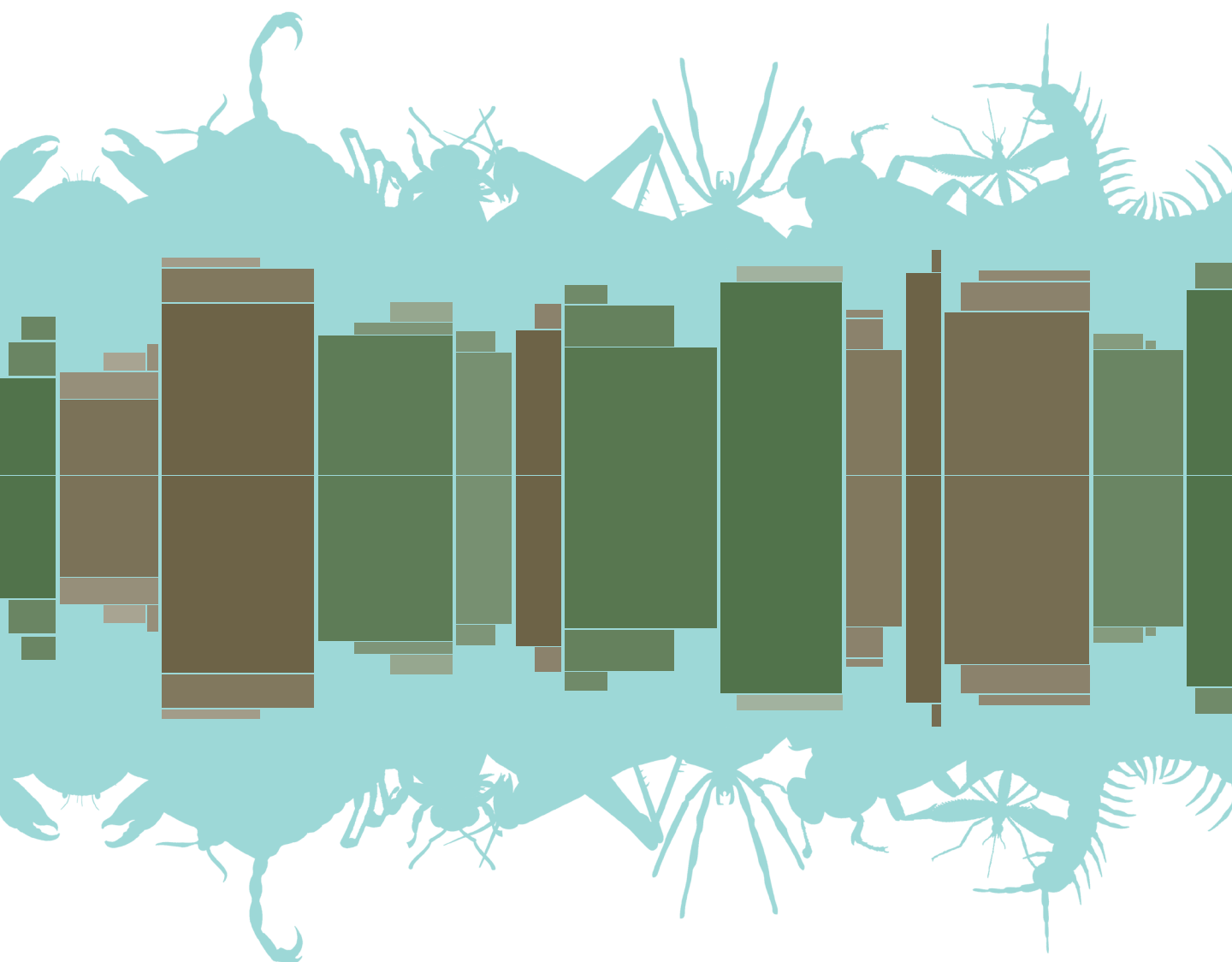


11th Annual

ARTHROPOD GENOMICS SYMPOSIUM (AGS)

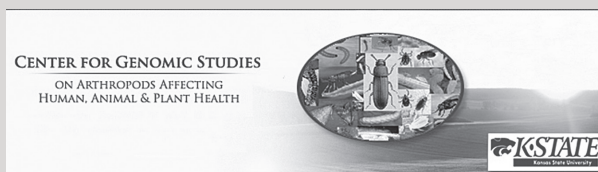




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Carl R. Woese Institute for Genomic Biology
Department of Entomology
School of Integrative Biology



WELCOME

11TH ANNUAL ARTHROPOD GENOMICS SYMPOSIUM (AGS)

Welcome to the 11th Annual Arthropod Genomics Symposium.

The University of Illinois at Urbana-Champaign is pleased to host the Arthropod Genomics Symposium, Thursday to Saturday, June 7-9, 2018. It has been 18 years since the sequencing of the genome of *Drosophila melanogaster*, and over three hundred arthropod genomes have been sequenced since then. The Arthropod Genomics Symposium is an opportunity to reflect on recent progress and explore future directions.

We have planned an array of sessions that reflect the diversity of arthropod genomics. We hope you will join us for a productive exchange of ideas and viewpoints, and we look forward to seeing you here.

—Hugh Robertson and Gene Robinson, co-chairs

AGENDA

*Alice Campbell Alumni Center
601 South Lincoln Avenue, Urbana, IL 61801*

Thursday, June 7, 2018

12:30 PM - 1:00 PM **Registration opens for Pre-symposium
Workshop registrants**

1:00 PM - 5:00 PM **Pre-symposium Workshop**

Monica Poelchau
NAL/USDA Maryland

Monica Poelchau
NAL/USDA Maryland

Robert Waterhouse
University of Lausanne

5:00 PM **Registration opens and Reception**

7:00 PM **Welcome**
May Berenbaum
University of Illinois

Plenary lecture
Michael Lynch
Arizona State University
“The 5000 Daphnia Genome Project”

Friday, June 8, 2018

7:30 AM **Coffee/Tea**

7:30 AM - 8:00 AM **Poster set-up for odd-numbered posters**

8:00 AM - 10:00 AM **i5k Session**
Session Chair - Adam Dolezal

Thomas Mathers
John Innes Centre

“Comparative analysis of 21 aphid genomes to gain insights into the evolution of grass feeding and extreme host range expansion”

Julian Gutekunst
German Cancer Research Center

“Clonal genome evolution and rapid invasive spread of the marbled crayfish”

Surya Saha
Boyce Thompson Institute

“Insights into genome organization and non-coding genes of *Diaphorina citri*, the vector of citrusgreening disease”

Stephen Richards
Baylor College of Medicine

10:00 AM - 10:30 AM

Break

10:30 AM - 12:30 PM

Microbiome Session

Session Chair - Katy Heath

Bryony Bonning
University of Florida
“The insect virome”

William Landesman
Green Mountain College
“Drivers of variation in bacterial community composition among *Ixodes scapularis* nymphs in the northeastern U.S.”

Emily Jennings
University of Cincinnati
“Viviparous reproduction delays microbiome acquisition in a live-bearing cockroach, *Diploptera punctate*”

AGENDA CONT.

Seth Bordenstein
Vanderbilt University
“Phage genes alter gametes and
kill males in drosophila”

12:30 PM - 2:00 PM

Lunch on your own

2:00 PM - 3:00 PM

Poster Session for odd-numbered posters

3:00 PM - 5:00 PM

Vector Biology Session

Session Chair - Brian Allan

Mara Lawniczak
Sanger Institute
“The malariagen vector observatory: present,
past, and future”

Heather Eggleston
Texas A&M University
“Identifying heme importers & exporters through
rna seq analysis in *Aedes aegypti*”

Robert Waterhouse
University of Lausanne
“Curation is key: Quantifying the impact of manual
gene annotation improvements”

Michel Slotman
Texas A&M University
“The genetics of human host preference in the
Anopheles gambiae complex”

5:00 PM

Remove odd-numbered posters

5:00 PM - 6:30 PM

Optional Campus Tours

Additional registration required

7:00 PM

Optional Dinner*Additional registration required*

May Berenbaum
University of Illinois

Saturday, June 9, 2018

7:30 AM

Coffee/Tea

7:30 AM - 8:00 AM

Poster set-up for even-numbered posters

8:00 AM - 10:00 AM

Social Insects Session

Session Chair - Andrew Suarez

Matt Webster
Uppsala University
“A hybrid *de novo* genome assembly of the honey bee, *Apis mellifera*, with chromosome-length scaffolds”

Beryl Jones
University of Illinois
“Reproductive worker honey bees:
A glimpse of ancestral sociality?”

Sarai Stuart
University of Illinois
“Brain transcriptomic changes associated with colony defense in the stingless bee, *Tetragonisca angustula*”

Hua Yan
New York University
“Generating genetic tools in ants to study social behavior and neural development”

10:00 AM - 10:30 AM

Break

AGENDA CONT.

10:30 AM - 12:30 PM

Comparative Genomics Session

Session Chair - Sydney Cameron

Heather Hines

Pennsylvania State University

“How the bumble bees got their stripes: understanding mimicry in bumble bees using a comparative genomic approach”

Kamil Jaron

University of Lausanne

“Genome wide consequences of asexuality in *Timema* stick insects”

Sarah Lower

Cornell University

“Firefly genomes illuminate parallel origins of bioluminescence in beetles”

Kevin Johnson

Illinois Natural History Survey

“Phylogenomics of the hemipteroid insects”

12:30 PM - 2:00 PM

Lunch on your own

2:00 PM - 3:00 PM

Poster Session for even-numbered posters

3:00 PM - 5:00 PM

Population Genomics Session

Session Chair - Alexander Lipka

Richard Clark

University of Utah

“Learning from long haplotypes: Selection and genetic diversity in an extreme generalist herbivore”

Andrew Mongue

University of Kansas

“Sperm competition drives molecular evolution in Lepidopteran sperm”

Scott Geib

USDA ARS

“Utilizing comparative genomic resources coupled with rapid, low cost targeted resequencing to develop robust diagnostic tools to characterize pest tephritid fruit fly invasion pathways”

Marcus R. Kronforst

University of Chicago

“Characterizing the link between mimicry and mate choice in heliconius butterflies”

5:00 PM

Closing Remarks

5:15 PM

Remove even-numbered posters



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ABSTRACTS | INVITED SPEAKERS

**THE INSECT VIROME**

*Bryony C. Bonning, Department of Entomology & Nematology,
University of Florida, Gainesville*

The number of virus-derived sequences identified from insect genomic and transcriptomic sequence data has increased exponentially since the advent of Next Generation Sequencing. Some of these sequences are derived from viruses that are actively replicating, either as independent entities or with genomes fully integrated into the host genome. Other sequences derive from endogenous viral elements, i.e. sequences integrated into the genome that may be inactive or may function in anti-viral immunity. Based on our work with virus sequence discovery in Coleoptera, Hemiptera, and Lepidoptera, this talk will provide an overview of the insect virome not only for insects in the lab and from the field but also in insect cell lines. The presentation will address the implications of the presence of insect viruses and virus-derived sequences for research.



PHAGE GENES ALTER GAMETES AND KILL MALES IN *DROSOPHILA*

Seth Bordenstein, Department of Biological Sciences, Pathology, Microbiology, and Immunology, Vanderbilt University

The obligate intracellular symbiont, *Wolbachia pipientis*, selfishly alters fly gametes (cytoplasmic incompatibility) and male embryos (male killing) to increase the fitness of infected females relative to uninfected females. These modifications enhance *Wolbachia*'s maternal spread through host populations and can significantly impact arthropod speciation and vector control. However, the genes underlying the cunning adaptations remain mostly elusive. In this presentation, we report the discovery of three genes (*cifA*, *cifB*, *wmk*) in the eukaryotic association module of prophage WO that recapitulate *Wolbachia*'s capacity to cause cytoplasmic incompatibility and male killing. The discovery of these genes reveals the legacy of a bacteriophage in shaping animal reproduction, a worldwide endosymbiosis, and ongoing vector control efforts.



THE MALARIAGEN VECTOR OBSERVATORY: PRESENT, PAST, AND FUTURE

Mara Lawniczak, Wellcome Sanger Institute

Anopheles mosquitoes are incredibly adaptable and particularly good at thwarting vector control efforts. We are sequencing many wild individuals from different vector species to help understand how mosquito genomes evolve. The first wave of wild genomes are from *Anopheles gambiae*, and we have discovered patterns of widespread gene flow but also multiple origins of insecticide resistance and interesting patterns of gene duplication and selection at key

loci. Levels of variation in this species are extremely high and will continue to contribute to resistance of not only insecticides but also gene drive if we aren't very strategic in their implementation. To contribute to strategic vector control methods and to better understand how mosquito species vary and evolve, we have formed the MalariaGEN Vector Observatory, which is an open, collaborative network, leveraging genome sequencing technologies to undertake coordinated surveillance of malaria vector populations. As part of the MalariaGEN Vector Observatory, which aims to sequence 10,000 mosquitoes each year, we are also beginning to look at other vector species over space and time, in both Africa and Asia. We also have plans to look back in time by sequencing genomes from museum specimens that pre-date the widespread use of insecticides, giving us an empirical understanding of vector genomes prior to our malaria-control interventions. Genomic data on this scale will help us develop the best combination of strategies given no single method on its own will be resistance-proof.



THE GENETICS OF HUMAN HOST PREFERENCE IN THE *ANOPHELES GAMBIAE* COMPLEX

Michel A Slotman, Department of Entomology, Texas A&M University

The sibling species *Anopheles gambiae* and *An. coluzzii* are among the most important malaria vectors in sub-Saharan Africa. A major reason for their high vectorial capacity is their strong preference for human hosts, a trait modulated by the olfactory system. Both mosquitoes belong to a species complex that also includes the non-vector *An. quadriannulatus*, a mosquito with a preference for feeding on cows. We used a three-pronged approach towards elucidating the genetic basis of human host preference in *An. gambiae* and *An. coluzzii*. First, we conducted a QTL mapping analysis of human host preference in backcrosses between the anthropophilic *An. coluzzii* and the zoophilic *An. quadriannulatus*. Second, we used RNAseq on the main olfactory organs, the antennae and the maxillary palps, to identify olfactory genes whose expression is biased towards one species or the other. Third, we are conducting a molecular evolution analyses of chemosensory genes belonging to three families; olfactory receptors, ionotropic receptors, and odorant binding proteins, in six species belonging the *An. gambiae* complex. The goal of this analysis is to identify chemosensory genes that experienced positive selection in *An. gambiae* and/or *An. coluzzii* and thus may have been involved in the adaptation of these species to blood feeding on humans. Integrating the results from these three approaches is used to identify promising candidate genes underlying human host preference in *An. gambiae* and *An. coluzzii*.



A HYBRID DE NOVO GENOME ASSEMBLY OF THE HONEY BEE, *APIS MELLIFERA*, WITH CHROMOSOME-LENGTH SCAFFOLDS

Andreas Wallberg^{1*}, Ignas Bunikis^{2*}, Matthew Christmas¹, Olga Vinnere Pettersson², Mai-Britt Mosbech², Anna Childers^{3,4}, Jay Evans⁴, Alexander Mikheyev⁵, Gene Robinson⁶, Hugh Robertson⁶, **Matthew Webster¹**

¹ Dept. Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden; ² Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden; ³ USDA-ARS Insect Genetics and Biochemistry Research Unit, Fargo, ND, USA; ⁴ USDA-ARS Bee Research Lab, Beltsville, MD, USA; ⁵ Okinawa Institute of Science and Technology, Okinawa, Japan; ⁶ Department of Entomology and Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
*equal contribution

The ability to generate long sequencing reads and access long-range linkage information is revolutionizing the quality and completeness of genome assemblies. Here we use a hybrid approach that combines data from four genome sequencing and mapping technologies to generate a new genome assembly of the honeybee *Apis mellifera* with exceptional accuracy and contiguity. We first generated contigs based on PacBio sequencing libraries, which were then merged with linked-read 10x Chromium data followed by scaffolding using a BioNano optical genome map and a Hi-C chromatin interaction map, complemented by a genetic linkage map. Each of these steps reduced the number of gaps and incorporated a substantial amount of additional sequence into the assembled chromosomes. The new assembly (Amel_HAv3) is significantly more contiguous and complete than the previous one (Amel_4.5). N50 of contigs is 100-fold higher (5.381 Mbp compared to 0.053 Mbp) and we anchor >98% of the sequence to chromosomes. All of the 16 chromosomes are represented as single scaffolds with an average of 3 sequence gaps per chromosome. The improvements are largely due to the inclusion of repetitive sequence that was unplaced in previous assemblies. Our assembly is highly contiguous across centromeres and telomeres and includes hundreds of *Aval* and *AluI* repeats associated with these features. The improved assembly will be of utility for refining gene models, studying genome function, mapping functional genetic variation, identification of structural variants, and comparative genomics. We demonstrate its utility by analyzing the breakpoints of a chromosomal inversion involved in environmental adaptation that were located in sequence gaps in the previous genome assembly.



GENERATING GENETIC TOOLS IN ANTS TO STUDY SOCIAL BEHAVIOR AND NEURAL DEVELOPMENT

Hua Yan, Department of Biochemistry and Molecular Pharmacology, NYU Langone Medical Center

Social insects, including ants, exhibit cooperative social behavior with an extensive dependency on communication. The perception of cuticular hydrocarbons (CHCs) as pheromones is mediated by odorant receptor neurons (ORNs). ORNs express specific odorant receptors (ORs) encoded by a dramatically expanded *Or* gene family in ants. The biological features in a few ant species, such as *Harpegnathos saltator*, allow CRISPR-Cas9 gene targeting to generate a germline mutation. This facilitates the genetic analysis of the *orco* gene that encodes the obligate co-receptor whose mutation should significantly impact ant olfaction. Our results show that Orco exhibits a conserved role in the perception of general odorants but also a role in social behavior, plasticity and reproductive physiology in ants. Surprisingly, and in contrast to other insects, the loss of OR functionality also dramatically reduces the development of ant ORNs and antennal lobe glomeruli. Taken together, the ant genetics will provide inroads towards understanding the function of genes in regulating developmental plasticity, reproduction, longevity, and complex social behavior.



HOW THE BUMBLE BEES GOT THEIR STRIPES: UNDERSTANDING MIMICRY IN BUMBLE BEES USING A COMPARATIVE GENOMIC APPROACH

*Heather M. Hines, Department of Biology, Eberly College of Science,
Pennsylvania State University*

Bumble bees exhibit exceptional diversity in their aposematic color patterns, driven largely by convergence and divergence onto multiple global mimicry complexes. The resulting intraspecific diversity and large number of convergent replicates are ideal for understanding the ways in which the genome can be targeted under selection. To identify these adaptive loci and develop this new system for evolutionary genetics, we utilized genome-wide association analysis of color morphs within two bumble bee species (*Bombus bifarius*, *B. melanopygus*) that undergo parallel changes in mimetic coloration across their North American range. Our data demonstrates that each species uses a different genomic target to generate these same phenotypes: one uses an upstream developmental selector gene and the other downstream effector genes. We use these loci compared to other markers in the genome to determine how these polymorphic colors evolved and to understand the selection acting at mimicry hybrid zones.



PHYLOGENOMICS OF THE HEMIPTEROID INSECTS

Kevin P. Johnson, Illinois Natural History Survey, Prairie Research Institute, University of Illinois

The hemipteroid insect orders (Psocodea, Thysanoptera, and Hemiptera) comprise a diverse assemblage of over 120,000 species with a diversity of feeding habits. New phylogenomic datasets are revealing insights into the evolutionary history of this group at a variety of timescales. At the deepest level, a transcriptome dataset of over 2300 genes for around 200 species provides support for backbone relationships within and among these orders. For small-bodied insects for which transcriptome sequencing is not feasible, whole genome shotgun sequencing approaches can yield gene assemblies to produce similar datasets. For example, within parasitic lice, genomic datasets assembled using aTRAM provide novel insights into relationships among major groups, including identification of a previously unrecognized large clade of mammal lice. Support for branches within avian feather lice is high, providing new insights into the evolution of this group which has previously been largely unresolved. Genes assembled using aTRAM from genomic sequences are also able to provide highly supported phylogenies within genera of lice. Within the dove louse genus *Columbicola*, nearly all branches are highly supported, dramatically increasing confidence compared to trees generated from targeted sequencing of just a few genes. Additional uses of these genomic datasets include read-mapping to identify polymorphic sites for population genomic analyses and assembly of bacterial symbiont genomes to uncover repeated replacement of these symbionts and consequences for molecular evolution.



LEARNING FROM LONG HAPLOTYPES: SELECTION AND GENETIC DIVERSITY IN AN EXTREME GENERALIST HERBIVORE

Richard M. Clark¹, Andre Kurlovs¹, Olivia Kosterlitz¹, Huyen Bui¹, Robert Greenhalgh¹, Astrid Bryon², Sabina Bajda², Thomas Van Leeuwen²

¹ University of Utah, Department of Biology, 257 South 1400 East, RM 201, Salt Lake City, UT 84112, USA; ² Ghent University, Department of Crop Protection, Coupure links 653, Ghent, Belgium

The two-spotted spider mite, *Tetranychus urticae*, has been documented to feed on plants in more than 100 families. Along with this exceptionally broad host range, the species has spread globally, and is well known for its rapid evolution of pesticide resistance. To facilitate understanding *T. urticae*'s ability to persist on many host plants, as well as to understand the basis of its pesticide resistance and global spread, we have sequenced the genomes of nearly 30 strains. Our population sample consisted of strains from multiple continents, including North America, Europe and Asia, although about half were collected from a geographically restricted region in the US West (the state of Utah). High levels of genetic diversity were observed at both local and global scales, and while genetic differentiation by distance was detectable, it was modest. As assessed with 17 strains that we inbred to near genome-wide isogenicity, linkage disequilibrium (LD) decays quickly in *T. urticae*. However, about 10 genomic regions harbor large, shared haplotypes in strains from dispersed locations (including from different continents). Unexpectedly, only one of these putative sweep regions was associated with a known target-site pesticide resistance mutation. However, with bulk segregant analysis (BSA) genetic mapping using multiple strains, we show that one of the most striking sweep regions is associated with dominant loss of diapause. Diapause in *T. urticae* is associated with overwintering, and is a trait known to be lost at low latitudes and in greenhouse environments in which *T. urticae* is a major pest. Our findings suggest that in addition to selection for plant host use and pesticide resistance, response to the abiotic environment should not be overlooked as a factor shaping genetic diversity in broadly distributed generalist herbivores.



CHARACTERIZING THE LINK BETWEEN MIMICRY AND MATE CHOICE IN *HELICONIUS* BUTTERFLIES

Marcus Kronforst, Department of Ecology & Evolution, University of Chicago

Genetic covariance between mating cues and preferences, or genetic coupling, is widespread and evolutionarily important but its underlying molecular genetic cause remains unknown. By combining fine-scale genetic mapping, genome-wide association studies, gene expression analyses, population and comparative genomics, and genome editing with CRISPR/Cas9, we have characterized the molecular basis of a wing color mating cue in *Heliconius* butterflies and its genetic association with mate preference. Here, I will present our recent results showing that color diversity in the butterfly *Heliconius cydno* is due to alternate haplotypes at a narrow cis-regulatory element (CRE) downstream of a tandem duplication of the homeodomain transcription factor *aristaless*. Mate preference is associated with a tightly-linked but separate gene. Contrary to predictions, we find that genetic coupling is a result of linkage and selection and not pleiotropy or structural genomic variation. These observations reveal the functional molecular mechanisms responsible for genetic coupling and the origin of species more generally.



EVOLUTIONARY GENOMIC INNOVATION WITHIN THE PHYLUM ARTHROPODA

Stephen Richards, Department of Molecular and Human Genetics, Baylor College of Medicine

Arthropods are the largest and most diverse phylum on Earth. With the availability of low-cost whole genome sequencing we are beginning to be able to answer wide-ranging questions across large sets of taxa. Here, we present an analysis of genomic changes in the evolutionary history of the phylum Arthropoda. We sequenced and annotated whole genomes of twenty eight arthropod species as a pilot project for the Insect 5,000 Genomes Initiative (i5K). We combined these 28 genomes with 49 previously sequenced arthropod genomes generating a sample of 76 species spanning 22 arthropod orders. From these data, we reconstruct the history of arthropod evolution by presenting a well-resolved phylogeny of these species that we use to analyse gene content and protein domain content change in the phylum. We find that the last insect common ancestor (LICA) had a genome containing roughly 14,600 genes and that the transition to the insect lifestyle can be detected among gene families that emerged in LICA. We also find that the emergence of novel gene families is more prevalent during the diversification of the insect orders rather than more ancestral transitions, specifically during the evolution of Lepidopterans in which we find 1,308 emergent gene families, the most among all lineages examined. We also find specific examples of adaptive gene family and protein domain evolution that coincide with phenotypic adaptations and transitions, and unusual patterns of DNA methylation across arthropods, especially in more ancestral insects and spiders. Together, these results show the utility of such large-scale comparative genomics in our ability to generate hypotheses regarding the link between phenotype and genotype. Additionally, the data generated provide an unparalleled resource for studying Arthropod evolution.

COMPARATIVE ANALYSIS OF 21 APHID GENOMES TO GAIN INSIGHTS INTO THE EVOLUTION OF GRASS FEEDING AND EXTREME HOST RANGE EXPANSION

Thomas C. Mathers, Department of Crop Genetics, John Innes Centre, Norwich, UK

Aphids are a diverse group of insects with over 4,000 species spanning over 100 million years of evolution. Many aphid species are economically important crop pests that cause direct feeding damage and act as vectors for plant viruses. Since the publication of the first aphid genome in 2010, only 5 additional aphid genomes have been released, limiting genomic insights into the evolution of this important group of insects. To speed up the generation of genomic resources for aphids I have developed an approach to sequence and assemble aphid genomes at low cost from a single individual collected directly from the field. In this talk, I will present the analysis of 16 new aphid genome assemblies to investigate two significant transitions that have occurred during aphid evolution – the colonisation of grass species and the evolution of exceptionally broad host ranges (generalism).

CLONAL GENOME EVOLUTION AND RAPID INVASIVE SPREAD OF THE MARBLED CRAYFISH

Julian Gutekunst¹, Ranja Andriantsoa¹, Cassandra Falckenhayn¹, Katharina Hanna¹, Wolfgang Stein², Jeanne Rasamy³ and Frank Lyko¹

¹ Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center (DKFZ), Heidelberg, Germany; ² School of Biological Sciences, Illinois State University, Normal, IL, USA; ³ Mention Zoologie et Biodiversite Animale, Universite d'Antananarivo, Madagascar

The marbled crayfish *Procambarus virginalis* is a unique freshwater crayfish characterized by very recent speciation and parthenogenetic reproduction. Marbled crayfish also represent an emerging invasive species and have formed wild populations in diverse freshwater habitats. However, our understanding of marbled crayfish biology, evolution and invasive spread has been hampered by the lack of freshwater crayfish genome sequences. We have now established a de novo draft assembly of the marbled crayfish genome. We determined the genome size at approximately 3.5 gigabase pairs and identified >21,000 genes. Further analysis confirmed the close relationship to the genome of the slough crayfish, *Procambarus fallax*, and also established a triploid AA'B genotype with a high level of heterozygosity. Systematic fieldwork and genotyping demonstrated the rapid expansion of marbled crayfish on Madagascar and established the marbled crayfish as a potent invader of freshwater ecosystems. Furthermore, comparative whole-genome sequencing demonstrated the clonality of the population and their genetic identity with the oldest known stock from the German aquarium trade. Our study closes an important gap in the phylogenetic analysis of animal genomes and uncovers the unique evolutionary history of an emerging invasive species.

INSIGHTS INTO GENOME ORGANIZATION AND NON-CODING GENES OF *DIAPHORINA CITRI*, THE VECTOR OF CITRUSGREENING DISEASE

Prashant S Hosmani¹, Mirella Flores-Gonzalez¹, Angela Kruse^{1,6}, Sherry Miller², Teresa Shippy², Stephanie Hoyt³, Wayne Hunter⁴, Tom D'elia⁵, International Psyllid Annotation Consortium, Michelle Heck, Susan Brown², Lukas A. Mueller¹ and Surya Saha¹

¹ Boyce Thompson Institute, Ithaca, NY 14853; ² Division of Biology, Kansas State University, Manhattan, KS 66506; ³ Cornell University, Ithaca, NY 14853; ⁴ USDA-ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL 34945; ⁵ Indian River State College, Fort Pierce, FL 34945; ⁶ Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Sciences, Cornell University, Ithaca, NY 14853; ⁷ Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853

The Asian citrus psyllid (*Diaphorina citri* Kuwayama) is the insect vector of the bacterium *Candidatus Liberibacter asiaticus* (CLas), causal agent of citrus greening disease or Huanglongbing which has decimated citrus industry worldwide. This vector is the primary target of approaches to stop the spread of the pathogen. Accurate structural and functional annotation of the psyllid's gene models and understanding its interactions with the pathogenic bacterium, CLas, is required for precise targeting with molecular methods such as RNAi.

Single copy marker analysis of the previous genome assembly shows a significant proportion of 3,350 markers conserved in Hemipterans to be missing (25%). The manual genome annotation also identified a number of genome misassemblies and missing genes in the highly fragmented assembly. To improve quality of the assembly, we generated a *denovo* assembly with Pacbio long reads followed by Dovetail Chicago-based scaffolding to create an improved assembly (Diaci v2) with a contig N50 of 758.7kb and 1906 contigs. The assembly was polished with Pacbio and Illumina paired-end reads to reduce indel and SNP errors. We have also generated full-length cDNA transcripts from infected and healthy tissue from multiple life stages with Pacbio IsoSeq technology. This and other publicly available data sets were used to create an official gene set (OGS v2) with 20,793 mostly full-length protein coding genes. We have updated the *DiaphorinaCyc* pathway database with OGS v2 and characterized 172 pathways with 1477 enzymatic reactions. A publicly available gene expression atlas (Psyllid Expression Network) was created to identify co-expressed gene sets. We will present updates on manual curation of genes involved in pathways of interest such as chitin and melanin biosynthesis, as well as annotation of non-coding RNAs. Expression analysis of long non-coding RNAs originally shown to be differentially expressed in the gut, will be presented. We found them to be altered in the insect by host plant and allelic variation. Genomes of the endosymbionts, *Ca. Profftella armatura* DC and *Ca. Carsonella ruddii* DC, sequenced for the first time from a psyllid collected in FL, will also be presented. Details about a genome independent transcriptome with a comprehensive catalog of all genes in the psyllid will also be described. All resources are available on <https://citrusgreening.org/> which is our portal for all omics resources for the citrus greening disease.

DRIVERS OF VARIATION IN BACTERIAL COMMUNITY COMPOSITION AMONG *IXODES SCAPULARIS* NYMPHS IN THE NORTHEASTERN U.S.

William J. Landesman¹, Kenneth Mulder² and Brian F. Allan³

¹ Biology Program, Green Mountain College, Poultney, VT; ² Center for Quantitative Literacy, Green Mountain College, Poultney, VT; ³ Department of Entomology, University of Illinois, Urbana-Champaign, IL

The drivers of microbiome variation among ticks are understudied but this information may improve our understanding of how tick-borne diseases are transmitted. We performed two studies to determine the drivers of microbiome variation in nymphal-stage black-legged ticks (*Ixodes scapularis*) - the principal vector of Lyme disease in the northeastern United States. In the first study, we quantified the relationship between the *B. burgdorferi* infection status of field-collected *I. scapularis* and the bacterial community composition of these ticks. In the second study we characterized the microbiome composition of *I. scapularis* that fed as larvae on Raccoon (*Procyon lotor*), Opossum (*Didelphis virginiana*), Skunk (*Mephitis mephitis*), Red squirrel (*Tamiasciurus hudsonicus*) and Grey squirrel (*Sciurus carolinensis*). Microbiome analyses were performed using 16S rDNA gene amplicon (515f/806r) sequencing, followed by bioinformatics analysis with Qiime 1.9. We used Permutational Analysis of Variance (PerMANOVA) and Analysis of Similarity (ANOSIM) on the Unifrac distance matrix, followed by principal coordinates analyses for visualization of differences in bacterial communities. To maximize the between-host species distances we used a canonical analysis of principal coordinates (CAP), which provided a new metric multidimensional scaling using a subset of the principal coordinates (PCOs). This was followed by a “leave one out” approach to attempt to identify the host species of each sample based on the CAP coordinates.

We found no significant differences in bacterial communities between infected and uninfected *I. scapularis* (PerMANOVA; Pseudo-F: 0.96, $P = 0.54$). However, we found a significant effect of blood meal host identity on bacterial community similarity (ANOSIM; $R = 0.351$; $p = 0.048$) and a highly significant effect of individual hosts ($R = 0.432$; $p < 0.001$). A plot of the first two CAPs revealed clear visual separation among several host species and the “leave one out” approach correctly identified the host species of 52/88 (59%) of the samples. The shifts in community composition were driven by variation in the relative abundance of several dominant bacterial orders (*Actinomycetales*, *Rickettsiales*, *Rhizobiales*, *Pseudomonadales*, *Burkholderiales* and *Xanthomonadales*), suggesting that host blood altered an existing tick microbiome, rather than being a source of bacterial colonization for these dominant orders. Such a pattern may be consistent with a “filtering” effect due differences in the chemistry of host blood by species. Some of the detected taxa likely colonized the tick surface, suggesting that they were acquired due to close physical contact between vector and host during feeding. To the best of our knowledge, this is the most comprehensive analysis to date of the contribution of blood meal hosts to the *I. scapularis* microbiome. The findings are likely to improve our understanding of the role of the microbiome in the colonization of *I. scapularis* by pathogens such as *Borrelia burgdorferi*, the primary agent of Lyme disease in the northeastern U.S.

VIVIPAROUS REPRODUCTION DELAYS MICROBIOME ACQUISITION IN A LIVE-BEARING COCKROACH, *DIPLOPTERA PUNCTATA*

Emily C Jennings¹, Trinity L. Hamilton², Joshua B. Benoit¹

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Animals share their bodies with a diverse suite of microorganisms known as the microbiome. These microbes, have been shown to profoundly impact host biology, though processes ranging from nutrient metabolism to immune function. The extensive and complex interactions between mother and offspring during gestation and birth impact the life and health of offspring throughout their lives. The vertical transmission of microbes from mother to offspring, the importance of which has been repeatedly demonstrated, is an example of such interactions. The importance of the microbiome composition and transmission in development is not limited to humans. In fact, invertebrate systems offer opportunities to conduct studies on microbiome-development dynamics. One such invertebrate is the live-bearing cockroach, *Diploptera punctata*. Female *D. punctata* carry embryos in their brood sac, which acts as the functional equivalent of the uterus and placenta. In our study, 16S rRNA sequencing was used to characterize maternal and embryonic microbiomes as well as the development of whole body microbiome across nymphal development. We identified 28 phyla and 161 families overall. *Bacteroidetes* and *Firmicutes* are the most abundant phyla in mothers, while *Bacteroidetes* was the only phylum identified in embryos, with *Blattabacteria* being the only genus having significant representation (99.4% of reads from embryos map to this genus). This suggests that *D. punctata* offspring, like humans, likely acquire most components of their microbiome during and after birth.

IDENTIFYING HEME IMPORTERS & EXPORTERS THROUGH RNA SEQ ANALYSIS IN *Aedes Aegypti*

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After a bloodmeal, mosquitoes import heme into the midgut epithelium. Heme acts as an essential signal for oogenesis in *Aedes aegypti*. However, the mechanisms behind heme import in *Aedes aegypti* are largely unexplored. In this study, RNA sequencing data from 4 different *Aedes aegypti* cell culture experiments and 1 midgut experiment where exposure to an overabundance or deficiency of heme was examined to identify heme-responsive genes. Zinc mesoporphyrin (ZnMP), a heme fluorescent analog, was used to measure changes in heme uptake prior to mRNA sequencing. A soft cluster analysis was performed to identify genes encoding potential membrane bound importers and exporters based on expression profiles across the samples for each experiment. Stronger candidates were obtained by comparing genes in each dataset to each other. When comparing all cultured datasets to each other, 223 candidate genes with expression pattern changes consistent with importers were found to be heme-regulated in only 2 datasets, 46 were heme-regulated in 3 datasets and 2 was heme-regulated in all 4 datasets. In contrast, 114 candidate genes with patterns consistent with exporters were common to only 2 datasets, with just 11 present in 3 of the 4 datasets. 39 potential importers were located in the midgut heme exposure experiment, 16 of these were also found in at least 1 cultured cell dataset. On the contrary only 23 potential exporters were found with only 7 found in at least 1 cultured cell dataset. Future work will focus on reverse genetic analysis of the top candidate genes described in this study to confirm their status as importers or exporters in *Aedes aegypti*. Techniques to be utilized in this analysis include RNAi to knockdown candidates in both cultured cells and midguts, heme import gain of function experiments in a model system lacking heme transport and finally CRISPR/Cas 9 knockout of target genes in the whole organism to observe phenotypes of homozygous mutants.

CURATION IS KEY: QUANTIFYING THE IMPACT OF MANUAL GENE ANNOTATION IMPROVEMENTS

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Confident characterisation of gene evolutionary histories is required in order to build reliable hypotheses of their putative functional roles in different species. Resolving these histories requires good-quality sequence alignments for robust phylogenetic analyses to build gene trees that accurately trace the evolutionary relationships amongst genes from extant species. This in turn relies on complete and accurate gene model annotations. The process of automatically annotating gene features in assembled genomes has developed to be able to use multiple sources of evidence to produce well-supported gene models. Nevertheless, this task remains challenging and even mostly-well-supported annotations can fail to meet the criteria of being complete and accurate. Here we comprehensively assess the quality of annotations of genes associated with mosquito immune responses using the Apollo collaborative genomic annotation editor. We enumerate different types of edits performed to improve imperfect gene models, and we quantify the impact of these manual gene annotation improvements by comparing protein lengths, sequence alignments, and gene tree qualities before and after curation. This case study on mosquito immune genes highlights the strengths and weaknesses of automated annotations and demonstrates the importance of curation in the context of accurately reconstructing the evolutionary histories of gene families.

REPRODUCTIVE WORKER HONEY BEES: A GLIMPSE OF ANCESTRAL SOCIALITY?

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Several hypotheses suggest changes in gene regulation have been important in caste evolution, and there are recent reports of correlations between level of sociality and gene regulatory complexity. However, we still do not understand which molecular changes were important in caste evolution, and which followed eusocial origins. A promising approach to differentiate these signals is to compare regulatory mechanisms underlying variation in social forms. Honey bee colonies display complex eusociality, but if an *Apis mellifera* colony becomes permanently queenless, some workers will activate their ovaries. It has long been assumed these reproductive workers behave selfishly, but recently it was discovered that some workers with developed ovaries perform cooperative tasks. We used a novel behavior tracking system to automatically monitor egg-laying, foraging, and trophallaxis behaviors for every individual in seven queenless colonies continuously for one week. With these high-resolution data, we discovered previously unknown social organization among reproductive honey bee workers reminiscent of simple forms of eusociality. “Dominant” workers showed high levels of egg laying, low levels of foraging and were more likely to be recipients during trophallactic interactions; “subordinate” individuals showed the opposite pattern, and some bees showed a more generalized mixture of behaviors, suggestive of intermediate dominance status or an even more basal level of sociality. RNA-seq revealed that dominant laying workers show significant overlap in brain gene expression with queens of the facultatively eusocial *Megalotheba genalis*, and with queens and dominant individuals in the simple eusocial species *Bombus terrestris* and *Polistes canadensis*, respectively. By contrast, no significant overlap in brain gene expression was found with honey bee queens. ATAC-seq was used to study accessible chromatin patterns associated with these patterns of gene expression. Together, our results reveal surprising behavioral and brain molecular plasticity in workers for reversions to less complex forms of sociality. We propose that comparisons between sterile and reproductive honey bee workers may therefore be informative in understanding how gene regulatory changes led to the evolution of the worker caste from solitary or simple eusocial ancestors.

BRAIN TRANSCRIPTOMIC CHANGES ASSOCIATED WITH COLONY DEFENSE IN THE STINGLESS BEE, *TETRAGONISCA ANGUSTULA*

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Colony defense behaviors in social insect colonies have evolved to protect valuable communal resources. Despite divergent modes of defensive action in two independent lineages of eusocial bees --stingless bees (*Meliponini*) bite and honey bees (*Apini*) sting-- both lineages use alarm pheromones to trigger a robust defensive behavioral response in a subset of workers. It is unknown whether the convergent defensive behavior in the *Meliponini* and *Apini* is subserved by shared neuromolecular mechanisms. We exposed colonies of the neotropical stingless bee *Tetragonisca angustula* (*Meliponini*) to alarm pheromone and then performed brain transcriptomic analysis. We compared these results to a similar study performed on the Western honey bee *Apis mellifera* (*Apini*), which resulted in hundreds of differentially expressed genes as reported in a previously published study. Paired-end mRNA sequencing on 59 whole bee brains from 3 colonies using Illumina HiSeq 4000 resulted in over 2.8 billion reads. Because the genome of *T. angustula* has not been sequenced yet, we first had to create a *de novo* brain transcriptome. We present here the brain transcriptome as well as the results of our comparative transcriptomic analyses. Results will provide insight into the biological processes and molecular functions used in the defensive response of both eusocial bee lineages. We also expect that this transcriptome will provide a resource for future studies on the evolution of behavioral complexity and provide molecular insight into the fascinating social organization of eusociality.

GENOME WIDE CONSEQUENCES OF ASEXUALITY IN *TIMEMA* STICK INSECTS

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Asexuality is predicted to have profound genome-wide consequences due to the absence of recombination and of the need for chromosomal pairing. Predicted consequences of asexuality notably include the accumulation of deleterious mutations, divergence between haplotypes, genomic rearrangements and changes in the dynamics of transposable elements. Previous work has shown evidence for these consequences in several asexual organisms, however it remains unclear whether the observed patterns are due to asexuality or stem from lineage-specific peculiarities. This is because previous genomic studies used either a single asexual species, or multiple species but with a single origin of asexuality. To characterize the evolutionary consequences of asexuality without the confounding effect of lineage requires a study of species with multiple independent transitions to asexuality. This is found in the stick insect genus *Timema*. In *Timema* there have been at least seven independent transitions from sexual to asexual reproduction, each representing a biological replicate of asexuality. We sequenced the genomes of five asexual species and their sexual sister species to assess the consequences of asexuality on heterozygosity (SNPs and indels), transposable element abundance, palindrome frequency, and acquisition of horizontally acquired genes. Overall we find lower levels of heterozygosity in asexuals than sexuals, comparable loads of transposable elements, and no evidence for different levels of horizontally transferred genes or palindromes. Finally, we use genome-wide polymorphism data to develop insights into the origin of asexuality in *Timema* stick insects.

FIREFLY GENOMES ILLUMINATE PARALLEL ORIGINS OF BIOLUMINESCENCE IN BEETLES

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Fireflies and their captivating luminous courtships have inspired centuries of scientific study. Today, firefly luciferase is widely used in biotechnology; however, the evolutionary origin of their bioluminescence remains unclear. To shed light on this long-standing question, we sequenced the genomes of two firefly species that diverged over 100 million-years-ago: the North American *Photinus pyralis* and Japanese *Aquatica lateralis*, as well as the genome of a related luminescent click-beetle, the Caribbean *Ignelater luminosus*. A variety of sequencing and assembly strategies, including hybrid assembly of long PacBio and short Illumina reads and scaffolding with HiC long-range data, yielded excellent reference genomes. Subsequent analyses support two independent gains of bioluminescence between fireflies and click-beetles, and provide new insights into the genes, chemical defenses, and symbionts that evolved alongside their luminous lifestyle.

SPERM COMPETITION DRIVES MOLECULAR EVOLUTION IN LEPIDOPTERAN SPERM

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Reproductive proteins have long been observed to diverge faster than the rest of the genome; often this divergence is assumed to be adaptive and sexual conflict or speciation are invoked as mechanisms. More recently, however, theory has called this assumption into question by demonstrating that the patterns of divergence observed in reproductive proteins can also result from relaxed selection and increased drift instead of selection (1). Under this paradigm, reproductive proteins, unlike those involved in somatic function, only experience selection in one sex, and even then, sometimes only in certain contexts (e.g. alleles affecting sperm competition outcomes have no benefit without competition). This theory also lacks empirical support but makes a very simple prediction: adaptive evolution of sperm proteins should depend on the level of sperm competition in a species.

Lepidoptera (butterflies and moths) make an ideal test system for this hypothesis, as different species vary greatly in female remating rates. For instance, female monarch butterflies (*Danaus plexippus*) naturally remate up to 14 times, while Carolina sphinx moth females (*Manduca sexta*) are mostly singly mating. Furthermore, these two taxa have published proteomic datasets detailing the set of proteins expressed in sperm. Monarchs also already have natural population resequencing data to allow assessment of molecular evolution of these proteins (2). To complete comparable analyses in *M. sexta* we have generated the first set of whole genome resequencing data from a natural population of Carolina sphinx moths.

We find that molecular evolution of sperm proteins is strikingly different in these two species. In the monandrous sphinx moth, sperm proteins do not appear to evolve differently from the rest of the genome. In the highly polyandrous monarch however, sperm proteins show significantly more adaptive evolution, largely mediated by a decrease in non-synonymous polymorphisms in the sperm proteome. This pattern suggests stronger purifying selection in sperm genes. Additionally, we see the strongest signal for adaptive evolution in monarch sperm genes that contain an ortholog in *M. sexta* sperm, indicating that the same set of genes is truly experiencing different intensities of selection in the two species. These results support the newer paradigm of the importance of mating system in reproductive protein evolution and the data generated for *M. sexta* in this project will doubtless be a valuable resource for contextualizing laboratory research in this model insect.

UTILIZING COMPARATIVE GENOMIC RESOURCES COUPLED WITH RAPID, LOW COST TARGETED RESEQUENCING TO DEVELOP ROBUST DIAGNOSTIC TOOLS TO CHARACTERIZE PEST TEPHRITID FRUIT FLY INVASION PATHWAYS

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Each year, thousands of exotic and invasive species, particularly insects, plants, or diseases, are intercepted at ports of entry or detected in the environment. Crucial to management and eradication of these species, and prevention of their establishment, is rapid species identification, and determination of source of invasive material. Our goal was to develop a rapid, straightforward tool for determining the species and source population of invasive Tephritid fruit flies that are commonly detected in California, Florida, and South Texas. For many species, clear morphological tools for discriminating species do not exist, particularly at the immature level. Here we present an approach for phylogenomic locus selection in these species that takes advantage of a variety of genomic and transcriptomic data sources, focusing on conserved exonic regions in orthologous genes. This approach yielded a phylogenomic tool far exceeding the resolution of existing approaches, such as target enrichment approaches or traditional marker sequencing, as loci are uniquely selected based off of their diagnostic utility. Utilizing reduced representation sequencing of individual flies from through the geographic range, diagnostic loci for discriminating populations of specific invasive species were also developed. To allow for rapid and low-cost re-sequencing of these species and population level markers, we employed a highly multiplex, single tube amplicon sequencing approach, allowing targeting of thousands of loci at once, coupled with high throughput sequencing. The wetlab and analysis pipeline developed can analyze hundreds of individuals at a time, and return taxonomic and population level assignment in a matter of three days from sample collection. Our approach provides a novel way to combine diverse genomic and transcriptomic data sources across a breadth of taxa, particularly when at least one well-annotated data source is available, and can rapidly develop diagnostic tools for non-model systems that are scalable, cost-effective, and robust. Preliminary analysis of a panel of 350 predominately *Bactrocera* specimens used to generate a comprehensive phylogeny was able to resolve several species groups previously not resolved in previous studies using barcode or multigene phylogenies as well as characterize population structure of economically important groups for pathway and source determination.

NOTES

1. GFF3TOOLKIT — PYTHON PROGRAMS FOR PROCESSING GFF3 FILES

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Generating automated or manual annotations of genome assemblies is a standard task in the genome project life cycle. General feature format Version 3 (GFF3) is one of the standard file formats used for describing genomic features. The 9-column format is easy to manipulate, but also easy to break. To help scientists process annotations in GFF3 files, we developed the GFF3toolkit to handle common problems with GFF3 files. Currently, we provide the following functions: detect over 50 types of formatting errors in the GFF3 file (`gff3_QC`); fix 30 of these formatting errors (`gff3_fix`); sort GFF3 files (`gff3_sort`); merge two GFF3 files into a single, non-redundant GFF3 file (`gff3_merge`); and generate FASTA files from a GFF3 file (`gff3_to_fasta`). GFF3toolkit accepts both canonical (e.g. standard protein-coding genes) and non-canonical gene models, which makes it flexible to use. Here we show a common use case: updating annotations in GFF3 files to demonstrate several core features in the GFF3toolkit. The difficult parts of updating annotations are (1) dealing with formatting errors and (2) incorporating thousands of revised or newly added models into a reference gene set. With the `gff3_QC`, `gff3_fix`, and `gff3_merge` pipeline, obtaining non-redundant arthropod gene sets in high quality is no longer a difficult task. Also, `gff3_to_fasta` can be used to generate six different feature types of biological sequences from a GFF3 file for many use cases. The GFF3toolkit is freely available at <https://github.com/NAL-i5K/GFF3toolkit>.

2. DE NOVO GENOME ASSEMBLY OF *BACTROCERA OLEAE* THROUGH A COST-EFFECTIVE COMBINATION OF LINKED READ AND LONG READ TECHNOLOGIES

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Until now the best genome assemblies have combined multiple sequencing technologies which each require the construction different DNA libraries adapted for each technology. Short read libraries alone typically lead to heavily fragmented assemblies. The addition of mate pair libraries can improve the size of the assembly, while Pacbio or Oxford Nanopore reads can extend to longer than 10Kb and along with their scaffolding ability they bring sequence content and therefore do not contribute to gaps in the genome as mate pairs do. PacBio however remains significantly more expensive, while Oxford Nanopore still requires large amounts of input DNA for its libraries.

Here we show that a single 10x Genomics linked read library sequenced on the Illumina HiSeq X platform is able to outperform all the above technologies combined with a single type of library. Furthermore, we show how these other technologies can still be combined with 10x Genomics in an orthogonal fashion to further improve a 10X genomics only solution. Using a haploid olive fly genome size of 322Mb and comparing to the published reference we show that our 10x Genomics assembly (supernova v2.0) improves the NG50 scaffold size from 0.04Mb to 4.4Mb and the LG50 count 150 to 23. This assembly's NG90 length remains above the length of 1Mbp with 80 scaffolds (NG90 Length: 1.2Mb in 80 contigs). An Oxford Nanopore long read based assembly was also generated with an NG50 length of 320Kb in 289 contigs (LG50 count). Combining mate pair and long reads to the 10x Genomics assembly further improves the NG50 scaffold size and also gap closes the assembly. Additionally, since the 10x Genomics assembler is diploid aware the linked reads allow the construction of two assemblies at one time representing both alleles in the diploid genome. This results in a better organized assembly where the contigs originating from the different alleles in the same genomic location are kept separate, alternatively common genomic portions are shared in both assemblies. In the previous methods this haplotype splitting and merging would have to be achieved post-assembly and is typically left for later stages of denovo assembly projects. In addition, we integrated transcriptomic and cytogenetic data, as well as in identifying Y chromosome specific sequences. Y chromosome is notoriously difficult to assemble in all organisms due to its heterochromatic and repetitive nature. We show how these haplotype specific assemblies can be enhanced by integrating short or long read data, to generate high quality genomes in a cost-effective manner and will become the gold standard for *de novo* assemblies.

3. BOOST THE MAINTAINABILITY OF BIOINFORMATIC WEB PROJECT: A CASE STUDY ON GENOMICS-WORKSPACE

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Usually, a bioinformatic website project will be maintained for several years. Modern bioinformatics projects are becoming more complex, the result of multiple people efforts. Over time, contributors change, and it's a common issue that the maintainability of a bioinformatic project need to be enhanced. Here, as a case study, we presented the work done in genomics-workspace, which is one of core web services provided by the i5k Workspace@NAL. This application provides web interfaces for the well-known BLAST, HMMER, and Clustal algorithms. It also implements task queues and back-end admin for large-scale management. Here we briefly introduce genomics-workspace application and compare it with other software solutions available. We also discuss issues we encountered and solutions we adopted. In the end, we discuss future directions in a software engineering aspect.

4. GENOME SEQUENCING OF THE JUMPING BRISTLETAIL *LEPISMACHILIS Y-SIGNATA* (INSECTA: ARCHAEOGNATHA)

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Insects are the most diverse animal group, comprising more than one million species. The genomes of some insect groups, such as Diptera, have been thoroughly studied, because they contain species of medical and agricultural importance. As a result, comprehensive genomic resources, including genomic and transcriptomic data, are available for research on these species. In contrast, for other groups, such as Archaeognatha, Ephemeroptera, Odonata, and Thysanura, significantly less comprehensive genomic resources have been compiled. These clades, however, contain species that are critical for answering key evolutionary and systematic questions in entomology. In order to address this paucity of genomic information, we sequenced, within the framework of the i5k Initiative (5,000 Insect Genomes Project), the genome of the jumping bristletail *Lepismachilis y-signata*. *L. y-signata* belongs to the order of Archaeognatha, containing primarily wingless insects that are important for studying the evolution of various insect traits, such as wings, the nervous system, and chemosensation. More specifically, we sequenced the 2.88 Gbp genome of *L. y-signata*, using the Illumina platform, and generated 148.2 Gbp of raw sequencing data. Assembling these reads into contigs and scaffolds resulted in a draft assembly of 3.35 Gbp that contained more than 100,000 scaffolds. Even though the assembly is very fragmented, it appears to be relatively complete, as judged from the results of the BUSCO pipeline, which found 76.9 % complete genes conserved in insects. Results from gene annotation, functional annotation, and comparative genomics that are currently in progress will be reported.

5. THE I5K WORKSPACE@NAL - UPDATES TO A GENOME DATABASE

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Genome databases can serve as catalysts for the scientific community by providing access to relevant datasets, and tools to improve and connect them. The i5k Workspace@NAL is a database for the display, curation and dissemination of arthropod genome assemblies. As of May 2018, the i5k Workspace hosts 64 organisms and their genome assemblies. All our content is currently user-submitted. To facilitate submission, we have updated our data submission forms, and now allow users to upload files to our site. Community-based manual curation and Official Gene Set development are some of the keystone services that the i5k Workspace offers. On this front, we have implemented new naming guidelines to facilitate consistent naming across species. We have also released Official Gene Sets for 11 organisms, most recently using our updated GFF3Toolkit software (<https://github.com/NAL-i5K/GFF3toolkit/>). Genome projects are often dynamic, so we are currently developing workflows to update the organisms that we host to new genome assemblies (https://github.com/NAL-i5K/coordinates_conversion and <https://github.com/NAL-i5K/remap-gff3>). Finally, we hold webinars every other month on topics of interest to the i5k Workspace community.

6. APPLICATION OF EMERGING TECHNOLOGIES FOR LOW-COST NON-MODEL GENOME SEQUENCING

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In an era when endeavors such as the i5k initiative and the Earth Biogenome Project can be realistically achieved, biologists face an almost overwhelming number of approaches for genome sequencing and assembly, and methods that worked well for model systems may not transfer to arthropods. Genome size, genome complexity, body size, and budget must be taken into consideration when selecting a method and there is not a single solution which can be applied to all genomes. Here we will present genome sequencing strategies along with their pitfalls and challenges that we have implemented on insects from three different orders: Diptera, Hymenoptera, and Lepidoptera. Sequencing and assembly were performed using a combination of emerging technologies such as long-read sequencing (Oxford Nanopore), linked-read sequencing (10x Genomics), Hi-C mapping, and linkage mapping and resulted in chromosome-scale references that were of high contiguity and completeness. These draft reference assemblies now serve as foundational genomic tools which we are using to answer research questions in a wide breadth of biological fields ranging from conservation to pest management.

7. CHARACTERIZATION OF THE MIDGUT MICROBIOME FOR THE NORTHERN HOUSE MOSQUITO, *CULEX PIPIENS*

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The ability to undergo diapause in insect species has greatly influenced their evolutionary success in severe climates. *Culex pipiens*, the carrier for West Nile Virus, undergoes diapause to survive harsh northern winters, permitting them to quickly establish spring vector populations upon the return of favorable conditions. Although much is understood about the hormonal mechanisms and environmental cues that invoke diapause, little is known about the role of bacterial symbionts in this process. Mosquito gut bacteria have proven to be essential in both development and disease resistance, suggesting they may also play an important role in other aspects of mosquito physiology such as the preparation for and survival through diapause. To determine whether biological benefits are conferred by the microbiome, the number of bacterial symbionts comprising the microbiome will be reduced using pupal surface sterilization, followed by placement into a completely sterile environment or one in which they can be reconstituted by environmental bacteria. The impact of these treatments on both survival and the nutritional reserves necessary for diapause success will be assessed and compared to individuals with unaltered microbiomes. Overall, this project aims to determine whether a difference exists between the gut microbiome composition of diapausing (D) and nondiapausing (ND) *C. pipiens* and whether the microbiome provides physiological benefits during diapause preparation.

8. DIVERSITY AND COMPOSITION OF MIDGUT MICROBIOTA OF *Aedes albopictus* IN CENTRAL ILLINOIS DEPENDS ON LAND USE TYPE AND LOCALITY

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Microbiomes of mosquito midguts are known to be closely associated with specific mosquito life processes such as nutrition, reproduction, aggregation and defense against toxins. We investigated the bacterial community composition and diversity in midguts of adult female *Aedes albopictus* collected from Champaign and Coles County based on two different land use types: residential areas and woodlots (40 samples in each category). After extraction of DNA from dissected midguts, we used next generation sequencing (MiSeq V3) to obtain sequences spanning the V4 hypervariable region of the 16S rRNA gene. The bacterial sequences were analyzed with QIIME. After quality filtering and rarefying, we identified 551 operational taxonomic units (OTUs) from 114 samples. Of the top 30 most abundant OTUs, 31 genera were discovered in 22 families. According to an indicator species analysis, in Champaign County *Pseudomonas* (50%) and *Sediminibacterium* (63.5%) characterized the midguts of *Ae. albopictus* collected from residential areas and woodlots, respectively. For Coles County, the midguts of *Ae. albopictus* collected from residential area were well characterized by the OTU for *Bradyrhizobiaceae* (49.3%), and by *Janthinobacterium* (51.2%) for woodlots. In general, the composition of bacterial communities differed between both trapping locations and land use types, with some overlap occurring in the residential sectors. In contrast, alpha-diversity measures were largely similar across locality, but differed between land use types, with greater species richness (Chao1), heterogeneity (Shannon Index) and equitability in the midguts of mosquitoes collected from wooded areas.

In conclusion, the midgut bacterial community composition and diversity of *Ae. albopictus* varies by land use and location. Further studies on whether and how such differences in midgut biota influence variation in vectorial capacity traits are warranted.

9. NEXT GENERATION SEQUENCING FOR SURVEILLANCE AND DIAGNOSIS OF TICK-BORNE DISEASES: EXPLORING THE MICROBIOME OF INDIANA TICKS

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Ticks transmit a variety of bacteria, viruses, and parasites to humans and animals during blood feeding. In the U.S., Lyme disease is a tick-borne disease (TBD) that has high impact on human health, with an estimated 300,000 human cases/year (Center for Disease Control and Prevention). The control of TBDs is complicated by limited knowledge of the pathogens and parasites circulating in tick populations at regional scale, and comprehensive diagnostic tools. To address this need, we have launched “Tick INSiders”, a citizen science project to improve the prevention, diagnosis and treatment of TBDs in Indiana. We are investigating the microbiome of three major tick vectors of importance to public health - *Ixodes scapularis* (black legged or Lyme disease tick), *Dermacentor variabilis* (dog tick), and *Amblyomma americanum* (lone star tick). A next generation sequencing platform (MiSeq) is being used to explore the microbial complement of these species with a focus on the bacterial and viral repertoire of ticks collected around the state. DNA libraries are being prepared from pools of male and female ticks. Primers targeting the V3 and V4 variable region of the 16S ribosomal RNA gene and random hexamers are being used to prepare the 16S for bacterial microbiome and the cDNA for virome libraries, respectively. Data from 2017 and 2018 tick collections will be presented, together with our preliminary analyses of the microbiome. Temporal and spatial microbiome data will be made available via the Tick INSiders website. Data will be used to develop region-specific disease risk maps and educational modules for health care practitioners.

NOTES

10. PSYLLID EXPRESSION NETWORK (PEN), A TISSUE AND HOST-SPECIFIC EXPRESSION ATLAS FOR *DIAPHORINA CITRI*

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Citrusgreening or Huanglongbing (HLB) is a tritrophic disease complex involving citrus host, the Asian citrus psyllid (ACP, *Diaphorina citri*) vector and a phloem restricted bacterial pathogen *Ca. Liberibacter asiaticus* (CLas). HLB is considered to be the most devastating of all citrus diseases, and there is currently no adequate control strategy. The vector is the primary target of approaches to manage the disease. Growth and development as well as environmental and immune responses are controlled by programmed expression of a suite of genes at any given time, stage and tissue in an insect.

Psyllid Expression Network (PEN) is an open-access, interactive and user friendly web tool with proteomics and transcriptomics resources for the psyllid. PEN contains high-resolution expression data from CLas infected and healthy individuals across multiple life stages (egg, nymph and adult), tissues (whole body, terminal abdomen, gut and antenna) and sexes (male and female). Insects were collected from a variety of hosts (*C. reticulata*, *C. macrophylla*, *C. sinensis*, *C. medica* and *C. clementina*). The expression patterns were determined with transcripts per million reads (TPM) and only genes with more than 1 TPM in at least one tissue were retained. A total of 620 million raw reads were analyzed to identify all the expressed genes from official gene set 2.0 across 13 different tissue and host combinations for transcriptomics data. We also have proteomics data with spectral counts for 12 different tissue and host combinations in PEN. Gene transcription and translation patterns are critical for understanding how the underlying genome sequence translates into specific phenotypes at key developmental and infection stages. PEN allows visualization of the spatiotemporal context of gene expression and helps elucidate function. This facilitates effective data analysis by enabling simultaneous visualization of correlated genes to develop novel hypothesis in addition to candidate gene identification. This tool is a useful resource not only for citrusgreening research but also other Hemipteran pests. PEN is available at <https://pen.sgn.cornell.edu>.

11. THE CHEMORECEPTOR REPERTOIRES IN *LUTZOMYIA LONGIPALPIS* AND *PHLEBOTOMUS PAPATASI* PROVIDE INSIGHTS INTO SAND FLY EVOLUTION AND POPULATION STRUCTURE

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Phlebotomine sand flies are the only known vectors of *Leishmania* parasites, the causative agents of cutaneous and visceral Leishmaniasis. Despite the considerable body of work on the chemical ecology of sand flies, little is known about their chemosensory genome. Here, we annotated the odorant (ORs), gustatory (GRs) and ionotropic receptors (IRs) in the genomes of *Lutzomyia longipalpis* and *Phlebotomus papatasi*, two major phlebotomine vectors in the new and old world, respectively. The size of the OR repertoires is relatively large compared to other Diptera characterized thus far, with approximately 130 ORs in each species. Phylogenetic analysis of ORs in six dipterans showed that over 85% belong to a highly divergent, sand fly-specific clade. The GR repertoires are similar in size to the mosquitoes and *Drosophila* with 88-92 GRs. In contrast to the mosquitoes, which have three CO2 receptors, only two full length genes (Gr1 and Gr2) were found, thus revealing the loss of the highly conserved DmelGr63a lineage in the sand flies. The IR repertoires are comparatively small, with 26-27 IRs, which include at least one copy of the 14 antennal IRs that are conserved across the Diptera. We performed comparative analyses of the chemoreceptor repertoire from 51 individually sequenced *L. longipalpis* field isolates from Brazil representing two pheromone types. Clustering and discriminate analysis showed distinct clusters of individuals correlating to their pheromone biology.

12. MEETING THE CHALLENGE OF TICK-BORNE DISEASE CONTROL: A PROPOSAL FOR 1000 *IXODES* GENOMES

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As vectors of emerging and re-emerging diseases, ticks represent a global public health threat that has reached crisis proportions and must be met with strategic investment in priority research areas. Data-driven science offers our best hope yet to unravel the molecular complexities of pathogen transmission by ticks and identify novel solutions to control. At a joint ‘One Health’ Tick and Tick-borne Pathogen and Asia Pacific Rickettsia Conference in Australia in 2017, researchers convened and issued a “call to arms” with an ambitious proposal to sequence the genomes of multiple *Ixodes* ticks – a genus that is notorious for transmitting the bacteria that cause Lyme borreliosis and for its global impact on human and animal health. The initiative set forth an ambitious target - to sequence, assemble and annotate the genomes of 1000 individual *Ixodes* – and reflects the consensus position of researchers in the tick and tick-borne disease research fields. The vision of the *Ixodes* 1000 Genomes project (Ix1000G) identifies two priority areas for research investment. The primary goal is to generate high quality reference assemblies for six “node” species considered the most serious tick pests in North America, Europe, Africa, Asia and Australia – *Ixodes scapularis*, *Ixodes pacificus*, *Ixodes ricinus*, *Ixodes rubicundus*, *Ixodes persulcatus* and *Ixodes holocyclus*. A secondary goal is to investigate genetic diversity among natural populations comprising hundreds of *Ixodes* ticks collected from sites around the globe. The international collaboration will radically advance tick genome research and position scientists to harness data-driven strategies to prevent the transmission of tick-borne diseases.

13. PHYSICAL MAP VALIDATES HIGH-QUALITY OF THE *Aedes aegypti* L5 GENOME ASSEMBLY

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Aedes aegypti is the principal vector of dengue, Chikungunya and Zika viruses. High-coverage genome maps are important tools in identifying chromosomal positions of epidemiologically important traits and population genetics studies. Among mosquitoes, high-coverage genome maps were previously developed for the African malaria vector *An. gambiae* and Neotropical malaria vector *An. albimanus*, that cover 84.5% and 98.2% of these genomes, respectively. For *Ae. aegypti* a map integrating the linkage, chromosome and genome maps, which included 100 genetic markers, and a physical map, which placed 45% of the original draft genome to mitotic chromosomes, were also developed. The recent Hi-C-based scaffolding approach successfully assigned 93.6% of the genome assembly to three chromosomes thus creating the L4 assembly of *Ae. aegypti*. However, the genomic coordinates of L4 assembly lack correspondence with chromosomal bands and information about the male sex-determining M locus. To develop a fine-scale physical genome map anchoring chromosome-length genomic scaffolds to their chromosomal positions for *Ae. aegypti*, we compared the assembly coordinates of ~550 previously and newly mapped BAC clones from the NDL library with their positions in the chromosomes using multicolor fluorescence in-situ hybridization in the recent PacBio sequencing based L5 assembly containing data from both sexes. The genome coverage of this map equals 93.4%, representing the second highest genome coverage among mosquitoes. We used this map to identify mis-assemblies and validate the position of male sex locus M in RU3 strain of *Ae. aegypti*. In addition, two large deletions spanning ~2 and ~6 MB on chromosome 1q identified by HI-C were also validated using the map. The physical map that we developed here will provide genomic coordinates for the physical mapping of genes of epidemiological importance, genomic regions, transgenes, and used to study structural genome variations. These efforts will help to identify, target and manipulate epidemiologically important traits and to develop potential new strategies for the vector control in *Aedes* mosquitoes.

14. GENOMIC DIVISION OF LABOR DURING COLONY DEFENSE IN A GENTLE AFRICANIZED HONEY BEE

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The evolution of behavior results from complex interactions between genetic variation and selective forces that often must occur within ecological time scales. A system that highlights the complexity of behavior is aggressive colonial defense by social organisms such as honey bees. Honey bee colony defense is dependent on coordination between individuals and as a trait with high fitness cost, is expected to be subject to strong selection pressure. In past work, we used the remarkable, rapid reduction in aggressive behavior observed in gentle Africanized honey bees (gAHB) in Puerto Rico to explore associated signals of selection. Our results highlighted that an understudied evolutionary mechanism, the soft selective sweep, drove the rapid change in gAHB behavior. This rapid change was facilitated by high recombination rate, outcrossing, and high degree of standing variation. Furthermore, we identified 128 genomic regions implicated in this rapid evolutionary response. In the present study we continue to capitalize on this well-characterized system to directly associate genomic variation with variation in aggression. We measured the aggressive response at the individual and colony level for 200 gAHB and also sequenced the genomes of these 200 individuals. Using behavioral phenotypes and genomic information for this population we elucidate how selection has affected the landscape of genetic variation while simultaneously arriving at a set of genes correlated with colony aggressive defense. This information enables us to determine to what degree genetic components implicated in the evolution of aggression have been retained in the population, and further explore their distribution within the social structure of individual colonies.

15. THE ORGANIZATION AND PROTECTION OF INSECT PROCUTICLE IS ORCHESTRATED BY CHITIN DEACETYLASE (TCCDA)-LIKE PROTEINS

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Molting, the replacement of the old cuticle with a new one, is a complex developmental process that all insects must undergo to allow growth and development. During molting, chitinases degrade the old exoskeleton and recycle some of the resulting products for new cuticle synthesis. Intriguingly, chitinases are found not only in those portions of the old cuticle targeted for degradation and recycling, but also in the newly developing procuticle. We recently demonstrated that *Tribolium castaneum* *knickkopf* (TcKnk) encodes a chitin binding protein that selectively co-localizes to the new cuticle and protects chitin from the activity of molting fluid chitinases (TcCht-5). However, factors that aid the proper localization of TcKnk to the developing procuticle have not been systematically investigated. Here we demonstrate that *Tribolium castaneum* chitin deacetylase (TcCda)-like proteins play a crucial role towards the proper localization of TcKnk to the procuticle. Down-regulation of TcCda-like genes resulted in mislocalization of TcKnk within the assembly zone of the newly synthesized procuticle and caused molting defects at all stages of development due to reduction in chitin levels, consistent with the loss of Knk function. Due to conservation of Cda-like proteins in arthropods, it is likely that these proteins may have a fundamental role in mediating the proper localization of multiple procuticular proteins.

16. INVESTIGATING GENOME COMPOSITIONAL FEATURES OF *APIS* AND OTHER HYMENOPTERAN SPECIES

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Initial analysis of the honey bee (*Apis mellifera*) genome in 2006 revealed several interesting features compared to other metazoan genome sequences available at that time: a low but heterogeneous GC content, an overabundance of CpG dinucleotides and a lack of repetitive elements. The average GC content of the honey bee genome is only 33%, but GC content is highly heterogeneous, ranging from 11% to 67%, with a bimodal distribution. Furthermore, unlike genes in most other metazoans, honey bee genes are overly abundant in regions of low GC content (<30%). It is unclear whether any of these genome features are related to the evolution of eusociality and we lack satisfactory explanations for them more generally. Since publication of the *A. mellifera* genome, genomes of several other hymenopteran insects, including additional *Apis* species, have become available. In this study, we compare *Apis* genome compositional characteristics with those of other hymenopteran insects. Comparing genome composition and organization among species with different levels of social complexity may lead to insight into genomic structural changes associated with the evolution of eusociality. We used a recursive segmentation procedure to partition genomic sequences into GC compositional domains, maximizing the difference in GC content between adjacent subsequences. We compared the distributions of GC contents in GC compositional domains among 21 hymenopteran genomes ranging in social complexity from solitary to complex eusocial. We also analyzed one eusocial and four solitary outgroups representing diverse insect taxa. Bimodal distribution of GC content within the GC compositional domains was a characteristic of the complex eusocial bees (*Apis* and *Melipona*), but not solitary or simple eusocial bees. The *Apis* genomes had larger ranges in GC content compared to the other species. Genes were biased to lower GC content regions in all bees, with the strongest bias in the complex eusocial bees, and the weakest bias in the solitary bees, while gene distribution tended to show little or no bias to low GC content regions in the ant genomes and non-hymenopteran insect outgroups. Further investigation of these preliminary data will provide insight into whether genomic compositional features unique to *Apis* are associated with the evolution of eusociality.

17. GENE EXPRESSION RESPONSES TO DIET QUALITY AND VIRAL INFECTION IN *APIS MELLIFERA*

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Commercially managed honeybees have undergone unusually large declines in the United States and parts of Europe over the past decade. As honeybees are the leading pollinator of numerous crops, their marked loss has considerable implications regarding agricultural sustainability. Honeybee declines have been associated with several stressors that researchers generally agree influence the large-scale loss of honeybees in interactive fashions as the environment changes. Nutrition and viral infection are two broad factors that pose heightened dangers to honeybee health in response to recent environmental changes. In particular, honeybees are confronted with less diverse selections of pollen due to the urbanization and monoculture crop production, and the Israeli Acute Paralysis Virus (IAPV) has demonstrated higher infectious capacities than other honeybee viruses in certain conditions. In this study, we aimed to use transcriptomics to better understand how high quality diets protect bees from virus-induced mortality, a finding that has been documented in previous studies. Specifically, we used RNA-sequencing to examine how monofloral diets and IAPV inoculation influence gene expression patterns in bees. We found a major nutritional transcriptomic response, with nearly 2,000 transcripts changing in response to diet quality. The majority of these genes were enriched for functions like nutrient signaling (insulin resistance), metabolism, and immune response (Notch signaling and JaK-STAT pathways). Somewhat surprisingly, the transcriptomic response to virus infection in our experiment was fairly limited. We only found 43 transcripts to be differentially expressed, some with known immune functions, such as argonaute-2, as well as additional genes related to transcriptional regulation and muscle contraction. Additionally, we created contrasts to determine if the protective effect of good diet was due to direct effects on immune function ("resistance"), or if it was due to indirect effects on energy availability and vigor ("resilience"). We found an approximately equal number of resistance ($n = 125$) and resilience ($n = 122$) related candidate genes, suggesting both processes may play significant roles in dietary buffering from pathogen infection.

We also compared the main effect of IAPV exposure in our dataset to that obtained in a previous study that used bees from single-drone colonies to control for genetic variability and found significant overlap in the lists of differentially expressed genes. As RNA-seq data can be highly noisy, this comparison allowed us to characterize the repeatability and robustness of our results. Moreover, we used an in-depth data visualization approach to explore and validate our data and suggest such an approach can be useful for cross-study comparisons of RNA-sequencing data in the future.

18. HOW TO PERFORM META-ANALYSIS USING HYMENOPTERAMINE

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HymenopteraMine (<http://HymenopteraGenome.org/hymenopteramine>) is a data mining resources for the Hymenoptera Genome Database (HGD) which maintains genomic data for 12 bee species, 11 ant species and 1 wasp. HymenopteraMine allows users to integrate custom genomic datasets and perform cross-species analyses. It relies on InterMine platform to integrate genome assemblies and gene sets with data from a number of repositories including NCBI, RefSeq, Ensembl, Gene Ontology, UniProt, InterPro, OrthoDB, KEGG, dbSNP, Reactome, BioGRID, Pubmed, and precomputed gene expression profiles based on publicly available RNAseq datasets. HymenopteraMine provides simple and sophisticated search tools, including a “Quick Search”, built-in “template queries”, and a “QueryBuilder” tool for creating custom queries. Query results can be exported in various formats (tab delimited, GFF3, Fasta, BED, JSON, and XML) to use for additional analyses.

The availability of high-throughput genomic technologies has accelerated the generation of massive quantities of genomic datasets. Researchers working on the hymenopterans very often wish to perform comparative analysis with their own datasets to the publicly available datasets. With our data mining tools, HymenopteraMine provides a unique opportunity to the researchers without scripting skills to perform meta-analysis by integrating their own datasets. Specially, the List tool in HymenopteraMine allows users to upload identifiers to create custom lists, perform set of operations such as unions and intersections, and execute template queries with lists. So, users can easily compare their results with published results by uploading genomic coordinates or IDs. HymenopteraMine is very useful for tracking gene identifiers across gene sets to facilitate meta-analysis. Here, we utilize publicly available genomic datasets to demonstrate meta-analysis using HymenopteraMine.

19. INTEGRATING DIVERSE DATASETS USING THE DATA MINING WAREHOUSE, HYMENOPTERAMINE

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With the vast amounts of sequence data being generated, there is a growing number of public genomic databases available allowing for large-scale comparative studies. HymenopteraMine is a data mining resource for hymenopteran insects, accessible through The Hymenoptera Genome Database (HGD; <http://hymenopteragenome.org>). HymenopteraMine houses genomic data for 24 species from multiple external sources within a user-friendly environment where researchers can perform complex analyses without any prior programming skills. Having a central data warehouse for hymenopteran genomic resources can expedite cross-species analyses for orthology and facilitate mining across gene sets from databases such as NCBI, RefSeq, Ensembl, UniProt, OrthoDB, Reactome, Pubmed, Gene Ontology and dbSNP that are all incorporated into HymenopteraMine. There is also gene expression information available that has been computed from RNASeq datasets and search tools that generate queries based upon genome coordinates.

One particularly powerful feature of Hymenopteramine is the ability to create integrated datasets. Users can implement simple searches with pre-defined query templates or conduct more elaborate inquiries with our “QueryBuilder” tool. The “QueryBuilder” interface allows researchers to generate queries that include endless combinations of ways to integrate any data type, creating more specialized datasets. Users can merge custom query results with their own data or upload a list of identifiers to gather associated functional annotations. Query output is in a tabular format that can be exported, saved or shared with others to be used in further downstream analyses. Here, we use the honey bee, *Apis mellifera*, to demonstrate the utility of Hymenopteramine and the different ways to integrate the many data types available.

20. LOOKING FOR VECTOR COMPETENCE GENES: TRANSCRIPTOME COMPARISON OF VECTOR AND NON-VECTOR DELTOCEPHALINE LEAFHOPPERS

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The leafhopper subfamily Deltocephalinae (Hemiptera: Cicadellidae) is the largest and most diverse subfamily of Cicadellidae. Its members can be found worldwide, some of them are cosmopolitan while others are locally distributed. Most of the leafhopper species that are vectors of economically important pathogens are located inside this subfamily. Several leafhoppers are competent vectors of either virus or mollicutes. Vector competence can be defined as the efficiency of a vector to transmit a pathogen over time or per transmission opportunity. It is thought that the ability to effectively acquire and transmit a pathogen may be genetically regulated in the insect vector; hence, the comparison of vector and non-vector genomes and transcriptomes can produce some information about the proteins or processes involved in vector competence. In order to find genes related with vector competence, the transcriptomes of four vector leafhoppers: *Dalbulus maidis*, *Exitianus exitiosus*, *Graminella nigrifrons* and *Macrostelus quadrilineatus*, and two non-vector leafhoppers: *Balclutha neglecta* and *Balclutha rubrostriata* were obtained through next generation sequencing (NGS) on an Illumina HiSeq X platform. The transcriptomes were assembled using the Trinity *de novo* transcriptome platform and the peptide sequences were obtained with TransDecoder. The sequences were clustered and compared using CD-Hit using different similarity thresholds (70%, 80% and 90%). With a similarity threshold of 90%, the difference in transcripts between the two non-vectors was the lowest: 72996 (77%) transcripts that were present in *B. rubrostriata* and were not present in *B. neglecta*. The biggest difference was among *E. exitiosus* and *B. rubrostriata*, with 95% of the transcripts of *E. exitiosus* absent in *B. rubrostriata*. Between the other species these differences were about 90%. Even with a similarity threshold of 70%, the percentage of transcripts that were present in one sequence and absent in the others was an average of 77%. The differences in the transcripts between the six species may be due the diversity that exists among the members of the subfamily Deltocephalinae. Even more, the species used in this study belong to different tribes and have different feeding patterns, *M. quadrilineatus* is a polyphagous leafhopper, whereas *D. maidis* is monophagous, so a difference in their transcriptomes was expected.

21. WHOLE-CHROMOSOME ASSEMBLY AND ANALYSIS OF HYBRIDOGNETIC LINEAGES OF THE DESERT ANT *CATAGLYPHIS HISPANICA*

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Assembling complete chromosomes of large genomes is a technical challenge presenting a number of long-standing issues, such as the bridging of gaps that can be found in draft genomes or the presence of repeated sequences that conventional assemblers have trouble resolving. As such, many large arthropod genomes are in an unfinished state, comprising many more scaffolds than the expected number of chromosomes. Here, we present sparseGRAAL, a fast, open-source program that uses chromosome conformation capture (Hi-C) data to scaffold contigs based on the collision frequencies between DNA sequences in the nucleus. SparseGRAAL builds upon and improves our formerly published program GRAAL, which uses a simple polymer model to represent the expected spatial contacts between these sequences and an MCMC method to maximize the likelihood of this model (Marie-Nelly et al., 2014). When applied to the genomes of two hybridogenetic lineages of the desert ant *Cataglyphis hispanica*, sparseGRAAL yielded completely assembled chromosomes and revealed large-scale structural differences that may account for their unusual reproductive strategies.

22. GENOMIC COMPARISON OF TWO *BALCLUTHA* LEAFHOPPERS (HEMIPTERA: CICADELLIDAE)

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The order Hemiptera contains several taxa that are known to contain identified vectors of plant pathogens that have significant impacts upon the agricultural economy. One taxon, the leafhoppers (Hemiptera: Cicadellidae), is very common in many different environments around the world. In this study we focused on two species of the genus *Balclutha*. *Balclutha neglecta* and *Balclutha rubrostriata* were selected and expected to be very genetically similar to each other. *B. neglecta* is not a known vector of any plant pathogens, while *B. rubrostriata* is a suspected vector of the mollicute *Candidatus Phytoplasma oryzae*, which causes white leaf disease in sugar cane, but its vector status has yet to be confirmed. The genomes and transcripts were sequenced on the next generation Illumina HiSeq X platform to obtain short read data. The genomes were then assembled using Meraculous assembler, and the transcripts were then assembled using Trinity *de novo* assembler. Peptide sequences were obtained from TransDecoder. The peptide sequences were then compared with CD-hit with 70%, 80%, and 95% similarity. The peptides were then demultiplexed by running them with a similarity of 98% against themselves to remove repeat sequences. The two transcriptomes were then compared again using 70%, 80%, and 95% similarity.

23. FINDING WINGS IN A NON-WINGED ARTHROPOD: INSIGHTS FROM CRISPR/CAS9-BASED GENOME EDITING IN A CRUSTACEAN

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The origin of insect wings is a biological mystery that has fascinated scientists for centuries. Through extensive investigations performed across various fields, two possible wing origin tissues have been identified; a lateral outgrowth of the dorsal body wall (tergum) and ancestral proximal leg structures. With each idea offering both strengths and weaknesses, these two schools of thought have been in an intellectual battle for decades without reaching a consensus. Identification of tissues homologous to insect wings from lineages outside of Insecta will provide pivotal information to resolve this conundrum. Here, through expression analyses and CRISPR/Cas9-based genome-editing in the crustacean, *Parhyale hawaiiensis*, we show that a wing-like gene regulatory network (GRN) operates both in the crustacean terga and in the proximal leg segments, suggesting that (i) the evolution of a wing-like GRN precedes the emergence of insect wings, and (ii) that both of these tissues are equally likely to be crustacean wing homologs. Interestingly, the presence of two sets of wing homologs parallels previous findings in some wingless segments of insects, where wing serial homologs are maintained as two separate tissues. This similarity provides crucial support for the idea that the wingless segments of insects indeed reflect an ancestral state for the tissues that gave rise to the insect wing, while the true insect wing represents a derived state that depends upon the contribution of two distinct tissues. These outcomes point toward a dual origin of insect wings, and thus provide a crucial opportunity to unify the two historically competing hypotheses on the origin of this evolutionarily monumental structure. Future comparative genomic and genetic analyses will provide further insight into the molecular basis underlying the evolution of insect wings.

24. POSITIVE SELECTION AMONG CHEMOSENSORY AND CYTOCHROME P450 GENES WITHIN THE AGRICULTURAL PEST THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*

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The Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, Say, is a significant agricultural pest on cultivated Solanum crops such as potato, tomato, and pepper (Grapputo et al., 2005). The *Leptinotarsa* genus comprises 41 known species distributed from North to South America, and while these species generally host on Solanaceous plants, only CPB is considered a significant pest. Host plant detection and insecticide resistance are associated with CPB's expansion to potato and status as a pest. Using the CODEML branch-site test of the PAML package, we identify genes in chemosensory and insecticide resistant families that show evidence of positive selection in the CPB lineage with respect to nine non-pest *Leptinotarsa* species.

25. VISUALIZING THE CONTRIBUTION OF TWO DISTINCT TISSUES TO THE FORMATION OF WINGS IN THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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Insect wings are often presented as a textbook example of evolutionary novelty; however, the origin of the insect wing remains a hotly debated mystery. Two contrasting hypotheses propose that the insect wing either originated from dorsal body wall (tergum) or ancestral proximal leg segments (corresponding to the pleural plates in extant insects). Through loss-of-function analysis of an important wing gene *vestigial* (*vg*), our lab has previously obtained data supporting a third hypothesis: the dual-origin hypothesis. This hypothesis states that both tergal and pleural tissues have contributed to the evolution of the insect wing. However, it is currently unknown to what degree each of the two tissues contributes to the formation of the wing. We reasoned that isolation of the enhancers responsible for the expression of *vg* in these tissues in *Tribolium* may provide a means to visualize each tissue separately, and monitor their behavior during wing development. Utilizing the previously obtained genome-wide tissue- and stage-specific chromatin profiles (FAIRE-sequencing), we identified nine possible enhancer regions from the *Tribolium* *vg* locus. We are currently evaluating the activity of these potential *Tribolium* enhancer regions in *Drosophila*, taking advantage of the sophisticated molecular and genetic tools available in this model system. Reporter constructs for the *Tribolium* genomic regions that show enhancer activity in the *Drosophila* wing will then be inserted into *Tribolium* for further evaluation. Visualizing the behavior of these tissues during wing development in *Tribolium* through this approach will further our understanding of the evolutionary contribution of the two distinct tissues to the insect wing.

26. PATTERN AND MECHANISM OF SEX CHROMOSOME DOSAGE COMPENSATION IN THE LEPIDOPTERA

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Evolution of heterogametic sex chromosomes creates a dosage problem for sex-linked expression, which different animals cope differently. WZ/ZZ taxa (such as birds and snakes) leaves their expression of Z-linked loci largely unbalanced between sexes, with a sole exception: the Lepidoptera that includes moths and butterflies. We have previously confirmed that the lepidopteran insects are the only WZ/ZZ species known thus far to equalize sex-linkage expression between sexes, which is a pattern that was previously believed to be exclusive to XX/XY taxa. Despite its importance in understanding the evolution of sex chromosome and dosage compensation, the mechanisms of dosage compensation in the Lepidoptera, however, have not yet been previously studied. To shed light on this, we used the monarch butterfly (*Danaus plexippus*) as a model and did analyses integrating transcriptome and epigenetics. We generated RNAseq and ChIPseq data and contrasted both gene expression and histone modification profiles between male and female, as well as between Z and autosomes.

27. COMBINING TRANSCRIPTOMICS AND PROTEOMICS REVEALS A MAJOR CONTRIBUTION OF ACCESSORY GLANDS TO THE SPERM PROTEOME IN LEPIDOPTERA

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Intuiting from the Central Dogma of biology, there should be a strong correlation between levels of mRNA expression and levels of protein abundance. However, unsuccessful attempts to correlate expression with abundance point to potential contributions of other factors to this relationship. Here we explore these factors, analyzing roles of tissue specificity and tissue of origin in relation to sperm protein abundance in two species of Lepidoptera: the monarch butterfly (*Danaus plexippus*) and the Carolina sphinx moth (*Manduca sexta*). We find that taking tissue specificity into account when correlating expression and abundance greatly increased the capacity of expression to predict abundance. However, a signature of genes with low expression in the testes that still had high abundance in the sperm proteome indicated that, for genes with low testes expression, there was likely an alternate tissue of origin for these sperm proteome genes. Heatmaps of expression revealed that, where testes gene expression was low, these sperm proteome genes have high gene expression in the accessory gland in *D. plexippus* and high expression in the malphigian tubules and fat body in *M. sexta*.

28. SEX-SPECIFIC RNA-SEQ ANALYSES OF ANTARCTIC MITE, *ALASKOZETES ANTARCTICUS*

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The Oribatid mite, *Alaskozetes antarcticus*, is one of the most abundant terrestrial invertebrates in maritime Antarctica. This mite can survive extreme temperature fluctuations, desiccation, and thrives even with a short growth season (1,2). There have been many studies examining ecology and physiology, but little is known about reproduction in this mite. In this study, we probe at molecular mechanisms underlying reproduction in *A. antarcticus*, utilizing sex- and development-specific RNA-seq analyses to identify differentially regulated transcripts. Pairwise comparisons between males, females, and tritonymphs revealed more than 4000 enriched transcripts when combined. More than 500 of these enriched transcripts were differentially upregulated by over 1000-fold within a comparison. Gene ontology-based analyses linked this transcriptional regulation to differences in reproduction, chemosensation, and stress response. Many of the highly enriched and sex-specific transcripts were previously uncharacterized with no known homolog, suggesting that many facets of *A. antarcticus* biology, and that of mites in general, have yet to be determined. Lastly, we compared the enriched sex-specific transcripts to those of other mites to develop putative gene sets. Our comparative approach allowed us to determine the sexually dimorphic expression of transcripts in *A. antarcticus* which we extended to examine that of other mites.

29. GENETICS AND GENOMICS OF HOST SPECIFICITY IN APHID PARASITOIDS

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Differences in parasitism success among potential host species can provide strong selection for divergence and speciation in parasitic Hymenoptera. Here we report research on the genomics and genetics of host specificity in *Aphelinus* species. We have sequenced, assembled, and annotated the genomes and transcriptomes of >10 *Aphelinus* species. Using coding sequences, we developed a robust phylogeny, onto which we mapped parasitism of diverse species of aphids. For some aphid species, parasitism was phylogenetically conserved, with closely related parasitoids showing similar levels of parasitism. For other aphid species, parasitism diverged between closely related parasitoids, consistent with host-driven speciation. To explore the genetic architecture of differences in host specificity, we crossed and backcrossed *A. atroplicis*, which readily parasitizes *Diuraphis noxia*, with *A. certus*, which rarely parasitizes this aphid. Using genetic markers from reduced-representation genomic libraries, we mapped quantitative trait loci (QTL) affecting parasitism of *D. noxia*. We found eight QTL (six of which interacted in their effects) that explained 39% of the variation in parasitism *D. noxia* among backcross females. To help identify candidate genes, we compared the genomes and transcriptomes of these parasitoid species to find proteins that diverged in sequence or expression, and we tested whether these divergent loci mapped to QTL affecting parasitism of *D. noxia*. So far, we have found 15 divergent genes that mapped to parasitism QTL or significantly affected parasitism by themselves. Using RNA probes, we have shown expression of candidate gene g6935 in both ovipositor and antenna sensilla. These are among the first results on the genetic architecture of host specificity in parasitic wasps.

30. TESTING FOR CONSERVED SEQUENCES ON THE LEPIDOPTERAN W SEX CHROMOSOME

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The female-determining W sex chromosome in the order Lepidoptera (moths and butterflies) lacks quality assemblies in most species due to difficulties with transposable elements and highly repetitive sequences, which are abundant on this chromosome. Likely candidates for W-linked genes or transposable elements in the sphingid moth *Manduca sexta* (tobacco hornworm) were identified bioinformatically through analysis of RNA-seq in male and female tissue samples. Primers for PCR validation were designed from these candidate W-linked transcripts. These have also been used to explore how conserved W-linked sequences are at the genus, family, and order levels. A methodology utilizing chloroform DNA extractions followed by a generic PCR protocol has shown most observed sequences to be restricted to *Manduca sexta* and closely related species within the genus. However, a few have shown amplification beyond the *Manduca* genus and one beyond Bombycoidea. Sampled species include *Manduca sexta*, *Manduca quinquemaculata*, *Manduca rustica*, *Hyles lineata*, *Bombyx mori*, and *Danaus plexippus*, with further specimen collections planned. This research looks to expand knowledge of the past evolution and current state of the Lepidoptera W chromosome. With further work in this area it is possible that a PCR-based test of genetic sex could be developed for at least a portion of W-bearing Lepidopterans.

31. EXPLORING THE ROLE OF DNA METHYLATION IN MECHANISMS OF NON-ASSOCIATIVE LEARNING IN THE TOBACCO HORNWORM, *MANDUCA SEXTA*

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Organisms respond to threatening stimuli with defensive behaviors and maintain heightened behavioral responses, a form of non-associative learning known as nociceptive sensitization. While this behavioral plasticity has been observed across the animal kingdom, it is unclear how conserved the molecular mechanisms underlying this response are. DNA methylation (DNAm) is a molecular mechanism that has been shown in different species to regulate memory formation and learning, including nociceptive sensitization in some cases. In insects, this phenomenon has only been examined in eusocial species that possess detectable orthologs of *de novo* DNA methyltransferase (DNMT3) in their genomes. DNMT3 is one of three known classes of enzymes that catalyze the transfer of methyl groups to DNA. The loss of specific DNMTs along insect lineages is associated with the loss of DNAm entirely in some species, but not in others, suggesting the existence of compensative roles of DNMTs and/or alternative molecular mechanisms for methylation. In this study, we hypothesize that DNAm is a critical regulator of nociceptive sensitization in the tobacco hornworm, *Manduca sexta*, which, in contrast to other insect species studied thus far, is a solitary species that lacks DNMT3. We have identified putative DNAm genes in the available *Manduca* genome, and use our established up-down protocol to determine changes in the threshold force to elicit a defensive striking response before and after a strong pinch, which acts as a threatening stimulus in this bioassay. Preliminary results indicate that coupling the pinch with an injection of RG108, a DNMT inhibitor, counters or blocks the decrease in nociceptive threshold typically seen in sensitized animals. Thus, inhibiting DNAm appears to have a direct effect on behavioral sensitivity to a harmful stimulus, demonstrating that DNAm may regulate nociceptive sensitization in *Manduca*. To corroborate these results on a molecular level, we will construct methylomes of control and sensitized animals, and perform bioinformatic analyses to quantify global changes in methylation and identify differentially methylated genes between these two groups. As there already exist methylomes of other insect species, this study will open doors for comparative methylomic analyses across different factors, including eusociality and presence of specific DNMTs, which will be valuable to gaining a more comprehensive understanding of the evolution and function of DNAm in insects.

32. PROTEO-GENOMIC ANALYSIS OF BUTTERFLY SPERM USING SHOTGUN MASS-SPECTRA DATA

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This research uses bioinformatic analysis of mass-spectra proteomic data to characterize the molecular composition of dimorphic sperm in Lepidoptera. Males of the Lepidoptera (moths and butterflies) produce two types of sperm. This dimorphic sperm is either nucleated or anucleated, respectively known as eupyrene or apyrene sperm. Apyrene sperm are unique because, along with having no nucleus, they also have no nuclear DNA, which seems counterproductive for it to be a reproductive cell. Previous studies have examined the protein composition of the sperm based on the official gene set of the Monarch butterfly (*Danaus plexippus*). However, these studies did not explore the potential for sperm proteins originating from currently unannotated genomic regions, which is a primary aim of our current research. To do this, a six-frame translation is performed on the entire genome of *Danaus plexippus*. Previously obtained mass spectra data are searched against all six-frame translations, and then cross referenced against the official gene set. This allows for the identification of previously unannotated peptides that are present in sperm. Identifying the function of the unique proteins found in apyrene sperm should illuminate the overall function of apyrene sperm.

33. CONSERVATION OF PAIN: A COMPARATIVE GENOMIC ANALYSIS OF *APLYSIA CALIFORNICA* SIPHON WITHDRAWAL AND MAMMALIAN CENTRAL SENSITIZATION TO LONG TERM SENSITIZATION IN *MANDUCA SEXTA*

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Chronic pain is a prevailing health problem ravaging the United States, where a predominance of research in this field is limited to vertebrate models. With the extensive ethical and financial limitations of vertebrate research, the development of an invertebrate model for preliminary therapeutic research would greatly broaden our understanding and treatment of chronic pain. The insect model, *Manduca sexta*, has a well-studied behavioral defensive strike to harmful stimuli that can be sensitized long-term. We have previously shown that maintenance of this mechanism is protein synthesis-dependent, like many pain models. With this model, we have the unique opportunity to study the conservation of nociceptive sensitization. Determining similarities between well-established invertebrate and vertebrate models with that of *M. sexta* will strengthen the development of the tobacco hornworm as a model to study chronic pain. In this project, the signal pathway for nociceptive sensitization in *M. sexta* was predicted using comparative genomics to determine similar *M. sexta* genes to those involved in the siphon-withdrawal of *A. californica* and central sensitization in mammals. This is in preparation to focus our analysis after RNA-sequencing of the central nervous system of sensitization *M. sexta*.

34. DETAILED ANALYSIS OF THE PROTHORACIC TISSUES TRANSFORMING INTO WINGS IN THE *CEPHALOTHORAX* MUTANTS OF *TRIBOLIUM*

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Despite the immense importance of the wing in the evolution and successful radiation of the insect lineages, the origin of this critical structure remains a hotly-debated mystery. Two possible tissues have been identified as an evolutionary origin of wings; the lateral expansion of the dorsal body wall (tergal edge) and structures related to an ancestral proximal leg segment (pleural tissues). Through studying wing-related tissues in the red flour beetle, *Tribolium castaneum*, we have previously presented evidence in support of a dual origin of insect wings, a third hypothesis proposing that wings evolved from a combination of both tergal and pleural tissues. One key finding came from the investigation of a *Cephalothorax* (Cx) mutant, in which the ectopic wing characteristic to this mutant was found to be formed from both tergal and pleural contributions. However, the degree of contribution of the two tissues to the wing remains elusive. Here, we took advantage of multiple Cx alleles available in *Tribolium*, and produced a variety of degrees and types of ectopic wing tissues in their prothoracic segments. Through detailed phenotypic scoring of the Cx phenotypes based on nine categories of mutant traits, along with comprehensive morphological analysis of the ectopic wing tissues, we found that (i) ectopic wing tissues can be formed at various locations in the prothorax, even internally, (ii) the lateral external ectopic wing tissues have tergal origin, while the internal and posterior external ectopic wing tissues appear to be of pleural origin, and (iii) the ectopic wing tissues of both tergal and pleural origin are capable of transforming into wing surface tissues. Collectively, these outcomes suggest that the evolutionary contribution of each tissue to a complete wing may be more complex than the simple binary view that is typically invoked by a dual origin model (i.e. the wing blade from the tergal contribution + musculature and articulation from the pleural contribution).

35. EXPLORING INSECT WING ORIGIN THROUGH *CIS* ANALYSIS OF *VESTIGIAL* IN *DROSOPHILA*

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Insect wings represent a classic example of morphological novelty, but their origin remains a chief conundrum in biology. Throughout the history of the insect wing origin debate, popular opinion has shifted between two contrasting hypotheses, the tergal-origin hypothesis and pleural-origin hypothesis, without reaching any consensus. Through the study of *vestigial* (*vg*) in the *Tribolium* beetle, we have previously obtained functional evidence supporting a “dual origin” of insect wings, which potentially combines the two hypotheses. *vg* is an important gene to trace the developmental and evolutionary history of wing structures. We reasoned that comprehensive *cis*-analysis for *vg* will provide novel insights into the origin of insect wings. In *Drosophila*, *vg* is expressed in several tissues, including wings and muscles. Our analysis revealed intricate *cis*-regulatory mechanisms operating at the *vg* locus. Intriguingly, one of the wing enhancers was also active in the larval tergum (i.e. these two tissues share a similar transcriptional regulatory landscape), suggesting an evolutionary connection between the two tissues (thus supporting either tergal or dual origin). Unfortunately, we could not evaluate the pleural-origin hypothesis through *cis*-analysis due to the derived dipteran body plan. Nonetheless, our study provides a framework to investigate the insect wing origin from a *cis*-perspective. Additionally, studying the *vg cis*-regulation in other insects, especially in those that have clear *vg*-dependent pleural tissues (such as *Tribolium*), will be fruitful to gain further insights into how *vg*-dependent tissues have contributed to the evolution of insect wings.

36. ANOPHELES GENOME ASSEMBLY IMPROVEMENTS INFORMED BY EVOLUTION

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Technological advances in whole genome sequencing have led to a steady increase in the public availability of genomic data from an ever-growing number and diversity of organisms. However, despite their tremendous impact in terms of reducing sequencing costs, second and third-generation sequencing technologies still present major challenges when aiming for chromosomal-level genome assemblies, all too often resulting in fragmented draft assemblies. Results from the *Anopheles* 16 genome project and other initiatives present the opportunity to assess the performance of three separate gene synteny-based methods to predict scaffold neighbours in 21 *Anopheles* mosquito assemblies to produce consensus sets of scaffold adjacency predictions. The improved assemblies generally show a 10% reduction in the total number of scaffolds for a 40% increase in the scaffold N50 length, with almost 43'000 additional predicted adjacencies. These results demonstrate that substantial improvements to annotated assemblies are possible with comparative genomics approaches only requiring gene orthology data across a set of closely related species. Without the associated costs required for experimental finishing or re-sequencing efforts, these approaches represent a handy new set of utensils in the genome assembly pipeline and highlight the utility of computational comparative genomics strategies.

37. HIGH QUALITY DRAFT GENOME ASSEMBLIES OF PESTS OF PRE- AND POST-HARVEST PESTS OF CEREAL CROPS FROM 10X CHROMIUM LIBRARIES

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The expansion of genomic resources for invasive and emerging insect pests has largely been hampered by cost, time required for inbreeding, and technical issues that can arise during genome assembly from pooling multiple individuals together for DNA isolation and library preparation. However, newer library methods, such as 10X Chromium libraries, largely overcome these issues in that sufficient DNA can be recovered from a single individual for library prep and allelic variants are assembled as separate phase blocks, eliminating the need for inbreeding. Using 10X Chromium libraries coupled with 150 x 150 bp HiSeqX sequencing to a depth of at least 60X coverage, we were able to develop high quality draft genome assemblies for eight different stored product insect species, including Dermestidae (*Trogoderma variabile*, *Trogoderma granarium*, and *Dermestes maculatus*), Tenebrionidae (*Tribolium confusum*), Anobiidae (*Lasioderma serricorne* and *Stegobium paniceum*), Bostrichidae (*Prostephanus truncatus*), and Pyralidae (*Plodia interpunctella*) and three aphid pests of bioenergy grasses, including *Melanaphis sacchari* (sugarcane aphid), *Sipha flava* (yellow sugarcane aphid), and *Schizaphis graminum* (greenbug aphid). Overall, BUSCO (Benchmarking Using Single Copy Orthologs) scores exceeded 95% in all assemblies with few fragmented or duplicated genes, suggesting a high quality assembly of the gene space. Further, scaffold N50s exceeded 1 Mb and high quality assemblies of symbiont genomes were recovered in some cases. Overall, this approach produced high quality assemblies for eight different insects and could be used to quickly and efficiently generate draft assemblies of invasive or emerging pests.

38. COMPARATIVE ANALYSIS OF SIX GRASSLAND LEAFHOPPER GENOMES

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Leafhoppers (Hemiptera: Cicadellidae) are some of the most numerous insects found in prairie and agricultural ecosystems. As such, they are important indicators of ecosystem health. Highly variable in habitat use, this family contains members that specialize in feeding from phloem, xylem, and/or mesophyll of host plants. This family also varies in host range specialization from monophagous to polyphagous. One subfamily of the Cicadellidae, the Deltocephalinae, contains many leafhopper genera that are grass specialists. These leafhoppers are also primarily phloem feeders. This group also contains the majority of identified leafhopper vectors of plant-infecting viruses. To better understand the underlying mechanisms of feeding habits and subsequent ability to transmit plant pathogens, we studied the genomes and transcriptomes of several Deltocephaline leafhoppers. Here we report the genome and transcriptome assemblies of six leafhopper species, *Exitianus exitiosus*, *Dalbulus maidis*, *Macrostelus quadrilineatus*, *Balclutha neglecta*, *Balclutha rubrostriata*, and *Graminella nigrifrons*. Five of the six leafhopper genomes are short reads obtained using Illumina HiSeq X, whereas one of the six genomes (*E. exitiosus*) was obtained by PacBio long read sequencing using a Sequel machine technology. Additionally, transcriptomes were obtained using HiSeq X; short read assemblies done with DE-BRUIJIN Graph assembly Megahit, and the PacBio assembly was done with Canu 1.7. All transcriptomes were assembled with the Trinity *de novo* transcriptome platform. Average genome size for all genomes was 1.15GB, they ranged from 1.08GB to 1.48GB. Completeness of transcriptomes was assessed using the presence of conserved eukaryotic genome markers and ranged from 96.0% to 100% and averaging 97.6%. Comparative analysis to other sequenced members of Arthropoda will be conducted using bi-directional blast. Unique and shared genes from all genomes will be calculated using cd-hit program. Salient differences between these genomes and holometabolous insects will be conducted using homolog search between groups of genomes.

39. MOLECULAR MECHANISMS UNDERLYING SYSTEMIC RNAI IN THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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RNA interference (RNAi) is a highly conserved cellular defense mechanism, in which double stranded RNA (dsRNA) triggers the degradation of homologous mRNA. Interestingly, in some organisms (including insects), RNAi can be induced systemically, i.e. systemic RNAi. Understanding the molecular mechanisms underlying this phenomenon will be crucial for the application of gene knock-down techniques to many insects and will also provide us with a route to establish RNAi-based pest management strategies. However, the investigation of the molecular basis for systemic RNAi has been a challenge in insects, largely because *Drosophila*, the most established insect model system, lacks a robust systemic RNAi response. We have previously shown that the red flour beetle, *Tribolium castaneum*, exhibits such a response, making this insect an ideal system to study factors involved in systemic RNAi. In this study, we have established a two-step *in vivo* assay system in *Tribolium*, allowing us to evaluate the involvement of genes in insect RNAi and also providing us with a new platform for genome-wide identification of novel RNAi-related genes. Utilizing this assay system, we are currently testing a set of candidate genes, whose orthologs in other organisms are known to be involved in systemic RNAi-related processes (such as dsRNA cellular uptake). Identification and characterization of molecules involved in insect systemic RNAi will be a crucial step toward the practical application of RNAi to pest management.

40. TRANSCRIPTOMIC STUDY OF RESISTANCE MECHANISM AGAINST BT TOXIN IN WESTERN CORN ROOTWORM

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The western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) is a coleopteran pest species that causes severe damage to maize. It is a highly adaptive species, having evolved resistance to Bt transgenic maize lines which were widely adopted as a management strategy in the U.S corn belt. The Bt resistance mechanism in WCR has not been well characterized. We conducted a transcriptomic study using PacBio and Illumina technologies to evaluate structural and expression differences related to Bt intoxication. Full-length transcriptome sequences and gene isoforms of WCR midgut were generated using the PacBio Iso-Seq Sequel system. Then, short Illumina reads from Bt-fed and control specific libraries were aligned to this transcriptome. We expect to detect SNP variation, alternative splicing events, and gene expression differences correlated to Bt resistance in WCR midgut tissue. The results may provide insight into the mode of action of Bt toxin and resistance mechanism of WCR against Bt toxins.

NOTES

41. *PHLEBOTOMUS PAPATASI* SAND FLY SALIVARY PROTEIN DIVERSITY AND IMMUNE RESPONSE POTENTIAL IN EGYPT AND JORDAN POPULATIONS.

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Phlebotomus papatasi sand flies vector *Leishmania* major parasites, one of the causative agents of cutaneous leishmaniasis (CL). Approximately 0.7-1.2 million cases of CL occur each year. CL produces scarring skin lesions for which, no vaccine currently exists. Hematophagous vector salivary proteins are pharmacologically active molecules that modulate inflammation, vasoconstriction, blood clotting, etc. for females that require a sanguineous meal for oviposition. Salivary proteins from multiple phlebotomine sand fly species have been widely studied and scrutinized to characterize their function in blood feeding facilitation as well as their ability to exacerbate or attenuate *Leishmania* infections and their potential as vaccine candidates. A successful sand fly salivary protein-based vaccine to combat CL largely depends on the genetic variability, expression profiles, and human immune response to the salivary proteins selected from geographically distant sand fly populations. The purpose of this study was to analyze 9 abundantly expressed *P. papatasi* salivary proteins as potential vaccine targets that are conserved across populations from three distinct ecotopes in Egypt and Jordan and demonstrate the potential to elicit an immune response.

42. BT RESISTANCE IN LABORATORY-SELECTED *HELICOVERPA ZEA* IS ASSOCIATED WITH GENETIC CHANGES AT A NOVEL CADHERIN

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Helicoverpa zea recently caused significant damage to ears of Bt-expressing sweet corn in Eastern Maryland, demonstrating that field populations have evolved Bt resistance. The molecular mechanisms underlying this Bt resistance in *H. zea* remain unknown, however. We performed whole genome resequencing of lab-selected Bt resistant and susceptible lines as a first step toward identifying polymorphisms associated with *H. zea* resistance. Our sequencing results showed that a previously undescribed cadherin gene underwent a selective sweep in the Bt resistant line as compared to the susceptible line. Future examination of this gene in *H. zea* larvae collected from Bt-expressing and conventional sweet corn will help us to identify whether this gene is relevant to field-evolved resistance.

43. VECTORBASE DATA, TOOLS AND RESOURCES FOR VARIATION AND POPULATION ANALYSES

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VectorBase.org is a free web-based resource, focused on invertebrate vectors of human pathogens, that hosts genomes, transcriptomes and proteomes, for 38, 40 and 2 species respectively. It also has data in the form of single nucleotide polymorphisms (SNPs), insertions or deletions (INDELs), microsatellites, chromosomal inversions, population abundance, infection status, blood meal source, and phenotypes and genotypes, for traits such as insecticide resistance. Data comes from more than 200 species, individuals and pools, from both peer-reviewed publications in public and private institutions that may have collected data for vector control purposes but may not intended to publish scientific papers. VectorBase is robust and flexible, and capable of storing field-captured data from many different types of studies from any species. We will present how to search, query, browse and download the all these different data types using different VectorBase tools such as the Genotype and Sample Explorers, Population Biology Map, Search and Advanced Search. To contact us please send a message to info@vectorbase.org. VectorBase is a National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) funded Bioinformatics Resource Center (BRC).

44. POPULATION GENETICS ANALYSIS WITHOUT THE POPULATION LABELS

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Insect population genetics analysis often starts with visualizing relationships between samples using Principal Component Analysis (PCA). Next, populations are identified via clustering (either using uncovered variants or PCA-projected coordinates). And lastly, variants of interest are identified by performing pairwise tests between pairs of populations. Insect populations can be highly introgressed, however, which can make it difficult to clearly define the populations and their memberships and hinder analyses.

We propose a new approach emphasizing *unsupervised* learning (no population labels). We use PCA to identify the latent variables that explain variation. We then apply single-SNP association tests between the samples' PC coordinates and variant genotypes to identify interesting SNPs.

We applied our approach to variants from 34 previously-published samples of *Anopheles gambiae* and *Anopheles coluzzii*. We found that 4 PCs explain most of the variation. When associated SNPs are plotted along the chromosome arms, PCs 2 and 4 identified known inversions on 2L and 2R. Genes containing variants associated with the PCs include a broad array of chemosensory receptors, cytochrome P450s, carboxylesterases, and various enzymes. PC3 was strongly associated with an insecticide-resistance mutation in *Rdl* as well as mutations in chemosensory receptors, suggesting that these variations may be driven (directly or indirectly) by the same underlying process.

We also applied our method to variants from 51 *Lutzomyia longipalpis* sand fly samples located in chemosensory receptors. The first 4 PCs explained most of the variation. PCA projection plots indicated that PC1 was able to separate the samples based on their sensitivity to different pheromones.

Our method provides an effective new way to identify SNPs associated with PCs. When applied to population genetics data, our approach provides an *unsupervised* way of discovering and interrogating the processes driving differentiation.

45. GENOME-WIDE ANALYSES OF SMALL NUCLEOTIDE POLYMORPHISMS (SNPS) PROVIDES INSIGHTS TO GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE LYME DISEASE TICK, *IXODES SCAPULARIS*

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The black legged or Lyme disease tick, *Ixodes scapularis* is the North American vector of the pathogens that cause Lyme disease, anaplasmosis, babesiosis and Powassan encephalitis. Single Nucleotide Polymorphism (SNP) markers offer an opportunity to evaluate the population genetics of *I. scapularis* and identify loci associated with pathogen transmission. Here we report a genome-wide analysis of SNPs in *I. scapularis* collected from eight locations in the U.S. - Florida, Indiana, Maine, Massachusetts, North Carolina, New Hampshire, Virginia and Wisconsin, and individuals of the Wikel strain (maintained by the University of Texas Medical Branch, Galveston, TX), the reference strain for the *I. scapularis* IscaW1 genome assembly (Gulia-Nuss et al., 2016). Restriction Site Associated DNA sequencing (RADseq) reads from 74 individual ticks were aligned to the IscaW1 assembly (using Bowtie2) and 745,760 SNPs were identified using mpileup function of samtools. After selection for bi-allelic SNPs and SNPs present in at least 80% of individuals sampled per location, we obtained a set of 56,542 SNPs for genotyping. Approximately 71.14% of these SNPs were identified in intergenic regions, 18.5% in introns and 28.8% in the coding regions of protein- and non-coding RNA genes, and a small percentage of SNPs were identified in splice-sites and UTR regions (0.015% and 0.29%, respectively). Using the program SnpEff, the effect of SNPs was classified as modifying (94.6%), low (3.93%), moderate (1.39%) and high impact (0.037%). Clustering was performed to genotype ticks and identify SNPs unique to ticks from the mid-west, north-east and south-east, or common among regions. Genes associated with 789 non-synonymous SNPs were analyzed to identify genes under positive selection and 71 SNPs were selected for confirmation by PCR in female and male ticks. Ongoing studies are focused on the identification of SNP markers associated with traits for tick host preference and vector competence.

46. SINGLE-CELL SEQUENCING OF THE HONEY BEE BRAIN

Traniello, I. M. and Robinson, G. E.

The Western honey bee *Apis mellifera* demonstrates a wide range of complex social behaviors generated by a brain of ~1,000,000 neurons. While high-throughput sequencing has been revelatory in profiling brain gene expression, less is known about the molecular identities of individual cells or regions within the honey bee brain. To develop a more finely resolved understanding, cellular integrity must be maintained throughout sequencing to uniquely characterize individual cells. Using the 10x Genomics Chromium platform, we performed single-cell transcriptomic analysis on thousands of neurons collected from either the whole-brain or mushroom bodies of adult worker honey bees. We performed gene expression-based clustering using the Cell Ranger analysis pipeline to predict distinct neuronal subpopulations within the honey bee brain. We present this data as a foundation for a honey bee brain atlas, which will complement existing anatomical studies and further our ability to perform comparative brain research between honey bees and other animals. In addition, these preliminary findings provide the groundwork for future behavioral studies in which the relative contributions of specific brain regions to behavior can be analyzed in unprecedented depth.

47. DE NOVO TRANSCRIPTOMES OF THE BURYING BEETLES *NICROPHORUS PUSTULATUS* AND *NICROPHORUS ORBICOLLIS*

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Most burying beetle species (family: Silphidae, Genus *Nicrophorus*) exhibit bi-parental care for their offspring. Adults of most species of burying beetles discover small vertebrate carcasses and bury them by excavating the soil beneath the carcass. After burial, adults create a brood ball by stripping hair or feathers and then secreting antimicrobial compounds to coat the carcass. After egg hatch, parental beetles provide regurgitated food to the developing larvae. Antimicrobial peptides (AMPs) found in the salivary and anal glands of *Nicrophorus* are important for the preservation of the carrion as well as the subsequent survival and growth of offspring. In order to develop molecular resources and create foundational knowledge for further study, we generated transcriptomes that are representative of the salivary glands of two *Nicrophorus* species, *N. orbicollis* and *N. pustulatus*. *De novo* transcriptome assemblies of the salivary glands of *N. orbicollis* and *N. pustulatus* were generated using Trinity and produced 97,224 and 86,475 transcripts, respectively. Of the assembled transcripts, 62,476 and 64,484 represent peptide sequences predicted to produce protein product. Out of these peptide sequences, 29,985 and 33,754 contain complete open reading frames. The transcriptomes generated serve as a substantial improvement of *Nicrophorus* genomic data and represent the first extensive molecular resource for these species. The two species were chosen because *N. orbicollis* has been shown to have AMPs, while *N. pustulatus*, a potential brood parasite has not been shown to have these compound. These data provide insights into *Nicrophorus* carrion preservation and the development of these unique beetles.

48. A TALE OF TWO BUMBLEBEES: PROSPECTS AND CHALLENGES OF UTILIZING GENOME-WIDE ASSOCIATION STUDIES (GWAS) TO INVESTIGATE THE GENOMIC BASIS OF ADAPTIVE TRAITS IN INSECTS

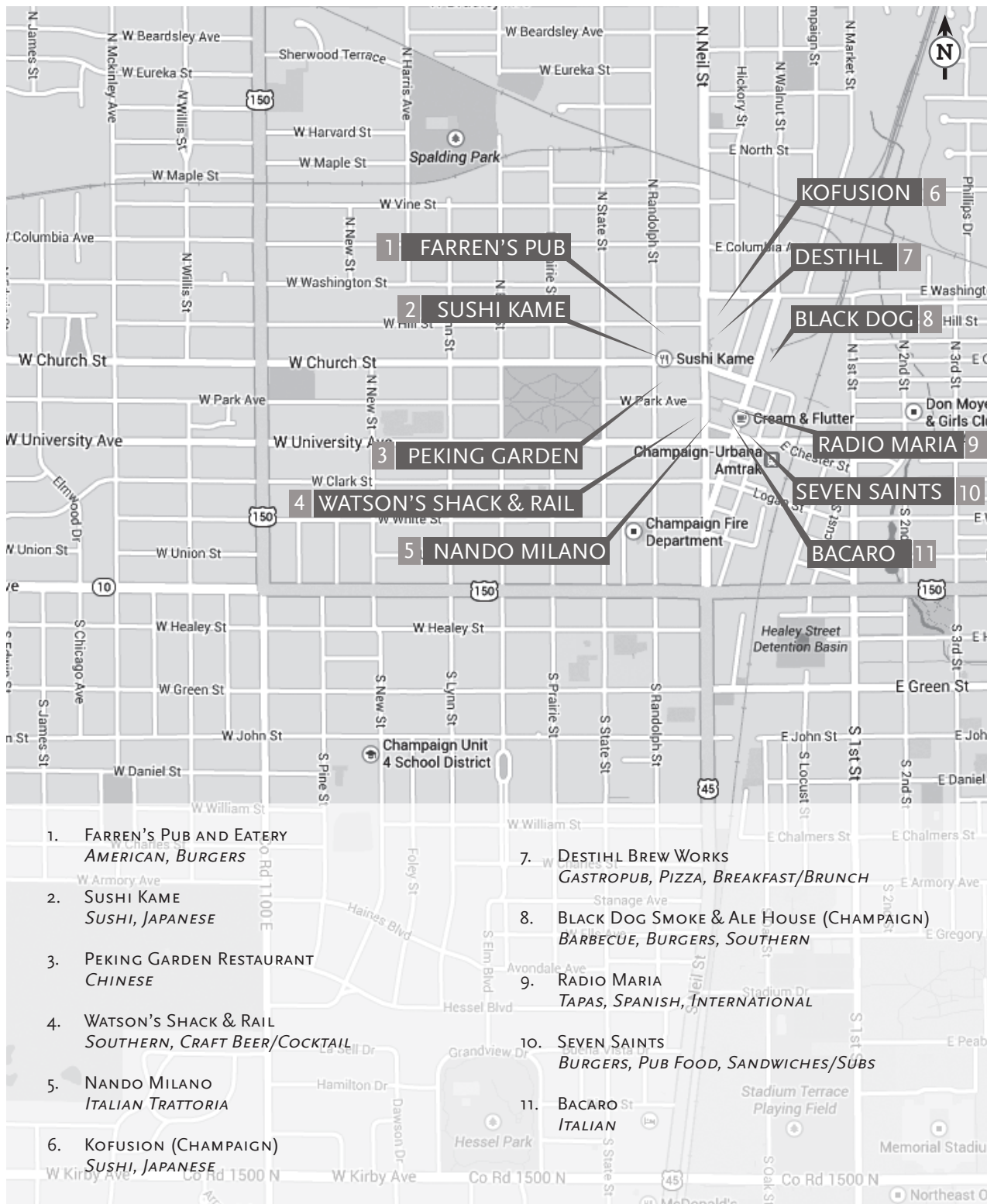
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Increasingly cheaper next generation sequencing techniques and fast-growing publicly available genomic resources are allowing researchers to conduct genotype-phenotype association studies in more diverse model and non-model organisms. We utilized GWAS to independently target the genetic basis of coloration in two bumble bee species that exhibit parallel mimicry patterns to determine whether the same or different loci may be involved during the generation of their similar red or black phenotypes. For the species *B. melanopygus*, which exhibits simple Mendelian inheritance and discrete color morphs, we were able to successfully identify a single peak of strong association in a Hox regulatory region of *abd-A*, suggesting that a cis-regulatory modification in this single gene is driving their phenotypes. In *B. bifarius*, which has intermediate red to black morphs we were unable to target clear peaks of association with the same sample size, suggesting much more complex gene regulation and/or additional complexity generated from population structure. Regardless, the Hox locus was not implicated in this phenotype, suggesting independent and likely more downstream evolutionary targets for color variation between these co-mimics. Our research has exhibited the potential of application of GWAS in non-model organisms to investigate the genomic basis of adaptive traits and addresses the underlying challenges (e.g. inadequate sample design, unknown inheritance patterns, population stratification) of conducting a successful genotype-phenotype association analysis.

NOTES

LOCAL EVENING DINING | DOWNTOWN CHAMPAIGN





LUNCH LOCATIONS | NEARBY RESTAURANTS

BASIL THAI RESTAURANT

Thai

BREAD COMPANY RESTAURANT

Sandwiches

CAFFE BENE

Sandwiches, Dessert, Coffee

ESPRESSO ROYAL CAFE

Coffee, Baked Goods

INTERMEZZO CAFE

Sandwiches, Soup

J. GUMBOS

Cajun, Creole

KOFUSION

Sushi, Stir-Fry

MANOLOS PIZZA AND EMPANADAS

Pizza, Empanadas

MERRY-ANN'S DINER

Sandwiches, Lunch Specials

ROSATI'S

Pizza, Italian

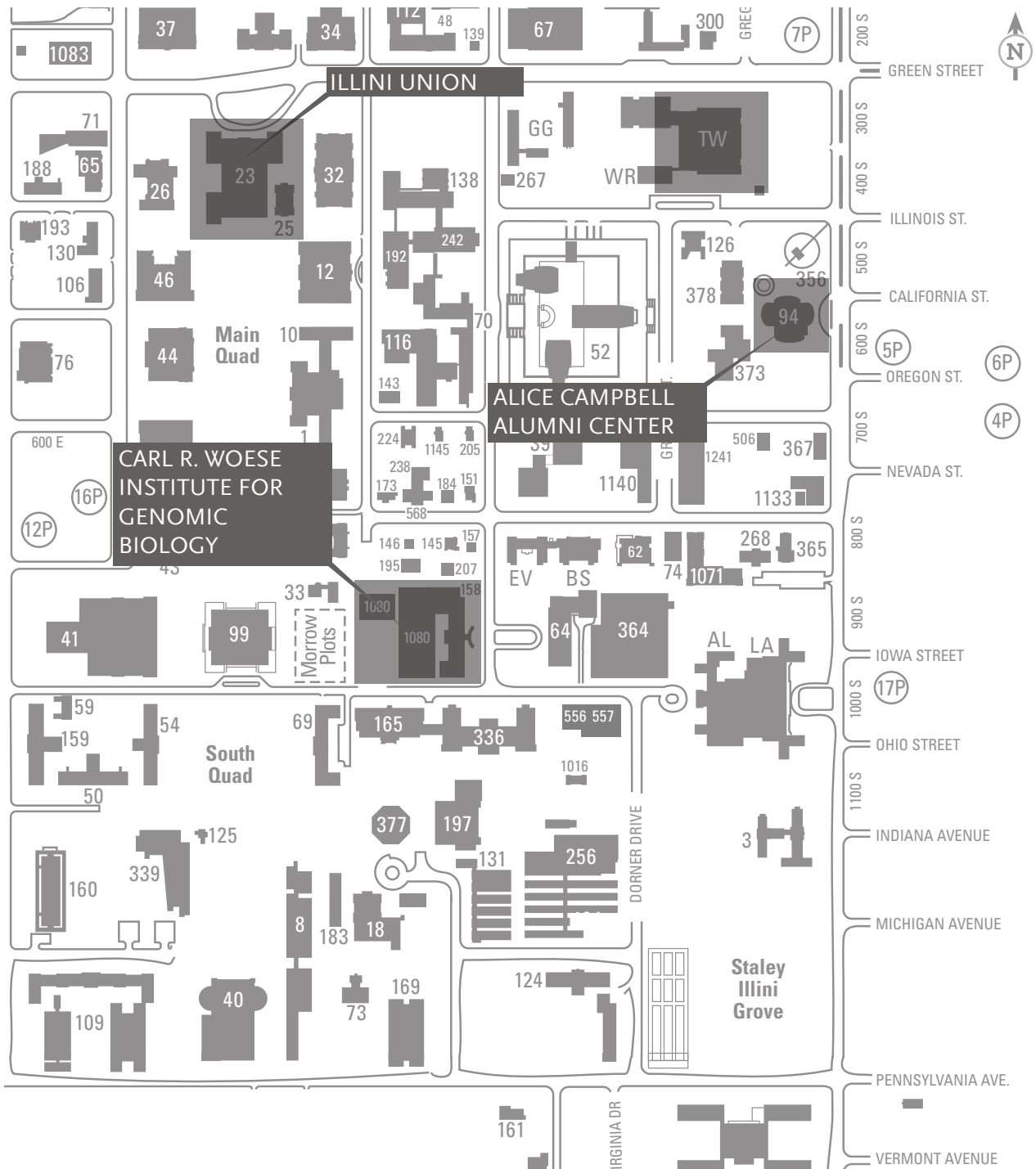
SUBWAY SANDWICHES

Sandwiches

TIMPONE'S ITALIAN RESTAURANT

Italian

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