton bollworm, *Helicoverpa armigera*

Song, Sue; Robin, Charles; Oakeshott, John

The cotton bollworm, *Helicoverpa armigera*, is one of the most damaging pests to agriculture. Its range of host plants is wide and includes several economically important crops such as cotton, corn, chickpea and tomatoes. Successful polyphagy coupled with resistance to several classes of insecticides motivates whole-genome sequencing to advance the search for candidate genes or even new gene families involved in detoxification and herbivory. One strategy is to scan the genome for signals of a selective sweep, which requires prior knowledge of levels of nucleotide diversity and the extent of linkage disequilibrium in the species.

To characterise the decay of LD, we adopted a design that would allow us to obtain longer reads and circumvent the issue of gametic phase. We sequenced amplicons on the Z chromosome from barcoded females as they are the heterogametic sex, therefore individual Z chromosomes could be given their own sequence identifier allowing pooled sequencing.

We find that *H. armigera* harbours extremely high levels of nucleotide diversity ($\pi=0.06$) and that LD decays over relatively short distances, in comparison to human genomes. Identification of candidate genes in *H. armigera* could achieve some degree of success as the finer resolution allows us to home in on the causal SNP while the low LD could facilitate detection of recent selective sweeps.

35 - Gene-omes built from mRNA not gDNA: new, accurate and simple methods are effective for several Arthropods

Gilbert, Don

For the last 2 decades, complete gene sets have been predicted from gene signal statistics in genomic DNA. The advent of high quality, high volume transcript sequencing provides data suited to constructing genes without statistical guesses, from biological gene products. Informatics methods now have caught up to this data, to construct biologically accurate, measurably complete organism gene sets, or transcriptomes. Recently improved mRNA assembly methods of the EvidentialGene project (<http://arthropods.eugenes.org/EvidentialGene/>) are presented with several Crustacean and Insect examples. These include species of locust, whitefly, beetle, waterflea and shrimp, as well as vertebrate and plant species. These methods are relatively simple, rapid and biologically valid; simpler, quicker and better than genome-based predictions. While not yet in general practice, these are recommended as they yield large improvements to published mRNA assemblies or genome-based predictions. RNA assembly combined with genome-based modelling gives more complete answers, but gene-centric projects will benefit by allocating more effort to transcript sequencing.

36 - Next-generation transcriptome and analyses of differential gene expression in head tissues of West Indies fruit flies (*Anastrepha obliqua*) across different sexes and reproductive stages

Rezende, Victor; Lima, André; Campanini, Emeline; Nakamura, Aline; Oliveira, Janaína; Chahad-Ehlers, Samira; Sobrinho Júnior, Iderval; Brito, Reinaldo

Tephritid fruit flies of the genus *Anastrepha* are of great economic importance because of the damage they inflict to the culture of diverse fleshy fruits. Some of the most important species in the genus belong to the “*fraterculus* group”. Species on this group are closely related and most are probably recently diverged. For several species in the group, the mechanisms of reproductive isolation are not yet consolidated, which facilitates the study of species differences and identification of important markers for the establishment of combat strategies. In order to investigate potential targets for evolutionary and pest control studies, we built several Next-generation sequencing (NGS) libraries from heads of male and female *Anastrepha obliqua* in different reproductive stages. These libraries were constructed from RNA extracted each from pools of 5 specimens and encompass the following contrasts: sexually mature virgin males and sexually mature recently-mated males, sexually mature virgin females, sexually mature recently-mated females, and post-ovipositing females. Each library was replicated with a separate pool of flies, generating 10 libraries which were used for analyses of transcriptome and gene expression among the five profiles considering differences in sex and reproductive stages. A total of over 160 million reads of paired-end sequences 100 bp long was generated by a single run on an Illumina HiSeq 2000. The Illumina reads were trimmed for quality in the 3' end for QV scores below 20, retaining reads with a minimum sequence length of 50, which trimmed between 7% and 11% of the reads. Furthermore, we only retained reads with more than 90% of the bases with an average QV over 20, which eliminated another 8% to 16% of the reads. The remaining 127 million reads were assembled together using the Trinity short read assembler and resulted in 100,973 contigs with an average length of 1,200 bp. Over 35,000 contigs had more than 1,000 bp and almost 20,000 had more than 2,000 bp, the longest of them being 17,802 bp long. These contigs were annotated using the Trinotate software, and blasted against several databases for functional annotation. We performed analyses of differential expression of the mapped reads on EdgeR version 3.0.8, which allowed us to detect more than 4,500 differentially expressed genes among the several contrasts we performed. Some of the important genes which are differentially expressed across reproductive stages are some odorant-binding proteins (such as Obp99c), Yolk proteins (Yp2), cholesterol and glutamate binding (mGluRA), protein binding (ninaE). These results have provided several important targets for investigation that will help us solve the problem of species differentiation of the group.

37 - Improving the Genome Assembly of the Mosquito *Aedes aegypti*

Juneja, Punita; Ho, Shwen; Ariani, Cristina; Pain, Arnab; Jiggins, Francis

The current assembly of the *Aedes aegypti* genome is composed of 4,758 supercontigs or scaffolds. The large size and repetitive nature of the *Ae. aegypti* genome has made it difficult to organize these scaffolds into chromosomes. We have produced the most complete chromosomal scaffolds to date by creating a genetic linkage map using RAD-seq to genotype individuals from a mapping population. Using this approach, we have assigned over 500 scaffolds and over 70% of genome sequence to chromosomes and ordered them based on our map. We have also identified a significant number of misassemblies in the current genome assembly. We demonstrate that our chromosomal scaffolds are accurate by QTL mapping *Ae. aegypti* resistance to *Brugia malayi* and recovering known peaks in expected regions.

38 - Genetics in Pink

Allen, Margaret

The lady beetle *Coleomegilla maculata* is a common generalist predator in most of North America. There is no concerted effort to sequence its genome, because it is neither a pest nor a biological control agent. However, the species is ecologically important and has become a useful subject of research. It is easy to find in nature and keep in the laboratory. To establish a foundation of gene sequence information, my lab has inbred a strain through seven rounds of isofemale selection. In the course of the selection process, interesting phenotypes have been observed, and some have been maintained in culture. These strains will be examined, along with progress toward transcriptome and genome sequencing.

39 - Genome size variation within and among *Collosobruchus maculatus* and its relatives

Johnston, J. Spencer; Gervais, Amy; Arnqvist, Göran

Collosobruchus maculatus (F.) is a serious pest of the cow pea (*Vigna unguiculata*), a staple food of East and West African countries. It is a particularly troublesome pest, as it feeds both on the plant and the stored seed. In an effort to help develop control measures for this pest species, the genome is being completely sequenced (GA, Uppsala, Sweden). We chose to complement this sequencing effort by estimating the genome size of males and females: first, in multiple strains of *C. maculatus* collected from throughout the world distribution of the species; and second, in multiple related species. An unexpected result to date is that the genome of *C. maculatus* is nearly twice that of closely related species. Pest species typically have small genomes, but this is clearly not true for *C. maculatus*. The two-fold increase in genome size suggests that *C. maculatus* may be the product of a recent polyploid event. If so, this possibility must be considered as part of the complete genome project. We hope further to extend the value of the collected data, by looking for correlations between the genome size and life history traits. We have found significant genome size variation among strains. We hypothesize that this variation will explain a significant proportion of the variation in life history observed across the world distribution of this species.

40 - Dipteran genome model resources

Minx, Patrick; Aksoy, Serap; Scott, Jeffery; McDowell, Mary Ann; Wilson, Richard K.; Weinstock, George; Warren, Wesley

Various species in the Dipteran order are of medical and economic importance. The *Glossina* (tsetse flies) are vectors of African trypanosomes, *Musca domestica* (house fly) is a pest species closely related to *Glossina*, that acts as a mechanical vector of important pathogenic microbes and Phlebotomine (sand flies) persist in transmitting protozoan, bacterial, and viral pathogens as a result of their blood-feeding behavior. To foster development of novel technologies to control disease transmission via these species it is necessary to build transcriptome and genome resources. Guided by NHGRI white papers we have selected, sequenced and in some cases assembled transcriptomes or genomes for 5 *Glossina*, and three related Dipterans, a non-vector obligate blood feeder (stable fly, *Stomoxys calcitrans*), a vector obligate blood feeder *Phlebotomus papatasi* (sand fly) and a non-blood feeding mechanical vector of numerous human pathogens *Musca domestica*. We will describe the methods to assemble these difficult genomes, and we examine repeat content, gene content, and provide basic assembly statistics for sand fly, house fly and *G. brevipalis*. The respective assembled genome lengths and N50 contig lengths were 363Mb, 5.8kb N50; 692Mb, 10.4kb N50; and 291Mb, 46.2kb N50. In addition, we have assembled transcriptome resource data from a variety tissue types from all eight Dipteran species described above.

We anticipate surveillance and health care programmes in the affected regions will greatly benefit from the insight gained in the study of these Dipteran genome models of disease transmission.

41 - *Spodoptera frugiperda* transcriptome analysis reveals conserved and specific developmental genes

Legeai, Fabrice; Gimenez, Sylvie; d'Alençon, Emmanuelle; Fournier, Philippe; Negre, Nicolas

Spodoptera frugiperda (Noctuidae) is a major agricultural pest throughout the American continent. Highly polyphagous yet commonly found devastating crops of importance such as corn, sorghum, cotton and grass. In addition, the Sf9 cell line, widely used in biochemistry for in vitro protein production, is derived from *S. frugiperda*. Many research groups are using *S. frugiperda* as a model organism to investigate questions such as plant adaptation, pest behavior or resistance to pesticides. In this study, we developed a reference transcriptome from various sources of RNA sequences obtained from *S. frugiperda* samples. We investigate the quality of this reference transcriptome and use it to detect different gene families involved in the early embryonic development. Expectedly we identified conserved developmental genes, but also identified genes that are specific of *S. frugiperda*. We demonstrated the effect of these genes in the embryonic development of *S. frugiperda* by RNAi treatment of the eggs. The reference transcriptome presented here will be a major resource to annotate the genes of the *S. frugiperda* genome currently in the assembly process.

42 - The foundation of subsociality and parental care in a beetle: Insights from the Burying Beetle genome and candidate genes

Cunningham, Christopher B; Meagher, Richard B; Moore, Allen J

The genomic basis of complex social behavior of insects has been addressed using several eusocial species. Despite what has been learned, a genome from an evolutionary intermediate between eusociality and non-social insects would be valuable but is not available. Our laboratory sequenced the genome of the subsocial beetle *Nicrophorus vespilloides* to investigate the molecular underpinning of parental care and complex social interactions, as well as bridge the gap between the eusocial and non-social insect genomes now available. Both sexes of *N. vespilloides* provide extensive care to offspring, including regurgitation of digested food into the mouths of begging larvae. Of an estimated 200Mb genome, we have assembled 66Mb with an N50 contig size of 25kb. This resource has allowed us to quickly pursue several candidate genes we predicted to influence parental care and social interactions in this species. Among other genes, we have been able to characterize the members of the octopamine system, a neurotransmitter that regulates aggression in many insects and arthropods. We have also begun to establish its pattern of expression during several breeding/caring behavioral stages. We believe that this species will provide important independent tests about the evolution and mechanisms of both sociality and parental care, and provide insight into the use of old genes in new behavior.

43 - Improved Photographic Map Of *Culex quinquefasciatus* Polytene Chromosomes As A Benchmark For Genome Physical Mapping

Unger, Maria F.; Sharakhova, Maria V.; Harshbarger, Adam J.; Glass, Patrick; Collins, Frank H.

Culex quinquefasciatus, a medically important mosquito, is the primary vector of several human pathogens including West Nile Virus, other arboviral viruses, and Lymphatic filarial worm. The JHB strain of *C. quinquefasciatus* originated from Johannesburg, South Africa, was used for sequencing. It was assembled into 3,171 supercontigs. However, the chromosomal location of most of these supercontigs remains unknown. We chose the 15 largest supercontigs, each spanning several million base pairs, and identified their genomic coordinates by means of Fluorescent in situ Hybridization (FISH). Two unique probes were designed to each supercontig, labeled with distinct fluorescent dyes and hybridized to polytene chromosomes. The results were then used to produce a physical map. The first photographic map of JHB *C. quinquefasciatus* polytene chromosomes from salivary glands was developed by McAbee et.al. in 2007. Using McAbee's work as a foundation we expanded on it by adding several new components: images of higher resolution were utilized to produce a map which show better details; more landmarks for each chromosomal arm were established to enable chromosome distinction; cytological inversions breakpoint boundaries have been determined; chromosomal arms have been straightened; and telomeres clearly shown. The improved map was created to help assign chromosomal coordinates to the supercontigs. The resulting set of supercontigs with assigned chromosomal coordinates and orientation (5'-3' or 3'-5') will be used to improve the *Culex quinquefasciatus* genome assembly. In the future, unified physical, genetic and optical maps will enable the creation of a golden path (sequence aligned to a chromosome, consisting of oriented supercontigs, with N-stretches inserted in the gaps). Other application of this study includes the development of markers to distinguish cryptic species in the *Culex pipiens* complex for population genetics studies.

44 - Genomic features of individual chromosomes in *Aedes aegypti*

Timoshevskiy, Vladimir; deBruyn, Becky; Severson, David; Sharakhov, Igor; Sharakhova, Maria

Widespread mosquito *Aedes aegypti* is the primary vector of the yellow fever and dengue viruses and also a convenient model for laboratory research. Among other mosquito species with sequenced genome, *Ae. aegypti* has the largest genome with the size of 1376 Mb and the highest density of repetitive elements in the genome. About 47% of its genome is represented by transposable elements (TEs). However, the distribution of various repetitive elements along the chromosomes of the mosquito remains unclear. Recently we reported the development of integrated linkage, chromosome, and genome map of 100 BAC clones carrying major genetic markers. BAC clones were placed and ordered on mitotic chromosomes using two-step fluorescent *in situ* hybridization (FISH) mapping. Here we present mapping data for additional 400 BAC clones. From all 500 BAC clones, which have been examined for their chromosome location, 449 we successfully hybridized and mapped to the chromosomes. A total of 294 genomic scaffolds or 619 Mb of *Ae. aegypti* genome were assigned to the particular bands on chromosomes. This study developed a low resolution chromosome map for 45% of *Ae. aegypti* genome: 70 (23%); 142 (48%); and 82 (29%) genomic supercontigs were assigned to the chromosomes 1, 2, and 3, respectively. Supercontigs were not oriented or ordered within chromosome bands. Using bioinformatics we examined the distribution of protein-coding genes, TEs and satellite DNA in three chromosomes of the mosquito. Chromosome 1 had the lowest gene density of 10.07 per 1 Mb and highest content of satellites (6.6%) and TEs (1715.1 per 1 Mb). Chromosome 2 had intermediate gene (11.87 per 1 Mb) and satellite (4.79%) densities and the minimal number of TEs per 1 Mb (1579.06). These values for chromosome 3 were 12.85, 4.68%, and 1604.90, respectively. Centromeric regions in all chromosomes demonstrated lower gene densities and higher content of satellites and TEs. These regions usually form small heterochromatic blocks on all three chromosomes. In addition, region 1q21-1q22 of chromosome 1, which is also characterized by bright staining with YOYO-1 iodide, demonstrated higher densities of satellites and TEs. We considered these 4 regions to be heterochromatin. Currently, the general picture of the distribution of genes, satellites and TEs is rather homogenous among the chromosomes. It does not display any extremely high peaks and low valleys. More detailed physical mapping is required for the better understanding of the relationship between DNA content and chromosomal banding patterns in chromosomes of *Ae. aegypti*. This information will contribute to our more complete understanding of the genome organization and function in the yellow fever mosquito.

45 -RNA-seq transcriptome analysis of *Spodoptera litura*: A preliminary study for identifying genes contributing to its polyphagy

Jouraku, Akiya; Kadono-Okuda, Keiko; Yamamoto, Kimiko; Konno, Kotaro; Yamamoto-Kihara, Mami

The genus *Spodoptera* (Lepidoptera: Noctuidae) contains more than 30 species, which is distributed widely in the world. Half of the species are destructive pests and most of them are polyphagous. The common cutworm, *Spodoptera litura* inhabits throughout Asia and Oceania from tropical to temperate zones. It is also found throughout the Pacific including Hawaii. It attacks a wide range of crops including soybean which is generally considered to be a most damaged crop by *S. litura* larvae in Japan. As a preliminary study to understand the mechanism of its polyphagy, we performed RNA-seq transcriptome analysis using two strains of *S. litura* with different degree of polyphagy. For each strain, we extracted nine RNA-seq samples of fifth instar larvae fed on different crops: artificial diets containing a protease inhibitor (PI) derived from soybean in concentrations of 0, 0.125, and 0.5 % (In *Bombyx mori*, the concentration of 0.125 and 0.5 % caused a serious developmental effect), two soybean strains which are susceptible and resistant to *S. litura*, cabbage, tomato, Taro, and sweet potato. After 48-hours feeding, the whole bodies of larvae were homogenized in Trizol (Invitrogen) for RNA extraction. High-throughput paired-end RNA sequencing was performed with Illumina HiSeq2000. The RNA-seq data from the above samples and other samples such as midgut, Malpighian tubules, and egg were de novo assembled for generating reference contigs. The reference contigs were used to identify differentially expressed genes of each whole body sample in comparison with PI-0% sample in each strain. The comparison revealed that (1) three gene families (serine protease, cuticle, and osiris) are remarkably up-regulated in most samples except for cabbage and Taro, (2) expression pattern of serine protease genes is especially different between crops.

46 - Preliminary data from the draft genome of the red-headed pine sawfly *Neodiprion lecontei*
Duong, Kim; Bagley, Robin; Leonberger, Adam; Linnen, Catherine

The red-headed pine sawfly, *Neodiprion lecontei*, is a well studied, herbivorous pest that occurs on multiple pine species throughout eastern North America. *Neodiprion* species are known to have an intimate and lifelong association with their host plants, and because many are considered forestry pests, detailed life history information is available for a large fraction of species. The genus is an emerging model system for research in evolutionary biology due to extensive variation within and between species in ecologically important traits like host use, morphology, and behavior. *Neodiprion* are also experimentally tractable and amenable to studies exploring the molecular and evolutionary mechanisms of these traits: species are relatively easy to find and collect in nature, colonies can be reared in the lab, and divergent populations and species are easily crossed. Here, we present preliminary data from the draft sequence of *N. lecontei*, assembled from Illumina sequencing reads obtained from the larval male offspring of a virgin female. Structural and functional annotation, genome wide analyses, and gene ontology analyses were performed on the genome and specifically for several gene families and functional classes of interest including those pertaining to social behavior, chemosensation, and digestion. The *N. lecontei* genome will be a valuable resource to help address fundamental questions in evolutionary biology and provides a basal Hymenopteran genome that can serve as an outgroup for more derived Hymenoptera like ants, bees, and wasps.

47 - Physical mapping of repeat-rich genomes of mosquitoes from subfamily Culicinae
Sharakhova, Maria; Timoshevskiy, Vladimir; deBruyn, Becky; Severson, David; Sharakhov, Igor

Culicidae family is subdivided into two subfamilies Anophelinae and Culicinae. Mosquitoes from both subfamilies are vectors of human diseases such as malaria; West Nile, dengue, yellow fevers; and lymphatic filariasis. To facilitate the development of new strategies for vector control, the genomes of several species of mosquitoes including *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*, have been sequenced. Mosquitoes from subfamily *Anophelinae* have well-developed polytene chromosomes that facilitate the successful physical mapping of their genomes: ~89% of *An. gambiae* genome was mapped to the chromosomes based on *in situ* hybridization. In contrast, mosquitoes from subfamily *Culicinae* are characterized by poor development of polytene chromosomes because of the high repeat content in their genomes. As a result, physical mapping of *Culicinae* genomes becomes challenging. The physical map developed for *Ae. aegypti* before our study included only 37 markers. This map was distance-based: each clone was placed to the chromosome depending on the distance from the telomere on the short arm (p-terminus), and it was the only physical map developed for the entire subfamily *Culicinae* in the past. However, because of genetic mapping effort ~31% of *Ae. aegypti* and ~9% of *Cx. quinquefasciatus* genome sequences were assigned to the chromosomes but without order and orientation. Here, we propose using mitotic chromosome band-based approach for the physical mapping of genomes from mosquitoes in subfamily *Culicinae*. We successfully used this approach for the placement of half of the *Ae. aegypti* genome to the chromosomes. Instead of previously used cell lines, which usually accumulate chromosomal rearrangements, our method utilized chromosomes from a live mosquito. We found that imaginal discs of 4th instar larvae are an excellent source of mitotic chromosomes. Idiograms for the mitotic chromosomes at early metaphase were developed based on the patterns of chromosomes stained with YOYO-1 iodide. Three chromosomes of the mosquito were subdivided into 23 regions and 94 subdivisions. Using fluorescent *in situ* hybridization (FISH), 500 BAC clones from the largest genomic supercontigs were assigned to the specific bands of idiograms. The BAC clone locations within the supercontigs were predicted by PCR analysis. BAC clones were directly labeled with Cy3 and Cy5 fluorescent dyes by nick-translation. Unspecific hybridization of the repetitive DNA was prevented by adding unlabeled C₀t1-3 DNA fraction to the probe in 1:20 ration. From all BAC clones, which were mapped, 106 were carrying previously mapped major genetic markers. These BAC clones were additionally ordered within each band by multicolor FISH because of the importance to link their genomic locations to the genetic locations of quantitative trait loci (QTL) related to pathogen transmission. The current study placed 45% of the *Ae. aegypti* genome to precise chromosomal positions and also for the first time combined cytogenetic, genetic and genome maps into one integrated physical map. Further application of this map will enhance the quality of the current genome assembly of *Ae. aegypti* and also will help to find the genomic locations of QTL that might be important targets for developing advanced genome-based strategies for vector/disease control. We propose using this mitotic chromosome band-based approach for the further physical mapping of *Cx. quinquefasciatus* and other repeat-rich mosquito genomes.

48 - Genome sequence and annotation of the primitively eusocial paper wasp *Polistes dominula* *Standage, Daniel*

Multiple origins of eusociality within the insect order Hymenoptera make these organisms an alluring system for investigating the evolution of social behavior. An increasing number of published genomes for both eusocial and solitary insects provide an unprecedented opportunity for comparative sociogenomics analysis on a grand scale. One significant limiting factor, however, is that only two of the major eusocial lineages in Hymenoptera (Formicidae--ants and Apoidea--bees) are represented in existing genomic resources, while the third (Vespidae--paper wasps) is not. Here we present the latest work on the *Polistes dominula* genome project, which produced the first complete genome sequence of a primitively eusocial paper wasp. We provide an assessment of genome composition and gene content, highlighting genes unique to the *Polistes* lineage. We also discuss a novel technique for leveraging genes with conserved exon/intron structures for training gene prediction tools and improving preliminary annotations produced by automated workflows. Finally, we discuss the tools we have developed for visualization, for community annotation/curation, and for managing the multiple sources of annotation associated with the project.

49 - VectorBase community submission system

Giraldo-Calderon, Gloria I.; Emrich, Scott J.; Collins, Frank; Consortium, VectorBase

VectorBase is a NIAID-funded Bioinformatic Resource Center that, since 2004, provides resources to investigate vector species involved in the transmission of etiological agents causing diseases such as malaria, dengue, yellow fever, filariasis and trypanosomiasis. Currently, VectorBase users have access to genomes, transcriptomes, proteomes, other "omics" data and, population data such as single-nucleotide polymorphisms (SNPs) and insecticide resistance phenotypes.

In order to help scientists in the process of improvement or development of new strategies for controlling or even eradicating vector borne diseases, as well as support basic science, VectorBase is committed to improve the system for community data submission, which has been divided in different sections:

- Gene annotation (models in GFF3 or fasta format) and metadata (gene name, symbol and description), which are meant to improve the organisms gene sets, through our Community Annotation Portal (CAP).
- Gene transcript and protein data coming from colonized or wild organisms, may be submitted for display on VectorBase using the expression browser and genome browser.
- Linking genes to publications allows researchers to share their papers with the VectorBase community, making them visible on the VectorBase genome browser.
- Phenotype data in the insecticide resistance database (IRbase), or variation data (*i.e.*, SNPs) in the population biology browser (PopBio).

To submit, go to our community section at either our website home page or navigation tab. Attend this poster for an overview of the submission system, ongoing developments to the submission system and discussion of suitability of this tool for the research community needs.

Website: www.vectorbase.org

Help, comments or suggestions: info@vectorbase.org

Tutorials: www.vectorbase.org/tutorials

50 - Annotation of genes involved in the immunology and digestion of phlebotomine sand fly species

Gomes, Bruno; Moraes, Caroline S.; Cuadrat, Rafael R. C.; Cardoso, Christiane; Díaz-Albiter, Héctor; Sant'Anna, Mauricio R. V.; Ferreira, Clélia; Terra, Walter R.; Brazil, Reginaldo P.; D'Ávila, Alberto M.; Genta, Fernando A.; Dillon, Rod J.

Leishmaniasis is a neglected disease that affects some of the poorest human populations worldwide. The maintenance of this disease in natural populations depends on the ability of the parasites of the genus *Leishmania* to survive in two different organisms: the mammalian/human hosts and phlebotomine sandflies.

For the past few years a collaborative effort has led to the sequencing of the Brazilian strain Jacobina of the vector of visceral leishmaniasis *Lutzomyia longipalpis*. The sequences of the genome of two sand fly species *Lutzomyia longipalpis* and *Phlebotomus papatasi* are now available via Vectorbase. The Jacobina strain used for the sequencing is now housed at Lancaster with a derived colony now housed at Fiocruz. Studies between the two labs focuses on immunity and digestion in both larvae and adults. The gut is a particular focus as the *Leishmania* development in the sand fly is confined to the gut.

Very little is known about immune associated genes in *Lu. longipalpis* although recent results suggest that manipulation of the immune system, by silencing Caspar gene expression leads to suppression of *Leishmania* infection in the gut (Telleria et al. 2012). Digestive enzymes are also another important component that may influence *Leishmania* infections. Besides that, digestive enzymes may be important targets for inhibition and control of natural populations of sandflies, specially at the larval stage. To get a better understanding of sandfly physiology and development, we conducted a 454 pyrosequencing of larval gut cDNA and annotation of 97% of the transcripts found in this tissue.

The sequencing of the sand fly genome will enable comparative analyses with other Diptera species and may allow the identification of genetic differences in the gene families associated with the immune system in insects such as Thioester-containing proteins and Peptidoglycan Recognition Proteins. This analysis will be an important step to identify genes that may have a role in the interaction between the sand flies and the *Leishmania* spp and feed into our work in understanding how *Leishmania* is able to survive and use the sand fly as a vector for transmission. Supported by CNPq, CAPES, FIOCRUZ, FAPERJ (Science Without Borders Program)

Epigenomics Session

51 - *Wolbachia pipientis* alters arthropod DNA methylation to induce embryonic arrest

LePage, Daniel; Jernigan, Kristin; Simmons, Alan; Bordenstein, Seth

Wolbachia pipientis is an obligate intracellular parasite known to infect ~40% of all arthropod species. This maternally inherited bacterium utilizes several mechanisms to disseminate through populations including a conditional embryonic arrest known as cytoplasmic incompatibility (CI). CI occurs when uninfected females mate with infected males and results in high levels of embryonic death. This lethality has been linked to defects of the paternal genome in the first mitosis, which include incomplete chromosome condensation, failed histone recruitment, delayed replication, and improper chromosome segregation. These defects in the paternal DNA occur without *Wolbachia* being present within the sperm and hint at underlying problems with chromatin structure and processes. We have found that *D. melanogaster* infected with the wMel strain of *Wolbachia pipientis* show a 64% increase in genomic methylation at cytosine residues within the testes. This increase is not seen in the ovaries of infected flies and host gene expression within the testes does not seem to be affected by the additional methylation (as measured by RNAseq and qRT-PCR). The removal of DNA methylation by 5-AZ treatment shows that the full rescue of embryonic lethality induced by *Wolbachia pipientis* is dependent on parental methylation levels. Finally, overexpression of the DNA methyltransferase Dnmt2 within females is able to partially rescue embryonic death in CI. These results show an interesting mechanism that *Wolbachia* could use to alter the paternal chromatin structure and suggest that disparate levels of DNA methylation in parents induce embryonic death in arthropods.

52 - Endoreduplication and underreplication of the genome in the thorax of *Drosophila melanogaster*

Johnston, J. Spencer; Schoener, Molly; MacMahon, Dino

Variation in levels of endoreduplication is a largely unrecognized epigenetic event. The discovery of endoreduplication in the majority of cells of the thorax of *Drosophila* has implications for genomics, transcriptome levels, chromatin structure and life history of these model insects. The ratio of 2C/4C DNA amounts is 2.00 for nuclei from the head, yet is 1.75 and 1.83 for nuclei from the thorax of wild type and suppressor of under replication (SuUR) strains, respectively. The latter ratios reflect under replication in the majority of nuclei from the thorax, which is only partially suppressed in the SuUR strain. The effect is age-dependent. Thoracic 4C DNA is significantly more under replicated in the nuclei of 10 day old than newly emerged flies. The consequences of under replication for the majority of thoracic cell nuclei, likely mimic those in highly endoreduplicated polytene salivary and nurse cell nuclei, which would affect expression levels in genomic, transcriptomic, methalomic and other studies based wholly or in part on *Drosophila* thoracic nuclei.

53 - A model of nuclear organization reveals new chromosome regions with significant affinity for the nuclear envelope

Kinney, Nicholas; Onufriev, Alexey; Sharakhov, Igor

We describe a method for modeling the three dimensional (3D) organization of the interphase nucleus, and its application to polytene chromosomes of *Drosophila* salivary glands. The model represents chromosomes as polymer chains confined within the nucleus. Physical parameters of the model are taken directly from experiment, no fitting parameters are introduced. The model is used to simulate chromosome tracing data and identify the statistically significant chromosome-nuclear envelope (Chr-NE) contacts in experimental chromosome tracing data. Using this approach, 33 new Chr-NE contacts are revealed. Most of these new Chr-NE contacts correspond to intercalary heterochromatin – gene poor, dark staining, late replicating regions of the genome; only three correspond to euchromatin – gene rich, light staining, early replicating regions of the genome. Analysis of regions least likely to form Chr-NE contacts reveals that these are mostly euchromatic, but may contain late replication regions or intercalary heterochromatin. These results reveal new details about the types of chromatin likely to form Chr-NE contacts. In addition, methods are developed to objectively quantify chromosome territories and intertwining, these are discussed in the context of the corresponding experimental observations.

Comparative Genomics

54 - Discovery of anonymous molecular markers, and de novo assembly of tissue-specific transcriptomes in spider species of the Infraorder Mygalomorphae

Frías, Cristina; Guirao, Sara; Sánchez, Alejandro; Almeida, Francisca C.; Arnedo, Miquel A.; Rozas, Julio

The spider infraorder Mygalomorphae (i.e. trapdoor spiders, funnel web spiders, tarantulas, etc.) includes about 3,000 species that frequently show high habitat fidelity, limited potential for dispersal and restricted distributional ranges. For this reason, the members of this group are highly attractive for fine scale phylogeographic studies. At the same time, because of the poor knowledge on the genomes of spiders, one of the most diverse animal orders, mygalomorphs provide a unique opportunity to investigate the genetic basis of *fundamental biological structures, such as the chemosensory system*.

By combining next-generation *sequencing* technology (454GS-FLX Titanium) with a reduced-representation library (RRL), we developed a novel approach for new marker discovery. We sequenced intra- and interspecific pooled data from a group of species of the mygalomorph families Nemesiidae and Ctenizidae. We obtained a total of ~400,000 reads (N50 of 440 bp) for each family that were subsequently assembled using the CAP3 software. Then, we applied an in-house built pipeline to identify new molecular markers to be used for phylogenetic and phylogeographic studies. The pipeline includes quality control (NGSQCToolkit), mapping of long reads to the contig assembly (BWA) and SNP calling (SAMtools, Picard and Gatk) steps. The pipeline also includes a filter step of SNP candidates.

Additionally, we use the *sequencing 454GS-FLX-based technology to sequence three* cDNA libraries (from legs, palps and ovary) of the funnel web spider *Macrothele calpeiana* (Hexathelidae). We generated a total of ~50,000 (N50 of 404 bp) reads for each library, which were assembled into 1300 (legs), 1680 (palps), and 833 (ovary) unigenes. We conducted a functional annotation of transcripts with Blast2GO in order to identify gene families involved in chemosensory reception.

55 - Population genomics of *Anopheles gambiae* and *An. arabiensis*.

Fontaine, Michael C; Smith, Hilary A; Love, Rachel; Steele, Aaron; Neafsey, Daniel E; Emrich, Scott J.; Besansky, Nora J

Anopheles coluzzii (formerly *An. gambiae* M form) and *An. gambiae* (formerly *An. gambiae* S form) are potent Afrotropical malaria vectors thought to be the products of very recent ecological speciation. These species have diverged in larval ecology and reproductive behavior through unknown genetic mechanisms, despite potentially high local levels of interspecific gene flow where their ranges overlap, in West and Central Africa. Moreover, their distributions overlap with additional members of the *An. gambiae* species complex, with whom introgression may also occur. In particular, *An. arabiensis* may have significantly contributed to the genetic makeup of *An. gambiae* and *An. coluzzii*. Whole genome sequencing of multiple individual genomes of *An. coluzzii* (N=12), *An. gambiae* (N=26) and *An. arabiensis* (N=12) sampled from several localities in West, Central and East Africa allowed us to compare the genome-wide pattern of nucleotide diversity and divergence at different geographic scales (local, regional and continental), to identify the genetic forces at play across the genome (selection, drift, migration) and to infer the demographic history of divergence with or without gene flow.

56 - Phylogenetic and Transcriptomic Analysis of Chemosensory Receptors in a Pair of Divergent Ant Species Reveals Sex-Specific Signatures of Odor Coding

Zhou, Xiaofan; Slone, Jesse; Rokas, Antonis; Berger, Shelley; Liebig, Jürgen; Ray, Anandasankar; Reinberg, Danny; Zwiebel, Laurence

Ants are a highly successful family of insects that thrive in a variety of habitats across the world. Perhaps their best-known features are complex social organization and strict division of labor, separating reproduction from the day-to-day maintenance and care of the colony, as well as strict discrimination against foreign individuals. Since these social characteristics in ants are thought to be mediated by semiochemicals, a thorough analysis of these signals, and the receptors that detect them, is critical in revealing mechanisms that lead to stereotypic behaviors. To address these questions, we have defined and characterized the major chemoreceptor families in a pair of behaviorally and evolutionarily distinct ant species, *Camponotus floridanus* and *Harpegnathos saltator*. Through comprehensive re-annotation, we show that these ant species harbor some of the largest yet known repertoires of odorant receptors (*Ors*) among insects, as well as a more modest number of gustatory receptors (*Grs*) and variant ionotropic glutamate receptors (*Irs*). Our phylogenetic analyses further demonstrate remarkably rapid gains and losses of ant *Ors*, while *Grs* and *Irs* have also experienced birth-and-death evolution to different degrees. In addition, comparisons of antennal transcriptomes between sexes identify many chemoreceptors that are differentially expressed between males and females and between species. We have also revealed an agonist for a worker-enriched OR from *C. floridanus*, representing the first case of a heterologously characterized ant tuning Or. Collectively, our analysis reveals a large number of ant chemoreceptors exhibiting patterns of differential expression and evolution consistent with sex/species-specific functions. These differentially expressed genes are likely associated with sex-based differences, as well as the radically different social lifestyles observed between *C. floridanus* and *H. saltator*, and thus are targets for further functional characterization. Our findings represent an important advance toward understanding the molecular basis of social interactions and the differential chemical ecologies among ant species.

57 - Functional analysis of the ATP-binding cassette (ABC) transporter gene family of *Tribolium castaneum*

Broehan, Gunnar; Kröger, Tobias; Lorenzen, Marcé; Merzendorfer, Hans

The ATP-binding cassette (ABC) transporters belong to a large superfamily of proteins that have important physiological functions in all living organisms. In insects, ABC transporters are of special interest in the context of insecticide resistance, because of the role they play in the elimination of xenobiotics and their metabolites. We have identified 73 ABC transporter genes in the genome of *Tribolium castaneum*, which group into eight subfamilies (ABCA-H). Thus, the ABC gene family in this coleopteran species is significantly larger than those reported for dipteran, hymenopteran, and lepidopteran species. Phylogenetic analysis revealed that this increase is due to gene expansion within a single clade of subfamily ABCC. To study the function of ABC transporters during development, we performed an RNA interference (RNAi) screen. In several cases, injection of double-stranded RNA (dsRNA) into larvae or pre-pupae caused developmental phenotypes, which included growth arrest and localized melanization, eye pigmentation defects, abnormal cuticle formation, egg-laying and egg-hatching defects, and mortality due to abortive molting and desiccation. Some of the ABC transporters we studied in closer detail to examine their role in lipid, ecdysteroid and eye pigment transport. The results from our study provide new insights into the physiological function of ABC transporters in *T. castaneum*, and may help to establish new target sites for insect control.

58 - Gene evolution of ionotropic receptors in tropical butterflies, on the scent of speciation
van Schooten, Bas

In a butterfly system famous for its visual mimicry, a trait that has caused ecological and sexual divergence on color pattern, chemical communication is probably as important to promote and maintain species boundaries. A recent study on the genome of *Heliconius melpomene* revealed a striking diversity in olfactory and gustatory receptors to an amount greater than seen in moths. However, little is known on pheromone communication in *Heliconius* butterflies. How does the sensory system evolve to detect these compounds? and How does it create prezygotic isolation? are two fundamental questions that need to be answered to further understand speciation. We are starting to characterize the members of the ionotropic receptor gene family. Ionotropic receptors fill a vital niche in the detection of olfactory and gustatory cues in an insect's life. We are investigating possible gene duplication of its members and their copy number variations across the *Heliconius* genus. The ionotropic receptors are taken from the *H. melpomene* genome and compared to other Lepidoptera. This data is complemented with RNA-seq analysis on antenna of several adult *H. doris* for both males and females. We will be presenting preliminary results that will shed light onto important candidate chemosensory gene that play a role and facilitate species recognition and prezygotic isolation.

59 - Sexually dimorphic gene expression in *Aedes aegypti* pupal brains
Tomchaney, Michael; Mysore, Keshava; Duman-Scheel, Molly

Most animal species exhibit sexually dimorphic behaviors, many of which are linked to reproduction. A number of these behaviors, including blood feeding in female mosquitoes, contribute to the global spread of vector-borne illness. However, knowledge concerning the extent of sexual dimorphisms in the structure of the central nervous system, the control of sex-specific behaviors by sexually dimorphic neurons, and the developmental genetic basis for sexually dimorphic behavior is limited in any organism, including mosquitoes. In this investigation, we performed a microarray experiment to investigate genes that are dimorphically expressed in the pupal brains of *Aedes aegypti* (the dengue and yellow fever vector mosquito). The array uncovered 2528 statistically significant differentially expressed genes. Significant genes were further analyzed with DAVID and GenMAPP, which grouped genes into statistically significant pathways and gene ontology (GO) processes. Genes upregulated in females were predominantly implicated in proteolytic and metabolic GO processes. Genes upregulated in males tended to be associated with metabolic GO terms, including polysaccharide metabolism. Dimorphically expressed genes were also linked to developmental, metabolic, and behavioral signaling pathways. Genes from these pathways will be prioritized for secondary validation experiments and further characterization. These studies will provide insight into the genetic differences underlying sexually dimorphic neural circuitries and behaviors that promote the spread of diseases as well as promote the elucidation of potential targets for intervention.

60 - Assembly of *Aedes aegypti* odorant receptor regulatory matrix

Mysore, Keshava; Li, Ping; Andrews, Emily; Duman-Scheel, Molly

Though the socioeconomic and medical importance of mosquitoes is profound, surprisingly very little is known about developmental mechanisms in the mosquito sensory system, including the olfactory system, a tissue of vector importance. Knowledge about the mechanism that specifies mosquito olfactory receptor neurons (ORNs) to express a particular odorant receptor (OR) from a large OR pool, an important step for odor detection and discrimination, is lacking. Here, we investigate this process in *Aedes aegypti*, the dengue and yellow fever vector mosquito. Studies in *Drosophila melanogaster* have suggested that the combination and levels of expression of various *cis*-regulators of transcription in ORNs generates the OR regulatory matrix, a code governing which particular OR gene is expressed and which are repressed in any given ORN. Although OR sequences have rapidly evolved in insects, our preliminary studies suggest that mosquito OR expression is regulated in a comparable manner. Here, we begin to examine the functions of eight transcription factors (TFs) that are hypothesized to function in the *Ae. aegypti* OR regulatory matrix. The TFs are expressed in a subset of *Ae. aegypti* antennal ORNs, and expression levels of each TF varies from neuron to neuron within this subset. Searches for consensus binding site sequences for the TFs in the 1 kB 5' flanking sequences of 115 *Ae. aegypti* OR genes known to be expressed in the adult antenna uncovered multiple consensus binding sites for each TF in the 5' flanking regions of *Ae. aegypti* OR genes. These data strongly suggest that the TFs will function in the *Ae. aegypti* OR regulatory matrix. To functionally test this hypothesis, we used chitosan/siRNA nanoparticles to target one of the transcription factors, *Ae. aegypti single-minded (sim)*, during olfactory development. The results of this investigation suggest that *Sim* regulates expression of a subset of OR genes and functions in the *Ae. aegypti* OR regulatory matrix. Our ongoing efforts will continue to assemble this OR regulatory matrix which is critical to the adult mosquito sense of smell.

61 - The Genetics of Maternal Suppression of Symbiont Titters in *Nasonia*

Funkhouser, Lisa; Bordenstein, Seth

Vertical transmission of bacterial symbionts through the maternal germ-line is pervasive in arthropods, with co-evolution between hosts and their symbionts producing unique host-microbe interactions. In arthropods infected with the reproductive parasite *Wolbachia*, endosymbiont titers are often strictly maintained at a density that is specific to host species and *Wolbachia* strain. In the parasitoid wasp *Nasonia*, *Wolbachia* maintains a low infection density in its natural host *N. vitripennis* but has an extreme and stable infection density 100-fold higher after transfer to the naïve host *N. giraulti*. We have utilized this interspecific difference in *Wolbachia* titers together with the powerful genomic tools of *Nasonia* to identify genes responsible for the regulation of *Wolbachia* titers. Here we report three key findings. First, the low *Wolbachia* density native to *N. vitripennis* is regulated by host factors that act dominantly through a maternal effect. Second, using selective introgressions, genotyping microarrays and a quantitative trait loci analysis, we report three significant QTL regions involved in *Wolbachia* density regulation located on *Nasonia* chromosomes 1, 2, and 3. Third, segmental introgression lines with QTL regions from *N. vitripennis* integrated into a *N. giraulti* genomic background have confirmed the effect of the QTL regions on *Wolbachia* titers and are currently being used to fine-map each region. Several candidate genes with important roles in innate immunity and oogenesis are located within these regions and will be individually tested for their effect on *Wolbachia* titers. RNA-seq of *Nasonia* ovaries has also been employed to identify candidate genes based on interspecific differences in transcript levels of genes located within the QTL regions.

62 - Tsetse fly lactation: comparative transcriptome analysis among *Glossina* females harboring developing intrauterine larva

Benoit, Joshua; Attardo, Geoffrey; Michalkova, Veronika; Aksoy, Serap

In tsetse flies, larvae undergo intrauterine development and are nourished exclusively by maternal lactation secretions derived from a specialized tissue called the milk gland. This study analyzes the *G. morsitans* lactation transcriptome and milk proteome to determine novel milk proteins, examine their function during lactation and provide a comparative transcriptome analysis in relation to lactation between tsetse species. Transcriptome analysis of lactating *G. morsitans* compared to dry (non-lactating) flies reveals that transcripts for protein synthesis machinery and secreted proteins are highly expressed during milk production. Specific transcripts increase during lactation including previously identified proteins: *milk gland protein 1*, *mgp1* (a lipocalin), *transferrin*, *trf*, and *acid sphingomyelinase 1*, *asmase1*. In addition, we identified a novel tsetse-specific gene family (*mgp2-10*) that increases during lactation and are localized to a 30kb genomic region. Proteomic analysis of tsetse milk recovered from the gut of feeding larvae reveals that MGP1-10, Trf and aSMase1 are the primary secreted components from the milk gland. Comparative transcriptome analysis with four other tsetse species revealed that high expression of *mgp1*, *trf* and *asmase1* during pregnancy is consistent among *Glossina*, but the number of genes within the MGP2-10 family varied among species. Expression patterns of *mgp2-10* are female-specific, localized to the milk gland and increase during larvigenesis followed by a drastic decline after parturition in *G. morsitans*. Knockdown of a single *mgp2-10* gene fails to alter fecundity, but tandem suppression reduces fecundity and extends pregnancy duration. Along with serving as the main protein resource, lipid emulsification assays after suppression of MGP2-10 revealed that these proteins are critical for maintaining lipid emulsification within the aqueous milk. Our study reveals a shift in metabolism during lactation to promote the generation of secreted proteins and a novel family of tsetse lactation-specific proteins that are the main polypeptide component of tsetse milk and promote milk homeostasis. This novel protein family likely serves a functional role similar to that of caseins within mammalian milk and could be utilized as a target for tsetse control since these genes appear to be *Glossina*-specific.

63 - Identification of genes specific to the lineage of the hymenopteran insect, honey bee (*Apis mellifera*)

Bennett, Anna; Elsik, Christine

Honey bees are important as key agricultural pollinators and models for social behavior and the evolution of eusociality. As social insects, honey bees live in close proximity to each other within hives and cooperate as a society with division of labor between castes. The honey bee genome, published in 2006 by the Honey Bee Genome Sequencing Consortium, had fewer gene predictions than expected, partially due to a lack of gene evidence in the form of transcriptome data and protein homologs from closely related species. As part of ongoing consortium efforts, we produced an improved official gene set (OGSv3.2) for honey bee with ~5000 more protein-coding genes than the first set. In search of genomic differences that contributed to diversification of the honey bee from other insects, we identified genes specific to *Apis mellifera* and to lineages within the insect order Hymenoptera. With numerous recently sequenced arthropod genomes, including two additional *Apis* species and two *Bombus* species, the hymenopteran clade is now well-sampled, providing the genomic and transcriptomic data necessary to allow for fine-scale, comparative analyses to detect lineage-specific genes.

We will present our approach to detect previously unknown genes in *A. mellifera*, to determine which genes were specific to the *A. mellifera* lineage, and to elucidate potential mechanisms for their emergence. We identified genes that were specific to the *A. mellifera* species, *Apis* genus, Apidae family, and Hymenoptera order, including those with tentative roles in brood care, immunity, defense and other processes important to hive health, and thus critical to the emergence of eusociality.

64 - Identification of A-to-I RNA Editing Sites in Honey Bee (*Apis mellifera*) Using RNA-Seq Data *Tao, Shu; Elsik, Christine*

Honey bee (*Apis mellifera*) is one of the most studied insects, due in part to its importance as a model to study molecular mechanisms related to social behavior. We performed computational identification of potential A-to-I RNA editing sites in the honey bee brain using the latest genome assembly and Illumina-generated RNASeq data from ten honey bee individuals (five nurses and five foragers, 2-3 replicates per individual). We chose the honey bee brain as the focus of this study because: *i*) it is important in controlling behaviors, and *ii*) studies in human and *Drosophila* have shown that A-to-I RNA editing is prevalent in brain. This is the first systematic investigation of A-to-I RNA editing in the honey bee brain. It will enhance the annotation of genes expressed in the honey bee brain, and provide candidate A-to-I RNA editing sites for further investigation.

Some of the candidate A-to-I editing sites in *A. mellifera* were found in genes with one-to-one orthologs in *Drosophila melanogaster* that were already shown to have A-to-I RNA editing sites. For example, we found two conserved A-to-I RNA editing sites in the gene orthologous to *quiver* (*qvr*) in *Drosophila melanogaster*. The first conserved A-to-I editing site changes codon from AGU to GGU, causing the conversion of serine to glycine, and the second conserved site recodes histidine (CAC) to arginine (CGC). Conservation of these specific amino acid conversions across 300 million years of evolutionary divergence indicates important biological functions.

Additionally, in an effort to develop an efficient method for testing our predictions, we searched for potential restriction enzyme recognition sites overlapping the predicted editing sites. We have found that the vast majority of predicted A-to-I RNA editing sites in coding DNA sequences and 3'-UTRs cause a change in a restriction site. Therefore, restriction enzyme digestion can serve as a complementary validation method to sequencing on A-to-I RNA editing site validation.

We will also report results from our investigation of clustered A-to-I editing, which has been found to occur in human transcripts. When extensive A-to-I RNA editing occurs in a cluster of adenosines in close proximity to each other, editing escapes identification through routine alignment of expressed sequences to reference genome. We have adapted a sequence transformation method used to study extensive A-to-I editing in human.

65- Combining Next-generation Sequencing and Comparative Genomics to Identify Novel microRNA in Hymenopteran Insects

Hagen, Darren; Elsik, Christine

Given the importance of microRNAs (miRNAs) in post-transcriptional gene regulation, it is likely that they play key roles in behavior adaptations, such as those exhibited in social insects, including ant and bee species, which are members of the order Hymenoptera. Small RNA molecules such as miRNAs pose a challenging computational problem. Bioinformatic algorithms struggle to identify these elements due to the reduced information of short sequences. Next generation sequencing technologies have identified novel miRNAs in many organisms, but short sequences often result in spurious mapping and inaccurate predictions. Furthermore, the analysis pipelines traditionally used fail to identify rarely-expressed miRNAs, whose signal is often drowned out by miRNAs with higher expression. Predicting which protein-coding genes are targeted by a particular miRNA is even more difficult, especially in animals. Mismatches, gaps, and incomplete matching found in known miRNA-messenger RNA (mRNA) interactions result in a tremendous amount of false positives.

The availability of several sequenced hymenopteran insect genomes has provided an unprecedented opportunity to identify hymenopteran miRNAs. Here we present our approaches based on next generation sequencing technologies combined with comparative genomics to identify conserved miRNAs, including the more rare transcripts, and to identify putative miRNA targets using orthology of both miRNA and protein-coding genes (the potential targets). In our analysis of seven ant species, honeybee, and parasitoid jewel wasp, we discovered 27 previously unidentified miRNA. Analysis of 3'UTR sequences resulted in improved target predictions for both conserved and novel hymenopteran miRNAs.

66 - Evolution of Axon Guidance Receptors and Cell-Cell Recognition/Adhesion Proteins in Arthropods

Seeger, Mark

Proper wiring of the nervous system is dependent upon the coordinated activity of axon guidance receptors, their ligands, and various proteins that mediate selective cell-cell recognition or adhesion. Many gene families that encode these different axon guidance proteins are evolutionarily conserved and found in organisms as diverse as planarians and humans. Unlike many of the genes that regulate embryonic patterning, redundancy in axon guidance mechanisms and gene function is often observed. It is not uncommon for mutations in various axon guidance receptors or cell adhesion genes to result in subtle phenotypic consequences.

The diversity of arthropods and the multitude of sequenced arthropod genomes provide an outstanding opportunity to study the diversification and evolution of axon guidance receptors and cell-cell recognition/adhesion proteins. I will present bioinformatic and functional studies of four genes, Neurotactin, Amalgam, Lachesin, and Fasciclin III, which display interesting patterns of variation in different insect orders. Neurotactin is a member of the cholinesterase-like adhesion protein family. Amalgam, Lachesin, and Fasciclin III are members of the immunoglobulin super family and can mediate homophilic cell adhesion.

67 - The transcriptome of the *Anopheles gambiae* embryos

Dennison, Nathan; Krzywinski, Jaroslaw

RNA-seq approaches have revealed unexpected extent of transcription and a large complexity among the RNA species in each studied eukaryotic organism. Using the 454 platform we probed the transcriptome of the African malaria mosquito, *Anopheles gambiae*, embryos. Approximately 500,000 reads were obtained from each independent sample of pooled male and female embryos, and assembled into 17,492 and 16,899 male and female contigs, respectively. Each transcriptome represents more than a half of the currently annotated AgamP3.7 genes. Over 6,000 contigs do not match the AgamP3.7 genebuild, but map to the *An. gambiae* genome. A small fraction of those are yet unannotated mRNAs, because they have clear homologs among protein coding genes in other insects; however, the status of the majority of these new transcribed regions (NTR) remains uncertain. Analysis of these NTRs is ongoing. One NTR, represented exclusively by male embryo reads, corresponds to two overlapping genes encoded on both strands of the Y chromosome.

68 - Examination of circadian driven alterations in transcript levels of visual system genes in *Aedes aegypti*

Leming, Matthew; O'Tousa, Joseph

The day/night light fluctuations and circadian rhythm of *Ae. aegypti* influence many behaviors, including biting, flight, oviposition, and sugar feeding. Light detection by photoreceptors is critical to both vision and entrainment of the circadian clock. In the adult eye, the major photoreceptor class expresses Aop1. In the dark Aop1 translocates to the rhabdomere, the phototransducing organelle of the photoreceptor cell. Here we show that this translocation results in increased sensitivity to light. Furthermore, Aop1 levels cycle during a 24-hour day, increasing in amount during the morning and day then declining in the afternoon and shortly after dusk. We demonstrate that the degradation of Aop1 is also regulated in a light independent manner, implicating the circadian rhythm as a potential regulator of the visual system. We will characterize the periodicity and circadian rhythm of mRNA transcripts within the visual system of *Ae. aegypti* to gain insights into these processes. Earlier work in *Anopheles gambiae* showed that the circadian rhythm regulates expression of many RNA transcripts. Thus our work makes possible a comparative study of these processes in the two species. RNA was collected in a 48-hour profile of mosquitoes reared in normal light conditions (LD) and in constant dark (DD). We will report on the expression levels of transcripts measured in LD with respect to DD. By using the JTK_CYCLE and COSOPT algorithms we will determine any statistically significant rhythmic gene profiles.

69 - Large gene structures of salivary gland secretion protein candidates from Pea aphid (*Acyrtosiphon pisum*)

Oh, Jonghee; Marimuthu, Murugan; Cilia, Michelle; Reeck, Gerald; Reese, John; Smith, Mike; White, Frank

Aphid species exhibit host specialization and their success in utilizing host plant nutrients is highly dependent on SG (salivary gland) secretions. Though plant responses to aphid feeding are well-studied, the components of aphid salivary fluids are not well characterized. Soluble insect saliva contains various secreted hypothetical proteins, enzymes, and other bioactive compounds that are likely to play roles in establishing and maintaining aphid feeding sites, including the induction of beneficial nutritional changes and the suppression of plant defenses. The pea aphid genes of SG secreted protein candidates were identified from salivary gland cDNA libraries using a combination of 454 and Sanger sequencing, and LTQ-FT MS protein identification. Hypothetical protein (gi|241896885) is encoded by a member of gene family and was identified in a synthetic diet solution. The size of the gene is approx. 45 Kbp with 37 exons (6bp-412bp) and introns (180bp-5Kbp). The gene structures of other members of the family will be presented.

70 - Transcriptome profiling of two male reproductive tissues of a major apple pest, the codling moth *Cydia pomonella*

Gu, Liuqi

The codling moth *Cydia pomonella* is a world-wide pest infesting apple and other fruit trees. It has not been characterized at the genomic level. In this study, we sequenced de novo the first transcriptomes of male testis and accessory gland. Our aim was to identify and catalog expressed genes potentially involved in spermatogenesis as well as putative seminal fluid proteins (SFPs) sequences, which are essential for mating success and considered to play potential roles in sexual selection and speciation. Reads from ½ picotiter plate per sample were generated by 454 pyrosequencing and assembled into 44,108 non-redundant unigenes (contigs and singletons) for testis and 19,522 for accessory gland. Surprisingly, accessory glands have only about 1.8 % of transcripts in common with testis to which they are connected, while 15.1 % are splice variants. BLAST analysis (e-value < 10⁻³) against non-redundant protein databases retrieved hits of only 35% of total unigenes for testis and 17% for accessory gland, with most hits and top hits both from the Monarch butterfly *Danaus plexippus*. Gene ontology analysis of both tissues revealed a broad class of biological properties such as reproduction, biological regulation/adhesion, developmental processes, response to stimulus, immune system process, and notably, viral replication. In testis, a large number of genes involved in spermatogenesis and copulation are identified, as well as several sex-specific isoforms of genes in the sex determination cascade. Over 300 putative SFPs were identified from accessory gland, with an additional small repertoire (13) from testis.

71 - *Anopheles gambiae* Bamako: A Whole-Genome Analysis

Love, Becca; Fontaine, Michael; Steele, Aaron; Coulibaly, Mamadou; Traore, Sekou; Emrich, Scott; Besansky, Nora

The Bamako taxon of the malaria mosquito, *Anopheles gambiae*, is distinguished from other forms of *An. gambiae* by its unique arrangements of chromosomal inversions, and by its preference for laterite rock pools as a larval habitat. This habitat is chemically distinct from more typical *An. gambiae* larval habitat and is limited to the vicinity of the Niger River in Mali and Guinea. Bamako co-exists there with other members of the *An. gambiae* species complex, but appears to be in near reproductive isolation with these other members.

In order to characterize this understudied taxon, as well as to answer ongoing questions about speciation in the malaria mosquitoes, we performed whole-genome sequencing of the Bamako form. Ten karyotyped female mosquitoes from the village of Kela, Mali, were individually sequenced, with an average depth of 10.7x coverage. The sequences were compared to other individual sequences of non-Bamako *An. gambiae* from across Africa. We present preliminary data about the Bamako genome, including regions of high and low divergence from other *An. gambiae* forms and regions that show evidence of selective pressure.

72 - Role of Microsatellites in Segmental Duplications in Insect Genomes

Behura, Susanta; Severson, David

The present study is a systematic investigation to determine genome-wide distribution of microsatellite sequences in segmental duplications in different insect species (n =20). Results show that specific microsatellite pairs are distributed in the genome in significantly non-random manner. These paired microsatellites are localized at multiple locations in the genome. Our data further shows that as much as 22% of these repetitive paired microsatellites represent segmental duplications (low copy repeats of length > 1kb and sequence similarities > 90%) in these insects. The genomic abundance of such microsatellite-associated segmental duplications is positively correlated with the frequency of paired microsatellites in the genome suggesting a “rich-gets-richer” mode of duplication shadowing of these sequences. Our data further shows that relationship of segmental duplications with the paired microsatellites varies in phylogeny independent manner suggesting that the microsatellites associated segmental duplications may be species-specific evolutionary innovation of insects.

73 - E-painting with k-mers

Blackmon, Heath; Demuth, Jeffery

Traditional in silico synteny mapping has been limited to homologous genes. I have developed an R package that allows in silico synteny mapping at an arbitrarily fine resolution. The core of this package is a function that slides across each linkage group in an existing completed genome and finds unique k-mers that can be used as in silico “paints”. Associated functions then apply these “paints” to the new draft assembly of a related species and produce a synteny plot showing features such as translocations and repetitive element expansion. Functions in this package are designed to analyze draft assemblies that contain hundreds of scaffolds rather than chromosome sized linkage groups. In addition to producing figures showing cross species chromosome painting the package also includes functions that can identify important aspects of assemblies such as patterns of translocations, ultraconserved elements, retrogenes, and breakpoint biases.

74 - Functional analysis of genes encoding peritrophic matrix proteins in *Tribolium castaneum*

Kelkenberg, Marco; Muthukrishnan, Subbaratnam; Merzendorfer, Hans

The peritrophic matrix (PM) is an extracellular barrier that lines the midgut epithelium of most insects and protects the intestine from invasion by microorganisms and parasites. It consists of chitin fibrils that are embedded in a matrix of proteins and glycoproteins. Some of them have CBM14-type chitin-binding domains and are referred to as peritrophic matrix proteins (PMPs). In the genome of the red flour beetle, *Tribolium castaneum*, eleven genes encode PMPs which differ in size and in the number of CBM14 domains. To examine the function of PMPs in the midgut of *T. castaneum*, we established a permeability assay based on fluorescein isothiocyanate (FITC)-dextrans. FITC-dextrans of defined sizes were added to a wheat flour-based diet and fed continuously to larvae. Next, cryosections were prepared to analyze the distribution of fluorescence signals in the larvae. While the PM of the anterior midgut has a high permeability for FITC-dextrans of even 2 MDa, the PM of the median and posterior midgut is almost impermeable for FITC-dextrans larger than 150 kDa. We silenced the expression of three genes encoding TcPMP3, TcPMP5-B and TcPMP9 by systemic RNA interference (RNAi). While injection of dsRNA specific for *TcPMP9* did not affect growth and development of the insects, those for *TcPMP3* and *TcPMP5-B* caused growth reduction, fat depletion due to starvation, and eventually lethality either at the larval-pupal molt or pupal-adult molt. The results indicate that the properties of the PM significantly vary in different midgut regions, and that some PMPs are required for PM integrity. Their loss leads to altered permeability, which in turn may compromise nutrition and fecundity. This study demonstrates for the first time the essential nature of PM proteins and sheds light on the mode of action of this class of proteins.

75 - Chitin synthases are tandemly linked genes in Noctuid moths

Shirk, Paul; Furlong, Richard B.; Perera, O. P.; Shelby, Kent S.; Popham, Holly J.

Two chitin synthase genes, chitin synthase 1 (ChS1), which is involved in the production of the cuticle, and chitin synthase 2 (ChS2), which is involved in the production of the peritrophic membrane, were characterized in the genomes of three noctuid moths: the corn earworm/cotton bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and the tobacco budworm, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae). In all three moths, the coding sequences for the two genes were arranged in tandem with the same orientation on the same strand with ChS2 5' of ChS1. Each chitin synthase gene had unique regulatory elements 5' to the individual transcription start sites. Sequence comparisons showed that each gene was highly conserved with the respective homologues from other species but that the genomic arrangement of the two genes being tandemly juxtaposed was unique. Transcript mapping of ChS2 and ChS1 in *H. zea* demonstrated that both genes were differentially spliced in various tissues and in the differing developmental stages of the larvae. A transcript comprised of all 23 exons of ChS2 was clearly present in the 1st-3rd instar larvae as well as in the foregut, midgut and hindgut of both 4th and 5th instar larvae. An alternatively spliced transcript of ChS2 involving exon 19 was observed in the 2nd, 3rd, 4th and 5th instar larvae but not the 1st instar larvae. However, non-splicing of the ChS2 transcript of exons 1-11 was also detected in 4th and 5th instar muscle, Malpighian tubules and fat body. A transcript comprised of all 23 exons of ChS1 was observed in muscle, fat body, hindgut, midgut and foregut of 4th instar larvae. It was also detected in muscle, and hindgut of 5th instar larvae but not in any of the other tissues. The tandem arrangement of these two genes is unique to the noctuids presents an opportunity to define regulatory elements influencing their transcription.

76 - Comparative analysis of commissureless family gene neurodevelopmental functions in dipteran insects

Sarro, Joseph; Andrews, Emily; Sun, Longhua; Behura, Susanta; Severson, David W.; Duman Scheel, Molly

Comparative neurodevelopmental studies have revealed that the arthropod embryonic ventral nerve cord is an excellent tissue in which to study mechanisms that contribute to neural diversity. Functional analyses of axon guidance genes suggest that the roles of orthologous genes have diverged during embryonic nerve cord development of different arthropod species. Comparative genomic studies have also revealed lineage specific duplications and expansions, as well as the loss of several genes known to be critical for *Drosophila melanogaster* ventral nerve cord development in the genomes of other insects. For example, despite a requirement for *commissureless (comm)* axon guidance gene function in the *D. melanogaster* ventral nerve cord, as well as the existence of two additional *D. melanogaster comm* family genes, *comm2* and *comm3* in *D. melanogaster*, the number of *comm* genes varies in other insects. Like *D. melanogaster*, some insects have three *comm* family genes, but this number is reduced in other insects, some of which lack a *comm* gene entirely. It was therefore hypothesized that the function of *comm* family genes has diverged in insects. Here, this hypothesis is explored through phylogenetic, expression, and functional analyses of *comm* family orthologs in vector mosquitoes. These studies revealed that although the malaria vector mosquito *Anopheles gambiae* lacks a *comm* family gene, *Culex quinquefasciatus* (West Nile and lymphatic filariasis vector) has three *comm* family genes, two of which are strongly expressed in the ventral nerve cord while axons are being guided to the midline. *Aedes aegypti* (dengue and yellow fever vector mosquito) has a single *comm* family gene, *Aae comm2*, that is an ortholog of *D. melanogaster comm2*. The function of this gene, which is broadly expressed in the embryonic CNS in axons that are being guided toward the midline, was assessed through siRNA-mediated silencing. These knockdown studies revealed that *Aae comm2* is required for embryonic nerve cord development in *Ae. aegypti*. The *Aae comm2* loss of function nerve cord phenotype was compared to the *D. melanogaster comm2* mutant phenotype, which was characterized for comparison. Although features of both the *D. melanogaster comm* and *comm2* mutant phenotypes are observed in *Ae. aegypti comm2* knockdown embryos, aspects of the phenotypes differ between the two species. The results of this investigation suggest that the developmental roles of *comm* family genes have diverged in dipteran insects.

77 - Genome Surveyor

Blatti, Charles; Kazemian, Majid; Hanson, Casey; Xu, Le; Sinha, Saurabh

Genome Surveyor (<http://veda.cs.uiuc.edu/gsl/>) is an online tool for analyzing regulatory sequences and discovering *cis*-regulatory elements in *Drosophila melanogaster* and other related species (including African malaria mosquito, parasitoid wasp, red flour beetle, and honeybee). Utilizing the GBrowse genome browser framework, Genome Surveyor specializes in the visualization of computationally predicted transcription factor (TF) binding targets based on hundreds of experimentally determined DNA binding specificities, or motifs, from curated databases such as JASPAR, TRANSFAC, and Fly Factor Survey. Users are able to select multiple TFs of interest and visualize their combined binding profiles, which have been shown previously to identify *cis*-regulatory modules (enhancers) in the genome. Additionally, features specific to regulatory analysis of the *D. melanogaster* genome include the ability to display predicted TF binding profiles as phylogenetic averages across the 12 *Drosophila* species and to filter binding profiles with chromatin accessibility data pertaining to early embryonic development. Genome Surveyor is also accompanied by a number of special purpose tools. The Motif Enrichment Tool (MET) predicts major regulators of user-provided gene sets by finding TFs whose regulatory gene targets are most significantly enriched in the input gene set. The Motif Cluster Search tool enables novel CRM discovery by searching genome-wide for regions with user-specified combinations of predicted TF binding. Finally, the Interacting Transcription Factors (iTFs) tool accepts a set of user defined sequences (or genomic coordinates) and a pair of DNA binding motifs and returns statistically significant spacing and orientation biases among predicted TF binding sites. Genome Surveyor is free and open to all users with additional species and motifs added regularly.

78 - Transcriptomic Analysis of *Aedes aegypti* response to Cry toxins

Canton Ojeda, Pablo Emiliano; Bravo, Alejandra; Soberón, Mario

The mosquito *Aedes aegypti* is important in public health because of its roles as a disease vector. The worldwide increase of dengue fever highlights the need to find ways to control these mosquito populations. An alternative to the use of chemical insecticides is the application of Cry toxins from *Bacillus thuringiensis* (Bt) at the mosquito's rearing sites. The advantages of these toxins is the specificity of their target insect, their lack of activity towards humans and their biodegradable nature due to their proteic origin. RNA-seq has developed as a powerful platform to study changes in whole genome expression. With this technique we are studying the progression of the response to Cry toxins in *A. aegypti* larval midgut tissue at different time intervals after exposure to the toxin. This information will allow us to identify clusters of genes that act sequentially in response to the toxins and shed light on the different pathways the midgut epithelial cells initiate to defend themselves against Bt. This knowledge will allow better and more efficient use of this biocontrol technology in open field applications.

79 - Low levels of genomic divergence among forms of the *Culex pipiens* under different ecological pressures

Gomes, Bruno; Wilding, Craig S; Weetman, David; Sousa, Carla; Novo, Maria T; Savage, Harry M; Almeida, António PG; Pinto, João; Donnelly, Martin J

The West Nile virus vector *Culex pipiens sensu stricto* is divided into two intraspecific forms termed *pipiens* and *molestus*, characterized by differing ecological traits. Whilst in northern Europe and the USA these forms occupy distinct habitats (aboveground and underground), in southern Europe they are found sympatrically aboveground. Previous molecular studies have shown common ancestry of geographically distinct populations of each form. However, the levels and patterns of genetic differentiation across the genome remain unknown. Here, an amplified fragment length polymorphism (AFLP) based genome scan was undertaken on samples collected from both sympatric and allopatric populations from Europe and USA in order to quantify the extent and consistency of differentiation between the two forms. The forms *pipiens* and *molestus* were clearly distinct but with major sub-structuring between continents within each form, and also more marked differentiation among European *molestus* than *pipiens* populations. Three outlier analyses applied to 810 loci showed low genomic divergence between *pipiens* and *molestus* (3.1%-3.2%), which is consistent with sympatric speciation with gene flow. Only two outlier common loci (0.25%) were detected in both Europe and the USA suggesting a low number of genomic regions involved in the typological traits (*i.e.* autogeny, stenogamy, ability for diapause) that influence the adaptation of *molestus* to anthropogenic habitats and the speciation process between *pipiens* and *molestus* forms.

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80 - Dynamic venom evolution in black widow spiders revealed by next-generation transcriptomics and genomics.

Garb, Jessica; Bhere, Kanaka; Hayashi, Cheryl; Ayoub, Nadia

The widespread application of next-generation sequencing technologies to ecologically important non-model organisms has revolutionized the pace of genomic discovery and evolutionary inference. Such approaches are especially useful in dissecting the complex molecular composition of animal venoms, the components of which play a key role in organismal ecology and have clear biomedical significance. The venoms of black widow spiders (genus *Latrodectus*) contain a unique family of neurotoxins capable of immobilizing invertebrate and vertebrate prey, which appear to facilitate their broad diet as generalist predators. Prior knowledge of black widow venom genes was largely restricted to a few sequences from a single species, limiting understanding of venom genomic diversity, evolution and its biomedical potential. We have assembled RNA-Seq based reference transcriptomes from the Western black widow spider *Latrodectus hesperus* and closely related, but less toxic species to determine a comprehensive inventory of venom-specific transcripts that allows for genomic-scale comparative analyses of venom evolution. Examination of differential expression patterns of ~100,000 unique *L. hesperus* reference transcripts across tissue types identified sets of transcripts that are exclusively or primarily expressed in venom glands, including many novel toxin sequences. We have found that black widow venom has far greater molecular complexity than previously reported, but is distinctly different from other characterized venoms, in part due to a dramatic expansion of a unique toxin gene family. We are expanding this work to annotate emerging i5K spider genomes with the aim of determining the genomic architecture underlying venom diversification and molecular evolutionary processes that have led to the extreme toxicity of black widow venom.

81 - Virtual Screening of the *Anopheles gambiae* Octopamine Receptor

Kastner, Kevin; Estiu, Guillermina; Shoue, Douglas; Izaguirre, Jesus; McDowell, Mary Ann

Malaria is the most prevalent vector-borne disease in the world, threatening about 40% of the world's population. *Anopheles* mosquitoes transmit this deadly disease via the *Plasmodium* parasites to humans and thus the use of insecticides has been very beneficial in controlling the spread of malaria throughout the world. However, insecticide resistance is increasing, requiring the development of new, effective insecticides with novel modes of action. Octopamine receptors perform essential functions in invertebrate-only biological pathways, making this class of G-Protein Coupled Receptors (GPCRs) a potentially good target for insecticides. As there is currently little structural and experimental data for this class of GPCRs, we developed homology models of the *A. gambiae* octopamine receptor, useful for *in silico* docking of small molecule libraries. In order to account for differences in antagonist and agonist conformations, homology models were generated using beta-2 adrenergic receptor bound with an inverse agonist (PDB code: 2RH1) as a template for the antagonist conformation and the active beta-2 adrenergic receptor-Gs protein complex (PDB code: 3SN6) for the agonist conformation. The models were further refined by 20ns Molecular Dynamics simulations of the structures in complexes with promethazine (antagonist) and octopamine (agonist). Virtual screening has been found to successfully identify new active compounds, even finding antagonists for GPCRs. We have used this method to discover new active compounds that were verified experimentally. The analysis of the primary ligand binding interactions observed in the computational models allowed us to uncover key residues that were confirmed by site-directed mutagenesis. In conclusion, using both computational and experimental methods, we discovered several potential antagonists that are being used as lead compounds for designing new insecticides.

82 - Genomic divergence between Kiribina and Folonzo karyotypes of *An. funestus* from NGS of pooled DNA samples

Witzig, Claudia; Fontaine, Michael C.; Steele, Aaron; Smith, Hilary A.; Guelbeogo, M; Sagnon, N. 'F.; Emrich, Scott; Besansky, Nora J.

The mosquito *Anopheles funestus* Giles, 1900, is one of the most important malaria vectors in Africa and worldwide. It is a highly anthropophilic, endophilic mosquito and its malaria transmission exceeds that of *An. gambiae* in many parts of Africa. *Anopheles funestus* display extraordinary abilities to adapt to a range of environments. Exploring ecological adaptation to better understand population substructure and disease epidemiology of this mosquito is an important step for vector control. Studies on the distribution of *An. funestus* chromosomal inversions have highlighted a great complexity.

In Burkina Faso, a deficit of heterozygotes and linkage disequilibrium among some chromosomal rearrangements has been observed. This strong departure from Hardy-Weinberg equilibrium was found to be temporally and geographically stable in the sampled area, indicating the existence of assortative mating. This led to the identification of two chromosomal forms, named Kiribina and Folonzo, which are characterized by contrasting degrees of inversion polymorphisms.

The two forms are morphologically indistinguishable but differentiated at the ecological level. Observed seasonal differences suggest different larval habitat preferences of Kiribina and Folonzo. Here, we used whole genome sequencing of pools ('pool-seq') of karyotyped females of Kiribina and Folonzo to compare the pattern of genomic diversity and divergence of these two chromosomal forms. Specifically, we addressed the following questions: (i) Are these two groups genomically distinct? (ii) If true, are these areas of genomic divergence widespread or localized? (iii) Does divergence result from genetic drift or selective process (iv)? Which genes or genomic regions have been affected by selection? (v) Can they be related to "ecotypic" divergences?

83 - Evaluation and Development of GPCR Classifiers for Vectors

Nowling, Ronald

We aim to inexpensively develop insecticides for disease vectors such as mosquitoes by incorporating bioinformatics and computational biology into all aspects of the drug development process. Due to the popularity of G-Protein Coupled Receptors (GPCRs) as drug targets, a GPCR classifier that perform well on vector proteomes and provides a prediction confidence score for each identified peptide is of great interest.

Still at the early stages of our project, we seek to identify a set of top drug target candidates from among the G-Protein Coupled Receptors (GPCRs), proteins which are popular drug targets, in the vector proteomes. We have evaluated two existing GPCR classifiers (GPCRHMM and PredCouple) on six genomes (*Ae. aegypti*, *An. gambiae*, *Ap. mellifera*, *Dr. melanogaster*, *Ho. sapiens*, and *Pe. humanus*). In addition, we have developed and evaluated an ensemble classifier that provides a probability for each sequence, enabling an intuitive way to control the trade off between sensitivity and accuracy. We show that our ensemble classifier provides greater or equal sensitivity with approximately equal accuracy.

84 - Transcriptome Analysis of Neuronal and Secretory tissues in Asian Honeybee (*Apis cerana*) using Illumina RNA-seq Technology.

Doori, Park; Hyung-wook, Kwon

Apis cerana, the Asian honey bee, is widely kept in eastern and southern part of Asia. *A. cerana* is known to have strong resistance against mites, wasps, and several pathogenic diseases. They also have a good ability in adapting to climate change and bring considerable economic benefits from their by-products comparing with western honeybee, *Apis mellifera*. With limited knowledge of molecular and physiological information in *A. cerana*, it has been overlooked the importance of biological models and effects of agricultural industry as potential pollinators. Therefore, in order to obtain the tissue-specific genomic information of *A. cerana*, we constructed cDNA libraries from three different tissues of *A. cerana*, brain, antennae, and hypopharyngeal gland, using high-throughput RNA-sequencing technology. Throughout the various homologous search against public database (NR, Swissprot, and GO), we obtained a significantly different gene expression information between *A. mellifera* and *A. cerana* such as metabolic process, biological regulation, and molecular binding protein categories. Further, we also focused more deeply on the genes related with neuronal and olfactory modulation and major royal jelly protein family. Our transcriptome data would bring us to the better knowledge of gene expression patterns in this honey bee species, which will in turn pave the way for the better understandings of gene functions in behavior and ecology of the honeybee.

85 - Functional Characterization of Mosquito GPCRs as Targets for Insecticide Development

Shoue, Doug; Lai, Tarissa; Ogino, Jayme; Fuerst, Megan; Wolford, Julia; Stayback, Gwen; Wadsworth, Mariha; Nowling, RJ; Abrudan, Jenica; Kastner, Kevin; Estiu, Guillermina; Markley, Lowell; Collins, Frank; Izaguirre, Jesus; McDowell, Mary Ann

With over one million deaths per year caused by mosquito-borne illnesses that include malaria, dengue, and yellow fever, mosquitoes are considered a major health concern worldwide. Vector control through insecticide treatment is a major tool for combating these diseases, however increased resistance to some insecticides has decreased their effectiveness. Consequently, prevention of these infectious diseases through new pesticides is a significant focus of research. Because G protein-coupled receptors (GPCRs) serve many key physiological roles, GPCRs are common targets for drug development. This research investigates GPCRs of interest as potential targets for the development of novel mosquito insecticides. Using cell-based assays to monitor cAMP and calcium changes, we have functionally characterized a number of GPCRs, Dopamine, Octopamine and Neuropeptide F receptors. Through their characterization we have gained insight into ligand binding and activity. In addition to identifying agonists and antagonist we have performed a screen to identify novel ligands. These compounds can be further developed as potential insecticides.

86 - Lepidopteran specific sequence near N-terminus of Distal-less.

Brown, Thomas

Lepidopteran specific sequence near *N*-terminus of Distal-less. *Brown, Thomas M. Genectar Com LLC, Whitefish, MT 59937. www.Genectar.com

Distal-less is among five regulatory genes expressed in the eyespot pattern region in butterfly wing imaginal disc development (Shirai et al. 2012. BMC Evolutionary Biol. 12:21). Upstream of the highly conserved homeodomain, peptide 66 QQNPHEA 72 was observed in all of several known lepidopteran distal-less sequences, but not among those of other families detected by BLASTp (Altschul et al. 1997. Nucleic Acids Res. 25:3389). A gap was inferred for most dipteran sequences when aligned. Results will be discussed in relation to lepidopteran pigmentation and to a native Montanan species displaying on forewings the white-black-yellow concentric ring eyespot pattern.

Systems Biology/Population Genomics

87 - Novel Discoveries in the Male Accessory Secretions of the Tsetse fly (A transcriptomic/proteomic analysis)

Attardo, Geoffrey; Scolari, Francesca; Benoit, Joshua; Michalkova, Veronika; Malacrida, Anna; Aksoy, Serap

Tsetse flies are the sole vectors of the human and animal forms of African Trypanosomiasis, neglected diseases that affect the health and development of marginalized populations in sub-Saharan Africa. Vector population control is one of the primary methods to prevent trypanosomiasis transmission. However, little is known about the reproductive biology of tsetse flies. A particularly important aspect of tsetse reproductive biology, male seminal secretions, remains unstudied. Based upon the knowledge derived from other insects such as *Drosophila* and various mosquito species, the proteins contained within these secretions play an important roles in regulating sperm storage, sperm motility, sperm competition, female sexual receptivity, egg production, ovulation, reproductive tract morphology, feeding behavior and other aspects of fly biology. To establish a foothold on this aspect of tsetse reproductive biology, we undertook a project to sequence the transcriptome of the male accessory tissue in tsetse and to sequence the proteome of the male seminal secretions found within the female after mating.

Material for transcriptomic analysis was derived from dissected adult male reproductive tracts at different time points. Samples were collected from teneral, 3 day old (reproductively mature), and 6-8 hours post mating male flies. Male reproductive tracts were dissected into two fractions, testes and accessory glands. RNA isolated from these samples was used to construct 6 illumina libraries which were sequenced using paired end sequencing technology. To complement the transcriptomes, samples of male produced spermatophores were collected for proteomic analysis from the uterus of newly mated female flies. Protein samples were analyzed by LC-MS/MS. Male accessory and testes illumina sequencing data was analyzed to identify the most abundant transcripts within each library, compared between libraries to identify temporal and tissue specific differential expression patterns and compared with transcriptome data from adult female flies to identify male specific transcripts. The results of these analyses were then compared with the proteomic data to confirm transcriptomic predictions of secreted accessory proteins and identify additional unpredicted proteins.

Analysis of these data sets resulted in the identification of a novel set of male accessory genes/proteins. Our initial analysis has identified a total of 25 putative accessory gland proteins via cross referencing our tissue specific transcriptome and the spermatophore proteome. Of these proteins, only one of the predicted genes (a serine protease inhibitor from the BPTI/Kunitz family) is orthologous to an accessory protein identified within *Drosophila*. Many proteins identified are tsetse specific and novel. Three of these novel proteins are the most abundant proteins in the spermatophore. These three proteins form a novel tsetse specific gene family that appears to have arisen through tandem gene duplication events. Further functional analysis will be required to identify the role that these novel proteins play in tsetse reproductive physiology. However, while many of the proteins we have identified are not true orthologs to other characterized accessory proteins, their function appears to have remained orthologous to those of accessory proteins from other Dipteran species. These functions include serine protease inhibitors, odorant binding proteins, antioxidants, immune proteins, glycoproteins, endocuticle proteins and sperm binding proteins.

88 - Towards ENCODE-like initiatives for unraveling biological adaptive traits in non-model organisms

Tagu, Denis; Negre, Nicolas

Forward genetics analysis has been classically successful in identifying and characterizing genes involved in major biological processes such as the regulation of development, cell cycle and cell signaling. However, the number of organisms supporting genetic approaches is very limited. Model organisms have mostly been chosen based on technical advantages. Indeed, genetics requires the capacity to easily rear or cultivate the organism in the lab in order to i) generate thousands of mutants, ii) produce several genetic crosses required for getting homozygous individuals at the mutated loci and iii) perform these genetic crosses rapidly thanks to short life cycles. What model organisms do not provide, however, is to capture the biodiversity of many life history traits. In fact, most of the living organisms that either participate to sustaining environment (e.g. the honey bees) or are responsible of most health, agronomical and environmental issues (e.g. human and animal disease causing agents, plant pests, invasive species) are non-model organisms. Here in, we would like to stress the opportunity to develop ENCODE-like initiatives on non-model organisms.

Fortunately, the democratization of high throughput analyses of the organic cellular molecules (mostly nucleic acids, but also proteins and metabolites) provides the possibility to heavily and even exhaustively describe the availability of different epigenetics DNA and chromatin states, and RNA molecules and post-transcriptional regulations, with no restriction for the type of species that can be retrieved from nature or reared in the lab. But what says a catalog of molecules without demonstration of their role in some particular biological contexts? Is it worth getting such an amount of purely descriptive data from non-model organisms without having access to functional assays such as forward genetics or transgenesis? Is it worth investing in the accumulation of terabytes of data from non model organisms?

We think the answer is “We have no choice”. In this paper, we will take example of the ENCODE and mod-ENCODE initiative to demonstrate how interdisciplinarity between genomics, bioinformatics, and modeling can open new tracks on the analysis of cell functioning. We will finally propose to develop ENCODE-like initiatives on non-model organisms, such as Arthropods.

89 - The University of Maryland Insect Transformation Facility: Providing researchers access to Transgenic and Non-Transgenic Insect Technology

Harrell, Robert; O'Brochta, David; Aluvihare, Channa

The University of Maryland Insect Transformation Facility (UM-ITF) provides functional genomics researchers access to transgenic and non-transgenic genome altering technologies. The techniques for altering insect genomes have been available for many years however they have not been widely used, mainly because the technology requires a high level of expertise and specialized equipment. The mission of the UM-ITF is to aid researchers in the creation of genetically modified insects through; fee for service microinjection of insects with developed genome altering protocols, collaboration to develop genome altering protocols for insects without such protocols, training for researchers who are interested in employing these technologies and access to "State-of-the-art" equipment for these purposes.

The key manner in which the UM-ITF assists researchers is through "fee for service" projects, mainly for *Aedes aegypti*, *Anopheles stephensi* and *Tribolium castaneum*, species actively maintained in the facility. The facility also works with other species as well but at the same high frequency as the species above. The facility offers "Full transgenesis" service for *Aedes aegypti* and *Anopheles stephensi* and "Microinjection only" services for *Aedes aegypti*, and *Tribolium castaneum*. "Full service transgenesis" includes client vector confirmation, microinjection of enough embryos to produce a contract specified number of G0 adults, G0 and G1 rearing and mating, G1 screening and transgenic confirmation by PCR, all for a reasonable cost. "Microinjection only" includes client vector confirmation and microinjection of a contract specified number of embryos; the injected embryos are then shipped to the client for rearing and screening. All injections include a quality assurance protocol that guarantees injections were of a quality to produce transgenic lines. The UM-ITF has developed a strong reputation with the success it has had in providing these "fee for service" transgenic services.

In an expansion of the facilities services, the UM-ITF recently has had success in creating an *Anopheles stephensi* "knockout" mutant by injection of TALENs and is now able to offer this as a "microinjection only" service for *Aedes aegypti*. We are currently working on a TALEN "knock-in" strategy which would improve the protocol by making the detection of mutants much simpler.

The UM-ITF assists researchers conducting research on insect which do not have developed transgenic and non-transgenic genome altering technologies by engaging in collaborative efforts to develop these technologies with these researchers. The UM-ITF collaboration with researchers at Cenicafe, Columbia's national coffee research center, is an example. Through this collaboration a protocol for the transformation of *Hypothenemus hampei*, the Coffee berry borer was developed.

The UM-ITF staff also provides training in all aspects of insect transgenesis technology ranging from single-day to week-long training sessions depending on the needs and experience of the client. Clients may also utilize the UM-ITF equipment. After an initial orientation session researchers may utilize the UM-ITF facilities at a reasonable per hour charge. The ability of researchers to use the UM-ITF equipment allows them access to "State-of-the-Art" equipment that they may not be able to afford or for which they only have limited needs.

90- Introducing Blacktie: a simpler way to do RNA-seq using Tophat/Cufflinks/CummeRbund *Dunn, Augustine*

Leveraging multiple fastQ files full of RNA-seq reads into a coherent picture of gene expression and transcript models is a multi-step process. It requires the organization and coordination of many files of different types through many different program calls and output steps. Each step might take hours or days depending on your input data. Then, as you are writing up your work, sometimes weeks/months later, you see that a new version of the programs you use has come out. Do you need to re-run your analysis? What settings DID you use back then?

The Tophat/Cufflinks/CummeRbund group of programs makes quality RNA-seq analysis doable once you understand the process. But what about when its time for you to leave the lab and you need to “train” someone else to repeat your process? It can be a nightmare. Especially if the trainee is not yet comfortable with the command line.

This is why I wrote the Blacktie pipeline software. Its goals are to streamline and simplify the complex task of analyzing full RNA-seq experiments using these programs; to automatically record settings used and program output messages in a way that users can track them to data later; provide a base set of functions and classes that will allow users to create custom pipelines easily by editing a single file (or if they want: writing their own custom scripts).

Some of Blacktie’s features include:

1. simple installation
2. simple command line interface that allows almost ANYBODY to fully automate and reliably repeat their analysis of RNA-seq data with Tophat/Cufflinks/CummeRbund
3. send email updates to the user
4. intelligently continue with the analysis if a single run fails
5. run multiple, complex tophat/cufflinks experiments at once using a single command
6. generates SGE qsub-able scripts for use with a computing cluster
7. automatic preliminary CummeRbund Quality Control, Basic Differential Expression, and Basic Pattern Discovery plots using CummeRbund

Dedicated bioinformatics personnel can be few and far between in the arthropod genomics field. Blacktie aims to bring automated, reproducible RNA-seq with built-in record keeping to more labs so that your valuable data does not fester on your servers, and you can publish sooner.

The code is available from the Python Package Index
or from its homepage: <https://github.com/xguse/blacktie>

Documentation: <http://xguse.github.io/blacktie/index.html>

91 - Splashes of color in an ocean of genes: the gene-rich transcriptomes of two highly color-polymorphic spiders.

Croucher, Peter; Brewer, Michael; Winchell, Christopher; Oxford, Geoff; Gillespie, Rosemary

A number of spider species within the family Theridiidae exhibit a dramatic abdominal color polymorphism. The polymorphism is inherited in a broadly Mendelian fashion and in some species consists of dozens of discrete morphs that are convergent across taxa and populations making these spiders fascinating examples of frequency-dependent balancing selection. Since few genomic resources exist for spiders, as a step towards identifying the genetic basis for this trait we have sequenced and *de novo* assembled the nearly complete transcriptomes of two species: the Hawaiian happy-face spider *Theridion grallator* and a convergently patterned mainland congener *Theridion californicum*. The gene complement of these species was mined for pigment-pathway genes and subject to differential expression (DE) analyses between unpatterned morphs (plain yellow) and patterned morphs (yellow with superimposed patches of red, white or black).

Deep sequencing both RNA-seq and normalized cDNA libraries from pooled specimens of each species enabled the assembly of a comprehensive gene set for both species that is estimated to be 98-99% complete. It is likely that these species express at least 33,500 protein-coding genes, perhaps 15% (ca. 5000) of which might be unique to spiders. DE analyses and mining for pigment-associated *Drosophila melanogaster* genes indicated the presence of the ommochrome pathway genes and suggested a previously unknown role for the pteridine pathway in theridiid color patterning.

The estimates presented here suggest that these spiders may express more protein-coding genes than described for any other Metazoan to date. It is likely that many of the novel genes and gene families are involved in venom and silk production. Our comprehensive assembly illustrates the continuing value of sequencing normalized cDNA libraries *in addition to* RNA-seq in order to generate a reference transcriptome for non-model species. The identification of pteridine-related genes and their possible involvement in color patterning is a novel finding in spiders and one that suggests a biochemical link between guanine deposits and the pigments exhibited by these species.

92 - Characterizing the infection-induced transcriptome of *Nasonia vitripennis*

Sackton, Tim

Understanding patterns of conservation and change in insect immune systems is a crucial component of work to reveal how innate immune pathways respond to evolutionary pressures imposed by pathogenic organisms. While key signaling components of the innate immune system appear to be broadly conserved in insects, the rapidly evolving recognition and effector components can be difficult to identify across diverse insect orders by homology alone. With the advent of RNA-sequencing, direct characterization of the transcriptomes of infected insects has become a feasible method for functional characterization of immune-regulated pathways in emerging genomic systems. Here, I present the infection-induced transcriptome of the jewel wasp, *Nasonia vitripennis*. To identify genes regulated by infection in *N. vitripennis*, I induced an immune response by pricking adult wasps with a bacterial culture containing common Gram-positive and Gram-negative entomopathogens. Eight hours after infection, treated and naïve wasps were collected for RNA-seq. Two replicates of each condition were sequenced using Illumina, generating between 5.2 million and 11.8 million mappable reads per replicate. Using the recently generated OGS v2 geneset in *Nasonia*, I was able to identify over 1,000 genes regulated by infection, which represent about 10% of all genes with detectable expression in this experiment. These genes include both known and novel immune components, and allow one of the few detailed examinations of the set of immune-responsive genes in a Hymenopteran.

93 - The genetics and evolution of hybrid incompatibility in the red flour beetle, *Tribolium castaneum*.

Watson, Eric

In sexually reproducing species, speciation is the process of converting variation within a species to differences between species through the evolution of reproductive isolation. It is a longstanding goal of evolutionary biologists to find “speciation genes”, which promote the divergence of two lineages by reducing the amount of gene flow between them. Taxon pairs exhibiting heritable intraspecific variation for interspecific hybrid incompatibility are a critical, yet overlooked component for understanding the early stages of speciation. Here, we investigate the genetic basis of polymorphic hybrid incompatibility occurring between recently isolated populations of the red flour beetle, *Tribolium castaneum*. Hybrids between wild populations collected from Tanzania (DES) and an outbred laboratory strain (c-SM) exhibit a neuromuscular disorder (NDD) upon eclosion, with affected individuals exhibiting muscular tremors, uncoordinated movement, complete ataxia, and premature death. We combine next-generation sequencing of pooled-DNA samples (pool-Seq) with a genome wide association (GWAS) approach using extreme discordant sib-pairs by choosing cases and controls from a single family. This approach increases the power to detect association greatly over case-control pairs selected at random while also controlling for population structure. We identify candidate speciation genes on chromosomes 3 and 9, which contain conserved domains related to oxidative stress which have recently been implicated as a common and fundamental mechanism in the pathogenesis of neurodegenerative disorders.

94 - Phenotypic plasticity in insects: How does one genome produce different phenotypes in response to the environment?

Duncan, Elizabeth; Dearden, Peter

All animals are able to respond to changes in their environment, but in some animals the environment has a profound effect on the biology and behavior of the animal. In the most extreme cases the environment is able to stimulate the production of discrete phenotypes.

This response, called phenotypic plasticity, is found in all major groupings of animal life, including the insects. But how is a single genome able to encode different, environmentally induced phenotypes? We are using two different examples of phenotypic plasticity seen in insects, the honeybee (*Apis mellifera*) and the pea aphid (*Acyrtosiphon pisum*) to understand how animals sense a change in their environment, how the responses are encoded in the genome, how the phenotypic changes are stabilised and how phenotypic plasticity has evolved.

In the female honeybee nutrition during early larval development determines caste; if larvae are fed royal jelly they develop into queens and if not they develop into worker bees. Queen and worker bees differ in reproductive capacity, size, physiology and behaviour. Normally the queen is the only reproductively active female in the hive, and worker bees have small quiescent ovaries. However, if the queen is lost from the hive, the bees sense this change in the environment and activate their ovaries laying eggs. We have used RNA-seq analysis, together with immunohistochemistry and functional analysis, to identify a key cell-signalling pathway that controls ovary activation in the honeybee.

The second insect we are studying is the pea aphid. The pea aphid usually reproduces asexually, but in response to environmental change (decreasing temperatures and short day lengths) it is able to reproduce sexually, producing eggs. The ovaries and early embryos of sexual and asexual aphids are morphologically distinct, but more remarkably there are also molecular differences in early development. Using these two insects we are beginning to understand how these environmentally induced phenotypes occur. We are now focusing on how these complex responses are encoded in the genome of these insects; to do this we are using analysis of DNA methylation, histone modifications, and chromosome structure.

95 - The use of next-generation sequencing for phylogenetic evaluation of the genus *Aphelinus*
Kuhn, Kristen; Hopper, Keith

Aphelinus wasp species are natural enemies of aphids, and they have a long history of use in the biological control of these important agricultural pests. The genus *Aphelinus* contains 87 species currently treated as valid, but many new species are being discovered, including both cryptic species and morphologically different species. In fact, one impediment to taxonomic progress in this genus has been the prevalence of cryptic species. These species differ slightly in morphological characters, but are phylogenetically distinct lineages that differ in their biology and are reproductively isolated from one another. Next-generation sequencing provides far more data than standard Sanger sequencing, and we have already begun to discover and genotype large numbers of single nucleotide polymorphisms (SNPs) distributed across parasitoid genomes. To get sequence data for the *de novo* assembly of reference genomes, we are using a combination of both Illumina paired-end libraries and PacBio RS data from genomic libraries prepared with standard kits and protocols. We are mapping the sequences from reduced representation libraries (RRLs) to the appropriate reference genomes to determine identity and homology of SNPs. *Aphelinus* genomes are about 300 megabases and this sequencing approach gives >100x coverage. 32 Gb of paired-end Illumina sequence data (117x average coverage) was used to assemble the *Aphelinus atriplicis* genome. This assembly was 267 Mb long with 47k contigs greater than 1kb, with 50 percent of the contigs being greater than 8kb. We have made and sequenced RRLs for 15 populations in 9 species of *Aphelinus*, with each library generating 1-16 million reads (100-1600 Mb). These reads were then mapped on the *A. atriplicis* genome assembly, giving consensus lengths of 2-9 Mb or 1-2 % of the reference genome with 10-65x average coverage. Using these mappings, we detected 287k SNPs (with a minimum of 20x coverage) that differ among these species and populations, 55k of which were phylogenetically informative. For comparison, in previous research using Sanger sequencing of known genes, we detected 303 phylogenetically informative SNPs in 2868 bp of sequence from 6 genes among 12 species and populations from these same complexes. Thus, Illumina sequencing of RRLs provided over 180x more phylogenetically informative SNPs than we found using Sanger sequencing of known genes.

96 - *Phlebotomus papatasi* salivary gland sequence variability in Middle Eastern field populations: potential impact for vaccine development

Wadsworth, Mariha; Stayback, Gwen; Bernard, Megan; Shoue, Doug; Abrudan, Jenica; Mukbel, Rami; Coutinho-Abreu, Iliano; Ramalho-Ortigao, Marcelo; Hanafi, Hanafi; Fawaz, Emadeldin; El-Hossary, Shabaan; Hoel, David; McDowell, Mary Ann

Hematophagous insects are not simply flying syringes, inoculating agents of human disease. Rather these vectors inoculate molecules to aid in blood-feeding that also have profound effects on the host immune system. The sand fly *Phlebotomus papatasi* is the primary vector for *Leishmania major* in northern Africa, southern Europe and the Middle East. Studies indicate that repeated exposure to sand fly saliva creates an inhospitable environment for the establishment of *Leishmania* infection, leading to attenuated *L. major* disease in rodent models. These data suggest that the incorporation of salivary molecules in multi-component vaccines may be a viable strategy for the development of anti-*Leishmania* vaccines. Here we investigated sequence variability of salivary gland proteins from three field populations of *P. papatasi* sand flies from the Middle East. Salivary gland cDNAs encoding nine secreted proteins were PCR amplified, sequenced and analyzed. Genetic analyses revealed little genetic diversity between populations. However, some limited selection was observed for some of the proteins analyzed. Mapping the genetic variability of sand fly salivary proteins in field populations is a crucial step in defining saliva-based vaccine candidates.

97 - RNA-Seq reveals early distinctions and late convergence of gene expression between diapause and quiescence in the Asian tiger mosquito, *Aedes albopictus*

Poelchau, Monica; Reynolds, Julie; Elsik, Chris; Denlinger, David; Armbruster, Peter

Diapause is a critical adaptation allowing insects to withstand harsh seasonal transitions. The pre-programmed developmental arrest of diapause is a form of dormancy that is distinct from quiescence, in which development arrests in immediate response to hardship. Much progress has been made in understanding the environmental and hormonal controls of diapause. However, studies identifying transcriptional changes unique to diapause, rather than quiescence, are lacking, making it difficult to disentangle the transcriptional profiles of diapause from dormancy in general. The Asian tiger mosquito, *Aedes albopictus*, presents an ideal model for such a study, as diapausing and quiescent eggs can be staged and collected for global gene expression profiling using a newly developed transcriptome. Here, we use RNA-Seq to contrast gene expression during diapause with quiescence throughout early, middle, and late stages of diapause to identify transcriptional changes specific to the diapause response. We identify global trends in gene expression that show gradual convergence of diapause gene expression upon gene expression during quiescence. Functionally, early diapause *Ae. albopictus* show strong expression differences of genes involved in metabolism, which diminish over time. Of these, only expression of lipid metabolism genes remained distinct in late diapause. We identify several genes putatively related to hormonal control of development that are persistently differentially expressed throughout diapause, suggesting these might be involved in the maintenance of diapause. Our results identify key biological differences between diapausing and quiescent pharate larvae, and suggest candidate pathways for studying metabolism and the hormonal control of development during diapause in other species.

98 - Genome-wide expression patterns during diapause induction in *Culex pipiens* mosquitoes

Hickner, Paul V.; Mori, Akio; Zeng, Erliang; Tan, John C.; Severson, David W.

Culex pipiens (L.), the northern house mosquito, is an important vector of several human pathogens including West Nile virus and filarial nematodes causing lymphatic filariasis. It is among the most geographically widespread mosquito species in temperate regions worldwide. Adult females are able to survive the adverse conditions associated with winter by entering diapause, a state of programmed developmental arrest. In *Cx. pipiens*, the environmental stimuli determining diapause are the lower temperatures and shorter photoperiods accompanying late summer or early fall. We performed comprehensive gene expression profiling in standard (25°C; 16h light/8h dark) and diapause-inducing (18°C; 8h light/16h dark) conditions at three time-points (8, 16, and 24 hours post-treatment) during the early pupal stage, an environmentally-sensitive period for diapause induction. Using ANOVA and the student's t-test we identified 1,132 differentially expressed genes (p -value ≤ 0.05 using Benjamini-Hochberg corrections). A gene network consisting of five modules was constructed based on correlated expression across the three time points. Genes in each of the five modules were used in pathway enrichment analysis in DAVID (KEGG). Network modules containing genes that were down-regulated during diapause induction were significantly enriched for genes involved in cuticle development and the circadian rhythm pathway, while modules that were up-regulated were enriched for genes in the TGF- β and Wnt signaling pathways. This analysis offers novel insights into the molecular basis of diapause by identifying potential mechanisms inducing diapause in *Cx. pipiens* mosquitoes.

99 - Morphological and molecular characterization of mealybugs (Pseudococcidae) in banana plantations (Musa AAA) from the Atlantic zone of Costa Rica and its comparison with mealybugs from the Neotropical Region

Palma Jiménez, Melissa; Blanco Meneses, Mónica

To determine taxonomic relationships between harmful insects in agriculture, it is important to use both morphological and molecular characterization techniques in reliable studies. Electron microscopy and light microscopy combined with molecular analysis allow insects proper classification. On the other hand, often prohibit correct classification, especially in female mealybugs. Thus the aim of this study was to apply the techniques of scanning electron microscopy and light microscopy and related it to the sequencing of specific genes: the 18S ribosomal DNA (rDNA) and the elongation factor 1 alpha (EF-1 α) of female mealybugs (Pseudococcidae) from 12 farms in Costa Rica and 7 countries from the neotropical region with a high incidence of crop pests in banana (*Musa AAA*). The study allowed the correct identification of the species causing the decrease in crop yield. In the present investigation, differences were obtained in the comparative analysis of genes and morphology of specimens in different geographic regions were performed. The genders present in banana plants were mostly identified like *Pseudococcus* and *Disymyococcus*.

100 - Changes in transcripts 3 hr after blood feeding based on Illumina Data with *Anopheles gambiae* with emphasis on cuticular proteins

Vannini, Laura; Reed, Tyler; Dunn, W. Augustine; Willis, Judith

We found significant changes of more than 2-fold in 764 transcripts 3 hr after a blood meal; 323 increased and 406 decreased. These transcripts were predominantly for genes associated with immunity and stress (23 increased, 80 decreased) and intermediate metabolism (58 increased, 35 decreased). Data available in Baker et al. 2011 (BMC Genomics 12:296) allowed us to learn that the midgut and Malpighian tubules were major sources for the affected transcripts. Thirty-one transcripts from genes coding for cuticular proteins (CPs) had RPKM>2 in non-blood fed mosquitoes even though the adults were taken 5 days after eclosion, when cuticle synthesis should have been completed. Only one (CPR26) increased and three (CPLCX2, CPAP3-A1b, CPR101) decreased significantly in transcript abundance after the blood meal. We examined the tissue localization of several of these CP transcripts with *in situ* hybridization. In almost all cases, no signal was detected in adults of this age, but younger animals revealed where the genes were expressed, and several of them had transcripts present exclusively or primarily in developing eyes. A comparison of our data with that from a microarray analysis by Marinotti et al. 2006 (Insect Mol Biol 15:1), using rank order of transcript abundance, revealed similar results for the non-blood fed mosquitoes, but massive differences in transcript abundance and even direction of change 3 hr after the blood meal. RT-qPCR analyses confirmed our Illumina data, leaving us uncertain about which of the seemingly slight differences between our conditions and those used by Marinotti et al. account for the huge differences between the two studies.

101 - A Comparison of Multicopper Oxidase-1 Orthologs from Diverse Insect Species

Gorman, Maureen; Lang, Minglin; Braun, Caroline; Brummett, Lisa; Dittmer, Neal; Kanost, Michael

Multicopper ferroxidases catalyze the oxidation of ferrous iron to ferric iron. In yeast, algae and mammals, multicopper ferroxidases facilitate the transport of iron across cell membranes. The functions of insect ferroxidases are unknown. *Drosophila melanogaster* multicopper oxidase-1 (DmMCO1) is a newly discovered multicopper ferroxidase. DmMCO1 is expressed in many tissues, but highest expression is in the digestive system and Malpighian tubules. DmMCO1 is located on the surface of these tissues, such that the enzyme is positioned to oxidize ferrous to ferric iron in the hemolymph. RNAi-mediated knockdown of DmMCO1 results in decreased iron accumulation in midguts and whole bodies. We predict that DmMCO1 has a role in iron transport. We have identified an MCO1 ortholog in all insect genomes analyzed to date. This observation suggests that MCO1 orthologs may have a conserved function in insects. To better understand the biochemical activity and physiological functions of MCO1, we have begun to compare the amino acid sequences, expression patterns, enzymatic activity, and loss of function phenotypes of MCO1 orthologs from *D. melanogaster*, *Anopheles gambiae*, *Tribolium castaneum*, and *Manduca sexta*. The similarities we have observed suggest that MCO1 orthologs from diverse species have a similar function. Thus, we can exploit the specific technical advantages of these four model insect species to learn more about iron transport in insects.

102 - TALEN-based gene disruption in the dengue vector *Aedes aegypti*.

Aryan, Azadeh; Anderson, Michelle; Myles, Kevin; Adelman, Zach

In addition to its role as the primary vector for dengue viruses, *Aedes aegypti* has a long history as a genetic model organism for other bloodfeeding mosquitoes, due to its ease of colonization, maintenance and reproductive productivity. Though its genome has been sequenced, functional characterization of many *Ae. aegypti* genes, pathways and behaviors has been slow. TALE nucleases (TALENs) have been used with great success in a number of organisms to generate site-specific DNA lesions. We evaluated the ability of a TALEN pair to target the *Ae. aegypti kmo* gene, whose protein product is essential in the production of eye pigmentation. Following injection into pre-blastoderm embryos, 20-40% of fertile survivors produced *kmo* alleles that failed to complement an existing *kh^w* mutation. Most of these individuals produced more than 20% white-eyed progeny, with some producing up to 75%. Mutant alleles were associated with lesions of 1-7 bp specifically at the selected target site. White-eyed individuals could also be recovered following a blind intercross of G₁ progeny, yielding several new white-eyed strains in the genetic background of the sequenced Liverpool strain. We conclude that TALENs are highly active in the *Ae. aegypti* germline, and have the potential to transform how reverse genetic experiments are performed in this important disease vector.

103 - Proteomic Analysis of Pharate Pupal Molting Fluid from the Tobacco Hornworm, *Manduca sexta*.

Dittmer, Neal; Rodriguez, Larry; Hiromasa, Yasuaki; Tomich, John; Kanost, Michael

Molting, the process of shedding of the old cuticle, is an essential and critical step in insect development. Following separation of the cuticle from the underlying epidermal cells, a solution of enzymes (molting fluid) is secreted into the newly created exuvial space that degrades the old cuticle prior to its being shed. While much has been learned of the various types of enzymes present (such as chitinases, proteases, esterases, and inhibitors), little is known of the specific proteins responsible for these activities. We have taken advantage of the recently completed sequencing of the *M. sexta* genome to perform a detailed analysis of molting fluid from the pharate pupal stage by coupling two dimensional gel electrophoresis with peptide mass fingerprinting mass spectrometry and using the *M. sexta* Official Gene Set as the database for protein identification. We have identified 86 proteins and grouped them in the following categories: 1) extracellular matrix proteins, 2) chitin catabolism, 3) carbohydrate catabolism, 4) metalloproteases, 5) serine proteases, 6) serine protease homologs, 7) serine protease inhibitors, 8) other enzymes, 9) lipid-binding, 10) immunity, 11) miscellaneous, and 12) hypothetical. This analysis has aided in the identification of specific proteins for further investigation of cuticle degradation.

104 - Linking adaptation, delimitation of evolutionarily significant units (ESUs), and gene function: a case study using hemlock looper ecotypes

Lumley, Lisa; Cusson, Michel

Developing genetic markers for the identification of recently diverged groups, such as ecotypes or species complexes, remains difficult due to challenges with incomplete lineage sorting, hybridization and introgression. Genome-wide scans of single nucleotide polymorphisms (SNPs) have proven useful for inferring patterns of genetic differentiation at the population level. In combination with a new analytical technique, the discriminant analysis of principal components (DAPC), and within the framework of iterative taxonomy, it may also be possible to extract a combination of SNPs as markers for the delimitation of closely related groups. In addition, since DAPC identifies the loci contributing the most to group clustering, it may be possible to link putative biological function to differences that define group boundaries. We tested this technique on two ecotypes of the hemlock looper (*Lambdina fiscellaria*), which differ in terms of number of larval stadia, developmental rate and fecundity. It was possible to separately cluster the two ecotypes with 95% correct assignment using 27 SNPs. We also determined that a storage hexamerin carried eight of these SNPs, including the two highest contributing loci, of which the top contributor was nonsynonymous. Other studies have found this protein to be highly expressed just before metamorphosis, pointing to a possible connection between its role in clustering ecotypes and its biological function. These SNP markers can now be further developed for high throughput delimitation of individuals of unknown ecotype identity.

105 - Genome-level comparison of mRNA transcripts in the anterior versus posterior portions of embryos of a midge, *Chironomus riparius*

Klomp, Jeff; Kwan, Chun Wai; Athy, Derek; Schmidt-Ott, Urs

In the common fruit fly, *Drosophila*, the gene *bicoid* has been found to play an important role in the specification of the anterior-posterior (AP) axis in early development along with a variety of other important developmental regulatory functions. However, this gene is absent from the genomes of many insects, including the mosquito *Chironomus riparius*. It is unknown whether other factors take the place of *bicoid* in the regulation of the AP developmental axis in these other insects. To identify potential localized mRNA regulators of the AP developmental axis in *C. riparius* we sequenced the transcriptomes of the anterior and posterior cell halves using high-throughput RNA sequencing. Using paired-end 100bp sequence reads, we assembled and annotated contiguous nucleotide sequences from these RNA pools. We conducted differential expression analyses between the sequence reads of the anterior and posterior halves to identify candidate axis specification genes. Our results confirm the expression of known anterior and posterior localized genes of early fly embryos and point toward other transcripts that may play a role similar to *bicoid* in *Drosophila*.

106 - Mitochondrial genome sequences reveal deep divergences within the *Anopheles punctulatus* group

Logue, Kyle; Chan, Ernest; Phipps, Tenisha; Reimer, Lisa; Halldin, Cara; Small, Scott; Siba, Peter; Sattabongkot, Jetsumon; Zimmerman, Peter; Serre, David

Members of the *Anopheles punctulatus* group (AP group) are the primary vectors of human malaria and lymphatic filariasis in Papua New Guinea. Given their public health importance it is critical that we understand the species diversity and evolutionary history of *Anopheles*, for example, to determine why only certain mosquito species can transmit malaria and other human diseases. Here, we present the complete DNA sequences of 13 mitochondrial genomes from 7 distinct species: 5 from AP sibling species and 2 from the *An. dirus* complex in Southeast Asia. We assembled four sequences directly from whole genome sequencing data, while the remaining 9 mitochondrial genomes were sequenced simultaneously on one lane of an Illumina HiSeq 2000 instrument (after individual adapter-based barcoding). Our phylogenetic reconstruction suggests that the ancestor of the AP group arrived in Papua New Guinea 25 to 54 million years ago. Our results also reveal a deep divergence between *An. punctulatus* s.s. and the *An. farauti* clade occurring 18 to 41 million years ago. This deep divergence within the AP group is interesting, as humans did not arrive in Papua New Guinea until 50 thousand years ago. We hypothesize that many malaria-related traits, such as human blood preference or the ability to carry human parasites, occurred independently in each *An. punctulatus* sibling species, which provides an opportunity to map these traits using comparative genomic methods. This deep divergence among AP mosquitoes also suggests that gene flow between species is limited and, therefore, that insecticide resistance is unlikely to spread from one species to another but instead would have to occur independently in each species.

107 - The transcriptome of an invasive mosquito vector, *Aedes japonicus japonicus*

Parker, Derrick; Emrich, Scott; Hellmann, Jessica

Globally, invasive insects represent a major threat to ecosystem and human health. In the U.S., West Nile virus, dengue and avian malaria are examples of foreign arthropod-borne diseases that are transmitted (or have historically been transmitted) by an introduced mosquito. The Asian bush mosquito, *Aedes japonicus japonicus*, is a recently introduced vector in North America that shows rapid geographic expansion and a high competency for West Nile virus, but no genomic analysis of this species has been performed. Such analysis sheds light on the relatedness of *Ae. japonicus* to other native and non-native mosquito vectors (e.g., *Ae. albopictus* and *Ae. aegypti*) and can be the basis for ecological studies on the extent of local adaptation and the number and origin of introduction. Toward these ends, we performed *de novo* assembly of the *Ae. japonicus* transcriptome using sequence data from the Roche 454 GS FLX+ platform. Non-normalized cDNA of pooled samples (n= 40) of all development stages were sequenced, drawn from two geographic regions in the U.S. These regions represent a putative introduction region (NY/CT) and the southern extreme (SC) of the invaded range, and each was sequenced on a ¼ 454 plate. In addition to an entire species assembly, comparisons were made between regions to identify regional genetic differences.

108 - Insights into the genetic basis of blood feeding rhythms in *Culex pipiens* mosquitoes

Mori, Akio; Hickner, Paul V.; Cunningham, Joanne M.; Lovin, Diane D.; Chadee, Dave D.; Severson, David W.

The daily cycle of blood feeding activity by mosquitoes is an important factor in the etiology of mosquito-borne disease transmission. For example, with lymphatic filariasis, the density of microfilaria in the peripheral blood of humans infected with periodic *Wuchereria bancrofti* coincides with the daily cycle of blood feeding activity of *Culex pipiens sensu lato*, the primary mosquito vector. Here we investigated the genetic basis of blood feeding activity by QTL mapping and gene expression profiling using two laboratory colonies differing in their blood feeding behavior. Shasta strain females readily feed any time of the day, while Trinidad strain females will blood feed only after dark. To assess blood feeding preference, 5 to 7 day old female F1 intercross progeny were provided access to an anesthetized rat for 30 min at 11:00 am and 8:30 pm during the same day. Day feeding mosquitoes were separated from those that later did feed at night later subjected to DNA extraction. Individual progeny were subsequently genotyped for a panel of microsatellite and SSCP markers developed from the *Cx. quinquefasciatus* genome sequence. Quantitative trait locus (QTL) analysis identified genome regions containing genes that influence day versus night blood feeding behavior. To investigate the role of the circadian rhythm pathway on blood feeding, we used qRT-PCR to measure the expression of seven genes in the circadian rhythm pathway at 5 time-points in 5 day old females.

109 - Efficacy of a mosaic Long Lasting Insecticide Net (LLIN); PermaNet 3.0, against wild populations of resistant *Culex quinquefasciatus* in experimental huts in Togo, West Africa

Dery, Dominic; Ketoh, Guillaume; Chabi, Joseph; Apetogbo, George; Clitho, Isabelle; Glitho, Isabelle; Baldet, Thierry; Hougard, Jean-Marc

Background: The efficacy of a new mosaic Long Lasting Insecticide Net (LLIN); PermaNet®3.0, against natural free flying *Culex quinquefasciatus* was evaluated in six experimental huts between February-March, 2008 in Akodésséwa district of Lomé in Togo. Primary endpoints of evaluation were deterrence, exophily, blood feeding inhibition and mortality of free flying *Culex quinquefasciatus*. In parallel, field susceptibility studies on local *Anopheles* and *Culex* species were investigated using WHO test kits and protocol.

Methods: The wash resistance of the new mosaic net and its efficacy on vectors was compared with commercially marketed net (PermaNet®2.0) in experimental huts. In parallel, field susceptibility and resistant status of *Culex quinquefasciatus* and *Anopheles gambiae* local species populations were assessed and the two species of mosquitoes were tested to Permethrin (1%), DDT (4%), Bendiocarb (0.1%), Deltamethrin (0.5%, 0.05%), Carbosulfan (0.4%) and Chlorpyrifos Methyl (0.4%) using WHO vertical test tubes and protocol. Subsequent evaluation of Kdr status was carried out in *An. gambiae* s.s.

Results: A total of 1,223 *Cx. quinquefasciatus* females were collected within six week evaluation period (one complete Latin square rotation). The new mosaic net (PermaNet®3.0) unwashed was able to deter 16.84% of the total *Culex* mosquitoes caught. At 20 washes, the net deterred 5.79% of mosquitoes compared to 6.84% deterrence by the unwashed conventionally used net; PermaNet®2.0. Also, the net induced mosquitoes to exit huts by 50.48% and inhibited blood feeding 70.97% in its unwashed state. At 20 washes, the net performed equally well by inducing 42.91% mosquitoes to exit and inhibited 67.06% of mosquitoes from blood feeding. The new mosaic LLIN PermaNet®3.0 was able to give 76% personal protection at zero wash and 69% protection at 20 washes. More so, the net retained almost equal its insecticidal effect at zero wash (7.1%) and at 20 washes (6.5%). *Anopheles gambiae* s.s populations in Akodésséwa were susceptible to Chlorpyrifos Methyl, Deltamethrin and Bendiocarb, but resistant to the organochlorine DDT. *Cx. quinquefasciatus* species were resistant to all classes of tested insecticides.

Conclusion: Field susceptibility test of *An. gambiae* populations showed resistance to DDT, Permethrin and Carbosulfan (12%, 61% and 77% respectively) but was however susceptible to CM (100% mortality) and Deltamethrin (100% mortality) in tested insects. *Culex quinquefasciatus* species however revealed resistance to all insecticides tested. The M molecular form of *An. gambiae* s.s was predominantly high (97%) with no S form detected. There was however one hybrid form detected (3%). The kdr resistant genotype frequency F(R) was 0.84 with approximately 70% homozygotes kdrRR. This evaluation clearly depicts the success of vector control innovations using pyrethroids and non-pyrethroids in combination on nets.

110 - Extensive Chromosomal Insertions Noted in the *Glossina* Genome

Falchetto, Marco

Wolbachia is an obligatory intracellular maternally transmitted bacterium that infects many arthropod and filarial nematode species. *Wolbachia* infections result in a number of reproductive alterations in the host, including parthenogenesis induction, male killing, feminization of genetic males, and cytoplasmic incompatibility (CI). *Wolbachia* has prompted research regarding its potential for control of agricultural and medical disease vectors, including *Glossina* spp., which transmits African trypanosomes, the causative agents of sleeping sickness in humans and nagana in animals. Several recent studies reported that *Wolbachia* genes have been horizontally transferred to host chromosomes including in *Glossina morsitans morsitans*. In our study we present the localization of three *Wolbachia* insertions on *G. m. morsitans* mitotic chromosomes. From the *in situ* hybridization results, it appears that the three *Wolbachia* sequences *16S rRNA*, *fbpA*, and *wsp* consistently show a biased location on X, Y and B supernumerary chromosomes. Southern-blot analysis were performed to determine the number of chromosomal insertions, using genomic DNA from tetracycline treated *G. m. morsitans* females and untreated normal females and males. Both the Southern blot and *in situ* hybridization analyses provide evidence that *Wolbachia* fragments are inserted into the chromosome, supporting the *in silico* data. The chromosomal location of the *Wolbachia* insertions may reflect the common evolutionary origin of the sex and B chromosomes. It remains to be assessed whether the inserted sequences play any functional role(s) in host physiology.

111 - Genetic analysis of variation in learning rate between *Nasonia* parasitic wasp species

Hoedjes, Katja M.; van Vugt, Joke J. A.; Vet, Louise E.M.; Werren, John H.; Smid, Hans M.

The ability to learn is universal among animals and can play an important role in optimizing behaviour. Female parasitic wasps can learn cues upon a host encounter, to improve foraging efficiency. Interestingly, large differences in learning rate and memory dynamics are observed between closely related species of parasitic wasps. For instance *Nasonia vitripennis* will form transcription-dependent long-term memory after a single host encounter, whereas *N. giraulti* will not. We study the genetic basis of this difference in learning rate using two different approaches. The first approach is by introgression of the low learning rate of *N. giraulti* into the background of *N. vitripennis*, followed by a genotyping array to identify the genomic regions involved in the regulation of this behaviour. We were able to identify a number of genomic regions that are likely involved in the regulation of learning rate using this approach. In addition, we have performed a brain transcriptome analysis to identify differential gene expression after a learning experience in these two species. We present our first results from this analysis as well.

112 - The Genetics of Sex Determination in the Parahaploid Predatory Mite *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae)

Pomerantz, Aaron; Hoy, Marjorie

Understanding the molecular process of sex determination in insects and other arthropods could allow for the manipulation of sex ratios and modes of reproduction in natural enemies used in biological control programs. The recent completion of the whole genome sequence of *Metaseiulus occidentalis* now permits the identification of genes involved in sex determination in this predator of phytophagous pest mites. Little is known at the molecular level about sex determination mechanisms in these parahaploid chelicerates. The main objective of this research proposal is to identify and characterize potential genes involved in the sex determination pathway in the parahaploid species *M. occidentalis* and to analyze the expression and functionality of sex-specifically spliced genes in males and females. A better understanding of genes involved in the sex determination system may help to resolve issues pertaining to the evolution and biology of this predatory mite family. This could lead to future genetic improvement and enhanced biological control programs using phytoseiid mites.

113 - Daily gene expression rhythms measured by microarray analysis correspond with rhythms in antennal proteins, electroantennogram response and behavior in the *Anopheles gambiae* mosquito

Rund, Samuel; Bonar, Nicolle; Champion, Matthew; Ghazi, John; Houk, Cameron; Collins, Frank; Syed, Zainulabeuddin; Duffield, Giles

Insect physiology and behavior are regulated on a 24 hr basis. *Anopheles gambiae* is the major African malaria vector, exhibits distinct daily rhythms in flight activity, mating, sugar and blood feeding, and egg laying. Previously, we reported microarray experiments revealing 1439 rhythmic transcripts under diel (light:dark cycle conditions) in female heads (Rund et al., 2011 *PNAS* 108 (32): E421-E430; www.nd.edu/~bioclock). These rhythmic transcripts include numerous olfactory genes including those encoding odorant binding proteins (OBPs), which are soluble odor carrying proteins found in the mosquito antenna and palps. Olfaction is of importance to the mosquito in detection of hosts for blood feeding, sources of sugar feeding and in oviposition site selection, and indeed daily rhythms of biting behavior in *An. gambiae* have long been known. Here we show in female sensory organs that 11 OBPs rhythmically expressed at the transcript level (among others) have corresponding rhythms at the protein abundance level, as measured by quantitative mass spectrometry. Further, electroantennogram (EAG) analysis in *An. gambiae* reveals time-of-day specific differences in olfactory sensitivity in antenna to major host odorants found in human sweat. Specifically, differences were observed for indole, nonanal and geranyl acetone (which are thought to require OBPs to be detected), but not for hexanoic acid (which is thought not to require an OBP). Finally, we find *An. gambiae* (Pimperena S-form) to exhibit diel and circadian (persists under constant dark conditions) rhythmicity in their blood-feeding behavior. We highlight our successful transition from the identification of rhythms using genomics approaches, to quantitative mass spectrometry for protein analysis, and to physiological and behavioral validation of rhythms: the pre-dusk/dusk peak in expression of OBPs detected by microarray analysis corresponds with peak protein concentrations, increased EAG odor sensitivity and increased biting behavior, each occurring during the night.

114 - Comparative genomics and evolution of memory dynamics in parasitic wasps

van Vugt, Joke; Smid, Hans; Vet, Louise

Parasitic wasps are particular suitable as model system for studying the ecology and evolution of learning and memory formation. When parasitic wasps find and parasitize their hosts, they learn to associate the host-environmental odors with the presence of suitable hosts. Their ability to learn and form a memory is therefore strongly related to their fitness. To be able to reveal the evolution of memory dynamics, we apply a comparative genomics approach. In the genus *Cotesia* we discovered natural variation in learning and memory between closely related species. Related species not only form different types of memory after an oviposition learning trial, but also their memory consolidation and retention time differs. To address the genetic origin of this natural variation we aim to identify the genes that are involved. One of the first questions that arises is whether the observed memory dynamics within and between these wasp genera involves different gene pathways, or whether the same gene pathways are used differently. Learning-induced gene expression levels will be compared through isolating and deep sequencing the mRNA of the heads with the HiSeq2000 Illumina platform, by varying the host species (constituting the reward), the number of learning trials and the sample time after learning. Our recent results will be discussed.

115 - Genome-wide patterns of genetic divergence during ecological speciation between sympatric host races of *Rhagoletis pomonella* flies

Egan, Scott; Ragland, Greg; Assour, Lauren; Emrich, Scott; Feder, Jeff

Understanding the process of speciation is a fundamental question in evolutionary biology. With the development of next generation sequencing methods, we can now survey the entire genome, rather than individual genes, for genetic differences that underlie population divergence during the speciation process. An important component of this quest involves determining how the genes involved in the evolution of new species are distributed and arrayed across the genome, which can affect the likelihood and rate of species formation. Herein, we examine this genomic architecture of speciation in the apple maggot fly, *Rhagoletis pomonella*, where hawthorn- and apple-infesting populations represent genetically differentiated and partially reproductively isolated host races, the hypothesized initial stage in speciation-with-gene-flow. We accomplish this by comparing three different SNP datasets generated by a genotyping-by-sequencing (RADtag) approach: (1.) a recombination distance map based on F1 test crosses, (2.) a genome scan of sympatric *R. pomonella* host races, and (3.) a set of selection experiments on an important axis of ecological divergence between host races, host plant associated prewinter timing. These studies allow us to identify SNPs associated with sympatric host race differentiation and map their chromosomal positions to determine how much of the genome exhibits host associated differentiation and what proportion of this genome-wide divergence can be accounted for by host associated selection on prewinter timing. The answers to these questions, in addition to helping discern the genetic architecture of rapid host-associated adaptation, have an important bearing on recent debate concerning the concept of 'genomic islands' of divergence and the roles that different types of genetic hitchhiking play in facilitating speciation occurring with gene flow.

116 - Which insecticide is appropriate for IRS in Ghana?

Dery, Dominic; Asante, Kwaku Poku; Owusu-Agyei, Seth; Abraham, Oduro; Asoalla, Victor

Of options for vector control, Indoor Residual Spraying with appropriate insecticides is a key intervention which can reduce vector populations in a given area and interrupt transmission. Insecticide susceptibility study on *Anopheles* vectors in order to recommend appropriate insecticides for spraying operations was initiated in ten selected districts in Ghana.

October 2012-January 2013, immature *Anopheles* were collected and reared to adults. Mortality from tarsal contact of females was assessed with 11 insecticides in four chemical classes: i) organochlorines, ii) organophosphates, iii) carbamates and iv) pyrethroids. Four replicates of 25 unfed *Anopheles* females, aged 3 days, were exposed to papers for 1 hour (Fenitrothion was 2hrs). Number knocked down was recorded every 10 min and mortality after 24 hours exposure. Pyrethrum Spray Collection was performed in 60 randomly selected rooms in each district.

Of 2,038 collections, species compositions were *An. gambiae s.l* (64%), *funestus* (22%), *Culex* species (9%), *Aedes* (4%), *Pharoensis* (1%) and few *Mansonia* and *rufipes* species. Sporozoite rate of 0.036 was computed and Malathion is recommended insecticide for spraying in seven districts, Fenitrothion in three districts and Propoxur in one district. Of 483 PCR, *An. gambiae s.s* was prevalent (475) and few *arabiensis* (5) detected in savannah arid districts in the north. The M-form was dominant (143) with no hybrids detected. The S-form was detected across the country though in low numbers (21). Few *kdr* susceptible strains were detected (14) but majority were homozygous *kdrRR* (120) resistant species and heterozygous were moderate in number (32).

Organophosphate is class of insecticide appropriate for Indoor Residual Spraying in Ghana. Rotation between different insecticides over time offers a practical solution for resistance management. Though reported that *Kdr* mutation is widespread in West Africa, results contrast observation that, frequency within S-form is much higher and the distribution is more widespread than within the M-form.

117 - Development of a tick-borne Langat virus-*Ixodes scapularis* ISE6 cellular proteomic screen: identification of novel targets for tick-borne flavivirus control

Grabowski, Jeffrey; Perera, Rushika; Roumani, Ali; Hedrick, Victoria; Inerowicz, Halina; Hill, Catherine; Kuhn, Richard

Tick-borne flaviviruses may cause hemorrhagic fever and encephalitis when transmitted to humans via a bite of an infected tick (Family Ixodidae). Langat virus (LGTV) provides an attractive model for the study of tick-borne flaviviruses and is antigenically similar to the highly virulent Tick-Borne Encephalitis Virus (TBEV), which causes thousands of cases of encephalitis per year. Significant effort has been directed at understanding flavivirus infection within mammalian systems; however, little is known about flavivirus infection within the tick vector. We have used tick-borne LGTV (TP21 strain) and an *Ixodes scapularis* ISE6 embryonic cell line to evaluate the tick proteome following virus infection. We characterized LGTV growth in ISE6 cells, established maximal infection of ISE6 cells, and optimized UV inactivation of LGTV for a necessary control group. Mass spectroscopy (LC-MS/MS) was conducted to analyze the protein/peptide profile of ISE6 cells exposed to LGTV, non-infectious UV-inactivated LGTV, and uninfected (mock) cells. Data were processed using a Proteome Discovery Pipeline (PDP) to identify peptides that were significantly differentially-expressed following infection. Protein identification was performed by comparison of protein/peptide sequences to the *Ixodes scapularis* WIKEL strain IscaW1.1 predicted protein set available at VectorBase. Proteins were categorized according to predicted cellular functions, including roles in environmental information processing, metabolism, genetic information processing, organismal systems, and cellular processes. We identified a significant number of differentially-expressed proteins that are involved in nucleic acid binding, lipid metabolism, energy, and carbohydrate metabolism which we are pursuing as candidates for functional analyses. Currently, validation with quantitative proteomics, biological, and functional experiments are underway. The long term goal of our research is to develop new protein targets for development of novel antiviral treatments.

118 - Molecular basis of caste determination: Using RNA-Seq for an emerging model organism

Berens, Ali; Hunt, James; Toth, Amy

The presence of queen and worker castes is a defining feature of insect societies, and a spectacular example of biological complexity since alternative phenotypes can be expressed by a single genome. How is this accomplished? *Polistes* live in small societies consisting of a queen and her daughter workers. They are considered to be “primitively eusocial” because caste differences are flexible in adults, although they are biased during early development. It has been proposed that there is a developmental switch (such as nutrition) that creates a caste bias in *Polistes* leading to adult phenotype of workers or queens. Advances in high throughput sequencing technologies afford us the opportunity to study the molecular basis of caste determination and the role of nutrition. We sequenced transcriptomes derived from four biological replicates of *Polistes metricus* fifth instar larval heads for two nutritional levels (restricted and ad libitum) and castes (queen- and worker-destined). Between castes, we identified 667 differentially expressed transcripts, of which 90% were up-regulated in queen-destined relative to worker-destined larvae. 70% of the 236 nutritional differentially expressed transcripts were up-regulated when nutrition was restricted compared to provided ad libitum. However, of the 39 transcripts that were differentially expressed for both caste and nutrition, 89% of the transcripts were up-regulated in queens-destined larvae and 85% were up-regulated with ad libitum nutrition. A previous candidate gene study (qRT-PCR) identified 16 caste differentially expression genes related to lipid metabolism, response to stress and heat, and locomotory behavior. Although we found similar expression patterns, few of these candidate genes were significantly differentially expressed between castes. It is unknown whether the mechanisms of caste determination are conserved between eusocial species. Comparisons of genes over- and under-expressed in queen and worker castes in different species have suggested there is a shared “toolkit” of genes or pathways involved in caste determination in several independently evolved social lineages. Comparative analysis of novel and conserved molecular signatures from a variety of social insects provides insights into the molecular mechanisms of eusocial evolution.

119 - Molecular responses in *Glossina pallidipes* to infection by *Trypanosoma brucei brucei*
Bateta, Rosemary

Tsetse flies are important vectors of trypanosomes, causative agents of African trypanosomiasis. The species *Glossina pallidipes* are more refractory to infection by the parasites than other related tsetse fly species, including *G. m. morsitans* which has been extensively investigated. Investigations were conducted to identify molecular factors/genes mediating this resistance phenomenon. The cDNA libraries were separately developed from midguts and carcasses of *G. pallidipes* challenged with *T. b. brucei* and sequenced on illumina platform. The qualities of the transcriptomes were validated using FastQC software, and the reads assembled using Trinity software. Differentially expressed genes were identified using edgeR software and annotated using Blast2go software. The differentially expressed putative immune genes will be validated using real-time quantitative RT-PCR, with *GAPDH* as housekeeping gene. Results of the differentially expressed putative immune genes will be presented, and should provide insight into interactions between the vector and the parasite that can be exploited in developing tsetse flies refractory to trypanosome infections and block transmission of the parasite.

120 - Genomic analysis of introgression in hybridogenetic ant populations
Helms Cahan, Sara; Zhou, Yihong

Hybridization between species provides the opportunity for two independently evolving genomes to recombine, which may have both negative consequences when co-adapted gene complexes are disrupted, as well as positive effects if globally adaptive alleles are able to cross the species boundary. The advent of whole-genome sequencing in non-model organisms has made it possible to dissect patterns of gene flow between species in hybrid zones at an unparalleled level of genetic resolution. We investigated the extent, directionality and genomic distribution of introgression between genetic lineages derived from two species of harvester ant, *Pogonomyrmex rugosus* and *P. barbatus*, that display an obligate hybridogenetic life history, interbreeding in every generation to produce sterile hybrid workers while reserving reproduction for non-hybrid progeny. We sequenced the transcriptomes of virgin queens of both parental species and each interbreeding lineage and mapped them to the published genome of *P. barbatus*, resulting in 1621 universally-expressed transcripts with sufficient sequence variation to resolve ancestry of each lineage. Despite continually interbreeding, overall genetic exchange between lineages was low, with less than 5% of transcripts indicative of introgression. However, the introgression that did occur was strongly unidirectional from the desert-adapted *P. rugosus* to the more mesic-adapted *P. barbatus*. Introgression was also not uniform across the genome, with five introgression "islands" detected. These results suggest that even hybrid zones in which reproductively viable hybrids are virtually absent can serve as potentially important genomic bridges facilitating gene movement across species boundaries.

121 - Identification of Single Nucleotide Polymorphisms (SNPs) in the mosquito *Culex pipiens*, West Nile Virus vector.
Sim, Cheolho; Kang, David

Single nucleotide polymorphisms (SNPs) are the most abundant source of genetic variation in animals and have become popular dense genetic markers. The *Culex pipiens* complex species are the major West Nile Virus vectors in the North America and yet, only small number of SNPs in this species has been reported. DNA fragments from 36 genes were amplified and sequenced from 20 specimens of two biotypes among *Culex pipiens* complex species. SNP markers were identified by analyzing sequence traces of PCR products, which were amplified from genomic DNAs of either *C. pipiens* form *pipiens* (*pipiens*-type) or *C. pipiens* form *molestus* (*molestus*-type). First, thirty six candidate genes were selected from the center of the supercontigs which include the known microsatellite or RFLP marker loci. Then, the primers were designed from exons and nested introns of the 36 candidate genes following by PCR amplification. Thirty three DNA fragments amplified from gDNAs of *pipiens*-type or *molestus*-type mosquito were then sequenced, pooling 10 of each form, revealing 116 SNPs in the coding regions, 78 SNPs in the noncoding regions, and 26 indels. Coding regions exhibited close to a 2:1 ratio of transitions to transversions, suggesting a lack of bias in functional in expressed transcripts, while non-coding regions a ratio closer to 1:1. Here we report a novel set of SNP markers for use in genetic studies on this important human disease vector.

122 - Understanding the transcriptomic variations that exist among the haplotypes of *Varroa jacobsoni* that differ in their reproductive success on the European Honey bee *Apis mellifera*.
Andino, Gladys; Hunt, Greg; Gribskov, Michael; Anderson, Denis

Varroa mites are considered the biggest health problem that honey bees face worldwide. Two species of the genus *Varroa* are known to cause damage to honey bees. *V. jacobsoni* was first described in the Asian honey bee *A. cerana*, in Java, Indonesia and *V. destructor* was first described in *A. cerana* in Asia more than a decade ago. *V. jacobsoni* is known to only live and reproduce in drone brood of *A. cerana* while *V. destructor* is known to live and successfully reproduce in *A. cerana* and in *A. mellifera*. However, we have samples of *V. jacobsoni* from a small island population found in Papua New Guinea that is highly destructive to *A. mellifera*, the primary species used for pollination and honey production. Therefore, these recently discovered populations of mites represent an enormous threat to apiculture around the world.

Our lab has sequenced for the first time the transcriptome of this parasitic mite *V. jacobsoni*. Currently, we are analyzing the RNAseq data of three different pools of *V. jacobsoni* populations that differ in their reproductive success on *A. mellifera*. Approximately, more than 300 million paired end reads were sequenced and after a detailed cleaning and trimming the total reads were assembled using Trinity into 225,778 contigs with a N50 of 2730bp.

In our preliminary differential expression analysis we found significant differences in transcripts level between the reproductive mite samples and the non-reproductive one. Blast and GO results revealed that some of the largest differences included cuticular proteins, secreted salivary gland and peritrophic membrane chitin binding proteins. In addition, we found histone methyltransferases, which are involved in epigenetic gene regulation and Spatzle a cytokine involved in development but also in immunity. A more detailed analysis of the differentially expressed transcripts is under way with the addition of two biological replicates for two of our previous samples of mites, which will account for honey bee hive and geographical variation. In addition, the transcriptome data obtained from this project will be also part of our contribution to the *V. jacobsoni* genome project lead by the Hunt lab.

Ecological Genomics

123 - Genetics determinants of malaria transmitting behaviours in *Anopheles arabiensis*

Marsden, Clare; Weakley, Allison; Han, Sarah; Kreppel, Katharina; Cornel, Anthony; Ferguson, Heather; Eskin, Eleazar; Lee, Yoosook; Gregory, Lanzaro

Anopheles gambiae s.s. is frequently referred to as the most important vector of malaria in Africa. As such it has been the main focus of malaria vector research to date. However, there is growing evidence that the sister species, *An. arabiensis*, outcompetes *An. gambiae* s.s to become the dominant malaria vector in areas of high insecticide treated net (ITN) coverage. Consequently the ecology, vectorial competence and population genetics of this somewhat neglected vector merit particular attention in preparation for future vector control scenarios. Aspects of mosquito behaviour, including host preference and resting behaviour, frequently represent the targets of malaria control campaigns. Variation in these behaviours likely have an underlying genetic basis that forms the means by which vectors evolve behavioural resistance to control endeavors. For this research, we are conducting association mapping on *An. arabiensis* whole genome sequences to identify genetic variants associated with resting behaviours (indoor vs outdoor) and host preference (human vs animal).

124 - In Synch: Genomic change associated with the evolution of seasonal synchrony via adaptation across the life cycle.

Ragland, Greg; Egan, Scott; Feder, Jeff; Hahn, Dan

Synchronization of complex life cycles with environmental fluctuations is a critical adaptation in seasonal environments. Insects typically regulate this synchrony through the modulation of the diapause response, timing exit from and entry into diapause with the beginning and end of the growing season, respectively. Any shift in seasonal timing, however, changes seasonal exposure of all life history stages, selecting for adaptations across the life cycle. Populations of the apple maggot fly, *Rhagoletis pomonella*, have recently shifted from a native hawthorn host to introduced apples, which fruit earlier in the year. These apple populations, which are in the early stages of speciation, have evolved differences in the timing of the termination of pupal diapause to adjust to earlier seasonal availability of their host fruit. As a consequence, diapause initiation also occurs earlier in the year when temperatures are warmer, exposing formerly covert variation for direct development (i.e., a second generation), which is a poor life history strategy. We have quantified genome-wide associations for traits associated with both diapause termination and initiation. We discuss how these associations relate to the process of rapid seasonal adaptation, genomic divergence and potential constraints limiting independent evolution across the life cycle.

125 - Elucidating the mechanism of pesticide resistance in a non-target amphipod, *Hyalella azteca*: Genetic and Functional Genomic Approaches

Poynton, Helen; Weston, Donald; Wellborn, Gary; Lydy, Michael; Blalock, Bonnie; Tuck, Padrig; Sepulveda, Maria; Colbourne, John

While pesticide resistance has been heavily documented in target pest species, few studies have investigated the evolution of resistance in non-target arthropods. We have detected one of the first examples of pyrethroid pesticide resistance in a non-target organism, the amphipod *Hyalella azteca*. *H. azteca* is a freshwater epibenthic crustacean and the primary species used for freshwater sediment toxicity testing in the U.S. Because *H. azteca* is a species complex which has diverged in North America over the past 11 million years, our initial identification of pesticide tolerant *H. azteca* in California prompted us to explore the genetic structure of the tolerant organisms. Our studies revealed three different species groups among the seven populations examined, while only one of the species groups, "Group D," displayed a tolerance to pesticides.

To understand the origin of pesticide tolerance in species group D *H. azteca*, we explored two potential mechanisms of pesticide resistance: metabolic and target site resistance. We investigated both whole transcriptome responses to pyrethroid pesticides in tolerant and sensitive organisms as well as targeted gene expression analysis of genes involved in pyrethroid metabolism. We also sequenced the voltage-gated sodium channel (*vgsc*) gene, whose gene product is the target of pyrethroid toxicity, in several populations and identified two single nucleotide mutations, which appear to be responsible for reducing pyrethroid toxicity 100-1000-fold and providing pesticide resistance. Transcriptomic analysis suggests that resistant *H. azteca*, eventually succumb to pyrethroid toxicity, at much higher concentrations compared with sensitive organisms, not through inhibition of the VGSC, but through oxidative stress.

126 - Metagenomics of mosquito gut ecosystem

Xu, Jiannong

Host associated microbes are ubiquitous, yet our understanding of the interactive relationships is very limited. The mosquito gut ecosystem accommodates a complex microbial assemblage. The dynamic gut microbiome profoundly affects various mosquito life traits, such as fecundity and immunity. Besides, bacteria may directly interfere with malaria Plasmodium development in the gut before invasion occurs. However, little is known about the genetic structure and functional repertoire of the gut microbiome. In this study we generated 5Gbp metagenomic DNA- and RNA-seq data from the guts of adult mosquito *Anopheles gambiae* under conditions with sugar meals or blood meals. Using an assembly-based data analysis pipeline, a 37.1 Mbp metagenomic reference was compiled, which included 29,000 contigs. Similarity based taxonomic classification recognized at least 6 phyla, predominant taxa included Proteobacteria (Enterobacteriaceae, Pseudomonadaceae and Acetobacteraceae) and Bacteroidetes (Flavobacteriaceae). This finding was consistent with documented microbial composition in the literature. The function annotation was implemented via SEED/Subsystems and COG/KEGG, which recognized 28,700 ORFs distributing in ~700 subsystems. Metabolic reconstruction was conducted based on the predicted protein features. In addition to serving the characterization of taxonomic composition, gene repertoire and metabolic configuration, the metagenomic reference was further used for mapping RNA-seq reads to decipher context dependent community functions, which was exemplified by metatranscriptomic analysis of sugar-fed vs blood-fed guts. In summary, the metagenomic sequencing data were organized into a metagenomic reference that could be utilized to characterize the taxonomic and functional diversity and dynamics of microbiome in the mosquito gut ecosystems.

127 - Current Studies of Interactions Between Wheat and the Wheat Curl Mite, *Aceria tosichella* Keifer

Chuang, Wen-Po; Marimuthu, Murugan; Malik, Renu; Whitfield, Anna; Fritz, Allan; Smith, C Michael

The wheat curl mite, *Aceria tosichella* Keifer, is one of the most important world arthropod pests of wheat and vectors wheat streak mosaic virus (WSMV), the most significant wheat virus in North America. Varieties with moderate levels of mite resistance or WSMV resistance exist, but none contain both. Further, mite virulence to wheat R genes exists in North America and Australia, further complicating the development of mite-WSMV management strategies. We have assessed population lineages of *A. tosichella* in North America and established their phylogenetic relationships with global *A. tosichella* populations. Two *A. tosichella* biotypes exist in North America based on internal transcribed spacer (ITS1) sequence variation. The Nebraska biotype is Homologous to the Australian biotype 2, and a biotype present in Kansas, Texas, and Alberta, Canada is Homologous and aligned to the Australia biotype 1. These results provide a platform for future studies to understand global *A. tosichella* movement and spread. Included in these are plans for *A. tosichella* whole genome sequencing to improve mite phylogenetic accuracy. We have used wheat SSR markers linked to *Cmc4* for *A. tosichella* resistance and markers linked to *Wsm2* for wheat streak mosaic resistance to identify wheat plants in F₄ populations with **both** mite and virus resistance. As *A. tosichella* virulence patterns in the U.S. hard red winter wheat production area have not been determined in 10 years, we are currently determining the *A. tosichella* virulence patterns to plants with **both** mite and virus resistance.

128 - Blood Meal-Induced Changes to Antennal Transcriptome Profiles Reveal Shifts In Odor Sensitivities in *Anopheles gambiae*

Rinker, David; Pitts, R Jason; Zhou, Xiaofan; Suh, Eunho; Rokas, Antonis; Zwiebel, Laurence

Olfactory-driven behaviors are central to the lifecycle of the malaria vector mosquito *Anopheles gambiae* and are initiated by peripheral signaling in the antenna and other olfactory tissues. To continue gaining insight into the relationship between gene expression and olfaction, we have performed cohort comparisons of antennal transcript abundance at 5 time-points following a blood meal, a key event in both mosquito reproduction and disease transmission. We found that more than 5000 transcripts were differentially abundant within *An. gambiae* antenna following blood-feeding, and cluster analysis revealed correlations between those differences. Although we observed a general reduction in the level of chemosensory gene transcripts, a subset of odorant receptors (AgOrs) showed modest increases in abundance. Integration of these data with previously characterized AgOr response profiles revealed potential changes in antennal odorant receptivity that correlated with the shift from host-seeking to oviposition behaviors in blood-fed female mosquitoes. We used this approach together with oviposition choice assays to identify the first unitary oviposition cue for *An. gambiae*. We posit that modest, yet cumulative alterations of AgOr transcript levels modulate peripheral odor coding resulting in biologically relevant behavioral effects.

129 - Plant-Insect Interactions Shape Plasticity of Asian Longhorned Beetle Gut Microbiome
Scully, Erin; Geib, Scott; Carlson, John; Tien, Ming; Hoover, Kelli

The Asian longhorned beetle (ALB; *Anoplophora glabripennis*) is an invasive, wood-boring pest capable of thriving in the heartwood of 25 deciduous host tree species in the United States where it faces a number of nutritional challenges, particularly digestion of lignocellulose and hemicellulose and nitrogen acquisition. Unlike other wood-boring beetles, which rely on cultivated fungal symbionts for the production of lignocellulose degrading and nutrient acquiring enzymes, we have demonstrated that the majority of the challenging reactions, including digestion of lignin, cellulose, and hemicellulose occur within the ALB gut. While midgut EST and transcriptome profiling revealed that ALB endogenously produces a number of glycoside hydrolases that facilitate digestion of major hardwood polysaccharides, cooperation with gut microbes is required to neutralize the majority of these nutritional challenges. Shotgun metagenomic profiling of gut microbes revealed a taxonomically diverse community with the metabolic capacity to completely degrade cellulose and hemicellulose, disrupt the major linkages in the lignin polymer, convert xylose sugars that dominate hardwood hemicellulose into compounds that can be utilized by ALB, fix atmospheric nitrogen, and recycle nitrogenous waste products. Thus, the metabolic potential of the gut community is complementary to ALB's endogenous metabolic capabilities and encodes a more extensive suite of enzymes, enabling ALB to complete development in such a broad host range. Currently, we are investigating the impacts of host tree resistance on the gut community in poplar using a combination of 16s amplicon sequencing and deep Illumina sequencing to study the impacts on expression of insect and microbial digestive genes.

130 - Function and evolution of aquaporins (AQPs) in the Lyme disease vector *Ixodes scapularis*
Tsujimoto, Hitoshi; Rasgon, Jason

Ticks are arachnid arthropods that are important vectors of viruses, bacteria and protozoan pathogens to humans, wildlife and domestic animals. Due to their lifestyle, ticks face significant challenges related to water homeostasis. Ticks obligately feed on vertebrate blood and are vulnerable to desiccation, meaning that water excretion and conservation are critical aspects of tick biology. We bioinformatically identified 4 putative AQP genes from the Lyme disease vector *Ixodes scapularis*. We cloned and functionally characterized the *Ixodes scapularis* aquaporin 1 gene (IsAQP1) as a functional water channel when expressed in *Xenopus* oocytes. The life-stage and tissue-specific expression profile of IsAQP1 was determined. Evolutionarily, all known tick AQPs form a monophyletic group with arachnid AQPs, and are distinct from known insect AQPs. The arachnid AQP clade clusters with aquaglycerolporins from protozoan parasites, indicating that horizontal gene transfer events may have driven AQP evolution in ticks and other arachnids.

131- Developing predictive models for non-additive effects of multidimensional environmental stressors: When do things just not add up?
Regan, Kerry; Coulborne, John; Pfrender, Michael

Predicting the responses of organisms to environmental challenges requires an understanding of the underlying physiological and genetic mechanisms mediating stress responses. Natural ecosystems are highly dynamic and natural populations are often exposed to complex mixtures of multiple stressors. Unfortunately, predictive models designed to gauge the response of natural populations to environmental stressors are typically based on the effects of single stressors and largely ignore the increased complexity of the combined effects of multiple stressors. In most systems we lack a sufficient understanding of the relationship between single and combined stressors to accommodate this increased complexity. For example, it is largely unknown whether exposure to multivariate stress creates an additive or multiplicative effect on an organism's fitness and the patterns of gene regulation as compared to single stressors. We used *Daphnia pulex*, a small freshwater crustacean found throughout North America, to develop testable predictions for transcriptional responses to multidimensional environmental stress using extensive existing transcriptome data. We use a multivariate approach to determine the degree of gene and pathway similarity in differential gene expression among univariate exposures to environmental stressors. Assessing the underlying similarities in transcriptional responses to environmental stressors is a critical first step to develop a predictive framework relating realistic environmental change to physiological responses.

132 - Extensive variation in genome size among populations and a novel sex determining mechanism in the gall wasp, *Belonocnema treatae*

Hjelmen, Carl; Ott, James; Egan, Scott; Johnston, Spencer

Genome size can vary by orders of magnitude between species. However, within species variation between populations and between individuals has received much less attention despite growing evidence of its prevalence in nature. We investigated variation in the genome size of the haplodiploid and cyclically parthenogenic gall wasp *Belonocnema treatae* that is distributed across the southeastern United States where it attacks different species of live oaks (*Quercus*) and appears to form locally adapted populations on specific host plant species. Due to the many peculiar characteristics of its life history and ecology, we asked whether there were differences between genome sizes among populations and individuals based on sex, host plant, generation (sexual or asexual), and geographic region. Not surprisingly, we found differences among sexes in the sexual generation consistent with haplodiploidy, but also found differences between females from the asexual and sexual generations. Moreover, we found differences among host-associated populations within geographic regions and, more generally, among geographic regions. Our results suggest changes in genome size associated with ecology and point to a novel sex determining system not previously documented.

133 - Investigating the transcriptional basis of diapause induction in the Asian tiger mosquito, *Aedes albopictus*

Huang, Xin

Insect diapause is a crucial ecological adaptation that enables insects to survive seasonally unfavorable conditions, such as the harsh conditions of winter. During diapause, insects typically reduce metabolic rates, enter a state of developmental arrest and elevate stress responses including cold and desiccation resistance. Despite extensive studies of the ecological significance and hormonal control of diapause, the molecular mechanisms underlying diapause are relatively underexplored. The Asian tiger mosquito, *Aedes albopictus*, is an excellent model to study the molecular underpinnings of photoperiodic diapause that can be easily manipulated in the laboratory. In the diapause response of *Ae. albopictus*, the photosensitive pupal or adult female perceives the signal of decreased day length in the fall and subsequently produces offspring in which the pharate larva enters diapause inside the chorion of the egg. Previous work in our lab using powerful next-generation sequencing techniques has elucidated the transcriptional profiles of *Ae. albopictus* during diapause preparation, diapause initiation and diapause maintenance. These studies reveal differential expression of genes involved in lipid metabolism, cell cycle regulation, stress response and additional fundamental physiological processes. Following up on these results, I am investigating the transcriptional basis of diapause induction in the adult female *Ae. albopictus*. We will examine transcriptional profiles from four experimental treatments: blood fed and non-blood fed mosquitoes exposed to both diapause inducing short-day photoperiods and diapause averting long-day photoperiods. Total RNA samples from whole bodies of 10-16 mosquitoes per treatment with three biological replicates were used for paired-end mRNA sequencing. Utilizing established bioinformatic algorithms in our lab, we will analyze the differential gene expression profiles during the diapause induction phase, expanding our understanding of the molecular basis of diapause. Integrating our previous transcriptome data with the data from the diapause induction phase, we will be able to understand the shared and unique molecular themes across multiple stages underlying this complex and crucial ecological adaptation.

134 - The Temporal Dynamics of Immune Gene Expression and Pathogen Load Reveal Resistance and Tolerance of Bacterial Infection by Trans-generationally Primed Flour Beetles

Tate, Ann; Andolfatto, Peter; Graham, Andrea

Immune priming allows individuals previously exposed to a pathogen to enjoy reduced susceptibility and higher survival probability upon re-exposure, compared to naïve cohorts. The protection can even be transferred across generations, but currently very little is known about the mechanisms that confer a primed immune response in invertebrates. Furthermore, it is not known whether trans-generational immune priming in insects increases ability to kill pathogens (resistance), ability to minimize or repair damage (tolerance), or both. In trans-generational priming experiments reported here, we infected the larval offspring of naïve, sterilely wounded, or bacteria-challenged (primed) adult female beetles (*Tribolium castaneum*) with the bacterial entomopathogen *Bacillus thuringiensis* (Bt) and used DNA microarrays and qPCR to investigate the influence of maternal treatment on the temporal dynamics of bacterial load and immune gene expression. Meanwhile, we monitored the survival and development of infected and uninfected larvae from these treatments to quantify the functional outcomes of priming in this system. Initially, primed larvae were better able to curb bacterial proliferation and they subsequently exhibited increased tolerance for high bacterial loads. Moreover, primed larvae had better survival odds and buffered developmental costs of infection. Taken together, these results suggest that invertebrate immune priming is not just an outcome of more efficient microbe killing, as previously supposed, but rather a two-pronged reduction in the overall negative consequences of infection for the host, with broad implications for the evolution of immune priming and pathogen virulence strategies.

135 - Speciation Microbiomes: The Consequences of Gut Bacteria on Hybrid Mortality in the Genus *Nasonia*

Brucker, Robert; Pauly, Rini; Bordenstein, Seth

Symbiosis is a process by which two or more distinct organisms interact, whereas speciation is the diversifying process by which one species splits into two. Symbiosis and speciation are not commonly discussed together and can seem to be odd partners in their capacity to operate synergistically in nature. However, the complex community of microorganisms that live in symbiosis within an animal species—known as the microbiome—has the capacity to confer new traits and selective pressures that drive speciation. To demonstrate the microbiome's influence in speciation, we present the following evidence using closely related insect species of the model *Nasonia*. First, the gut microbiome forms phylosymbiotic assemblages in which the relationships of the *Nasonia*-associated microbiomes parallel the evolutionary genetic relationships of *Nasonia*. Second, these phylosymbiotic assemblages become irregular in hybrids between the species. Third, the altered microbiome in hybrids is correlated with high rates of hybrid mortality. Fourth, when the hybrids are cured of their irregular microbiota, they are rescued from hybrid mortality. Finally, when the cured hybrids were reinoculated with bacteria, they recapitulated high rates of mortality. We conclude that in this animal complex, the gut microbiome is equally important as host genes in promoting hybrid mortality and thus advancing speciation.

136 - Genetic Architecture and Transcriptomic Basis of the Response to Exposure to Sub-Lethal Doses of Cadmium in the Waterflea *Daphnia magna*

Roy, Sayanty; Routtu, Jarkko; Dieter, Ebert; Thomas, William K; Colbourne, John K; Ridenhour, Benjamin J; Li, Jun; Pullins, Mellissa; Garfield, Erin; Pfrender, Michael E

Natural Populations are increasingly challenged by rapidly changing environmental conditions. Toxic compounds produced from industrial and urban activities present a significant challenge to aquatic organisms. Heavy metals, in particular, are common stressors that play a major role in environmental damage. Since aquatic systems are the major sinks of industrial effluents they are often more highly impacted by heavy metals than terrestrial ecosystems. Understanding the fitness consequences of heavy metal exposure requires knowledge of the physiological responses of organisms and the genetic basis of these responses. This information is critical to environmental scientists interested in predicting the long-term consequences of altered environments, and to regulatory agencies constructing predictive models to assess the impact of pollutants on human and environmental health.

We evaluate the underlying genetic architecture and transcriptional responses of the freshwater microcrustacean *Daphnia magna* to cadmium exposure in order to identify genes and pathways involved in the physiological response to this common environmental contaminant. We use QTL-mapping in recombinant lines derived from parental lines that differ in their response to cadmium exposure to detect genome regions associated with survivorship, and cadmium assimilation and depuration rates. In addition, we use a functional genomic approach with RNA-Seq data to examine transcriptional responses to cadmium exposure. Our investigation of organismal response to cadmium will enhance our understanding of the effects of stressful environments on a widely distributed and ecologically relevant aquatic organism, and will add valuable information to current ecotoxicology research.

137 - Putative F-box/LRR-containing effectors in a plant-parasite insect

Navarro Escalante, Lucio; Zhao, Chaoyang; Campos, Veronica; Benatti, Thiago; Chen, Ming-Shun; Stuart, Jeff

Like many plant pathogens, the Hessian fly (HF, *Mayetiola destructor*) uses a complex battery of effector proteins to attack its host plant, wheat (*Triticum* spp.). Included among the genes that encode these effector proteins are members of a large gene family (SSGP-71). With over 500 genes and gene fragments, family SSGP-71 constitutes the largest group of putative effector encoding genes in the HF genome. Sequence and predicted structural analyses of these genes and their predicted proteins indicated that most encode an N-terminal F-box domain followed by a series of leucine-rich repeats (LRRs). Although F-box/LRR containing proteins are common in the genomes of insects, several lines of evidence clearly indicated that SSGP-71 genes encode effectors: First, their abundance in the HF is over 10-times that of *Drosophila* and mosquitoes. Second, RNAseq transcriptome mapping and RT-PCR suggest that these genes are mainly expressed when the larvae attack the plants. Third, unlike the F-box/LRR protein encoding genes of other insects, SSGP-71 genes encode secretion signals. Fourth, genomic copies of SSGP-71 genes display evidence of rapid duplication and decay with over 50% of the genes located near the telomeres where high recombination rates augment gene diversification. Fifth, although this is the first example of F-box/LRR effectors in an insect, there is precedence for F-box/LRR effector proteins in other plant pathogens. And finally, mutations in the Hessian fly genome that allow the insect to escape plant resistance-gene mediated effector-triggered immunity are located in certain SSGP-71 genes.

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