Abstracts of papers presented
at the 2018 meeting on

BIOLOGY & GENOMICS OF SOCIAL INSECTS

May 5–May 8, 2018

Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2018 meeting on

BIOLOGY & GENOMICS OF SOCIAL INSECTS

May 5–May 8, 2018

Arranged by

Sarah Kocher, *Princeton University*
Seirian Sumner, *University College London, UK*
Amro Zayed, *York University, Canada*

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BIOLOGY & GENOMICS OF SOCIAL INSECTS

Saturday, May 5– Tuesday, May 8, 2018

Saturday	7:30 pm	Keynote Speaker I
Saturday	8:30 pm	**1** Health and Immunity of Social Insects
Sunday	9:00 am	**2** Emerging Genomic Tools for Non-model Organisms
Sunday	11:00 am	**3** Ecological Genomics of Social Insects
Sunday	2:00 pm	Keynote Speaker II
Sunday	3:00 pm	**4** Poster Session
Sunday	5:00 pm	Wine and Cheese Party
Sunday	7:30 pm	**5** Integrating Social Insect Theory with Sociogenomics
Sunday	9:00 pm	**6** Symbionts and Microbiomes
Monday	9:00 am	**7** Genomics of Social Plasticity
Monday	11:00 am	**8** Gene Regulation and Epigenetics in Social Organisms
Monday	2:00 pm	**9** Genome Evolution and Social Evolution
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Monday	6:00 pm	Banquet
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Mealtimes at Blackford Hall are as follows:

Breakfast	7:30 am-9:00 am
Lunch	11:30 am-1:30 pm
Dinner	5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

SATURDAY, May 5—7:30 PM

KEYNOTE SPEAKER

Christina Grozinger
Pennsylvania State University, University Park, Pennsylvania

“Bee health—From genomes to landscapes” 1

SATURDAY, May 5—8:30 PM

SESSION 1 HEALTH AND IMMUNITY OF SOCIAL INSECTS

Chairperson: **Seth Barribeau,** University of Liverpool, United Kingdom

Genomic approaches to understanding immunity in an aspiring model species
Seth Barribeau.
Presenter affiliation: University of Liverpool, Liverpool, United Kingdom.

Genomic tools for tracking invasive Africanized honey bees
Kathleen A. Dogantzis, Amro Zayed.
Presenter affiliation: York University, Toronto, Canada. 2

Transgenerational effects in honey bees—Focus on the egg
Esmaeil Amiri, Micheline K. Strand, David R. Tarpy, Olav Rueppell.
Presenter affiliation: University of North Carolina at Greensboro, Greensboro, North Carolina; U.S. Army Research Office, Research Triangle Park, Durham, North Carolina; North Carolina State University, Raleigh, North Carolina. 3

Thermal stress induces broad transcriptional changes in signaling pathways in response to tissue damage in the honey bee
Dunay Bach, Jonathan W. Snow.
Presenter affiliation: Barnard College, Columbia University, New York, New York. 4

SUNDAY, May 6—9:00 AM

SESSION 2 EMERGING GENOMIC TOOLS FOR NON-MODEL ORGANISMS

Chairperson: **Guojie Zhang,** University of Copenhagen, Denmark

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SESSION 3 ECOLOGICAL GENOMICS OF SOCIAL INSECTS

Chairperson: **Jessica Purcell,** University of California, Riverside

Selfish gene, social outcome—Exploring the genetic basis of a complex social trait
Jessica Purcell.
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Formica ants reveal a gene network that underlies speciation
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Presenter affiliation: University of Helsinki, Helsinki, Finland. 12

Conservation genomics of *Bombus terricola*
Nadia Tsvetkov, Clement Kent, Amro Zayed.
Presenter affiliation: York University, Toronto, Canada. 13

Transcriptomic impacts of developmental environment and "supergenotype" on the red imported fire ant, *Solenopsis invicta*
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Antoine Felden, Philip Lester, Monica Gruber.
Presenter affiliation: Victoria University of Wellington, Wellington, New Zealand. 15

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KEYNOTE SPEAKER

Jacobus Boomsma
University of Copenhagen, Denmark

"Social adaptation, natural selection and genomics"

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Isabel Fletcher, Thomas J. Colgan, Andres Arce, Richard Gill, Yannick Wurm.
Presenter affiliation: Queen Mary University of London, London, United Kingdom. 44

Honey bee males produce semen with unique DNA methylation profiles
Boris Yagound, Gabriele Buchmann, Benjamin P. Oldroyd, Emily J. Remnant.
Presenter affiliation: University of Sydney, Sydney, Australia. 45

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Presenter affiliation: The Rockefeller University, New York, New York. 48

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Chairperson: **Nancy Moran,** University of Texas at Austin

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Chairperson: **Amy Toth,** Iowa State University, Ames

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Chairperson: **Roberto Bonasio,** University of Pennsylvania, Philadelphia

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Presenter affiliation: Weizmann Institute of Science, Rehovot, Israel; University of Lausanne, Lausanne, Switzerland. 60

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Guy Bloch.
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SESSION 11 NEUROGENOMICS

Chairperson: **Andrew Barron,** Macquarie University, Sydney, Australia

AUTHOR INDEX

BEE HEALTH: FROM GENOMES TO LANDSCAPES

Christina Grozinger

Pennsylvania State University, Department of Entomology, Center for Pollinator Research, Huck Institutes of the Life Sciences, University Park, PA

Bees are critical pollinators of agricultural crops, but many populations of both managed and wild pollinators are experiencing declines. Multiple interacting factors are driving pollinator declines, including parasites, pathogens, and biotic and abiotic features of the landscape, such as the availability of forage, pesticide use, population densities, and climate. I will summarize our studies using genomic approaches, behavioral studies, and spatial ecology to understand how these factors interact to influence bee health and to build predictive models of bee health that beekeepers, land managers, growers, and policymakers can use to evaluate the quality of diverse landscapes for supporting bee populations and obtain recommendations for improving their landscapes and management practices.

GENOMIC TOOLS FOR TRACKING INVASIVE AFRICANIZED HONEY BEES.

Kathleen A Dogantzis, Amro Zayed

York University, Biology, Toronto, Canada

The honey bee, *Apis mellifera*, is an ecologically and economically important species contributing to pollination services worldwide. Consequently, it is essential that potential threats to honey bee populations are identified and mitigated to prevent losses to the beekeeping industry. Africanized honey bees (AHB) are a hybrid population composed of European and African ancestry and are considered undesirable for beekeeping due to their aggressive defensive behaviour. Given the large-scale trade and movement of honey bees, there is a concern that AHBs will spread from South America and the southern United States to the rest of North America, Australia, New Zealand, and Hawaii. Developing an accurate and cost effect assay to detect AHB is an important first step towards restricting the accidental importation of AHBs. Here, we used an extensive population genomic dataset composed of individuals from all known evolutionary lineages to assess the genomic composition of *Apis mellifera* populations, and patterns of genetic admixture in North and South American commercial honey bee colonies. Our genomic dataset includes over 150 newly sequenced individuals from at least 14 subspecies encompassing the known distribution of *Apis mellifera*'s native range. We used this data set to develop SNP assays that show high accuracy in assigning bees of unknown genetics as either African or non-African. The genomic resources will also be important for understanding the biogeographic history of the honey bee.

TRANSGENERATIONAL EFFECTS IN HONEY BEES: FOCUS ON THE EGG

Esmaeil Amiri[1,2,3], Micheline K Strand[2], David R Tarpy[3], Olav Rueppell[1]

[1]University of North Carolina at Greensboro, Biology, Greensboro, NC, [2]U.S. Army Research Office, Life Sciences Division, Research Triangle Park, Durham, NC, [3]North Carolina State University, Department of Entomology & Plant Pathology, Raleigh, NC

Transgenerational effects are important in many species but have been largely understudied in honey bees and other social insects. Several mechanisms produce transgenerational effects, and the fitness consequences can be positive or negative. The most prominent medium for transgenerational effects is the egg, which provides offspring with nutrients and other factors essential to start development but can also be exploited as a vehicle by pathogens. To explore both processes and relate them to each other, we studied the prevalence and mechanism of virus transmission through eggs, characterized the transcriptome of honey bee eggs with varying virus infections, and explored influences on egg size. We report that vertical transmission of Deformed Wing Virus—one of the most notorious bee pathogens—occurs intermittently and mostly through egg surface contamination. These findings are complemented with RNASeq data of freshly laid eggs that compare the transcriptomes of eggs with and without viruses. Lastly, we describe significant egg-size variation among honey bee stocks and individual queens, and we show that eggs size is increased in smaller or food-stressed colonies, which suggests that egg size may be functionally relevant for quantitative transgenerational effects and colony function in the honey bee.

THERMAL STRESS INDUCES BROAD TRANSCRIPTIONAL CHANGES IN SIGNALING PATHWAYS IN RESPONSE TO TISSUE DAMAGE IN THE HONEY BEE

Dunay Bach, Jonathan W Snow

Barnard College, Columbia University, Biology Department, New York, NY

A robust regenerative program in response to tissue damage is a critical component of stress resistance in most organisms. In the fruit fly, multiple processes underlying tissue regeneration in the digestive tract have been characterized. As the honey bee digestive tract differs significantly from that of the fruit fly in terms of morphology, organization, and function, distinct features in the regeneration program are likely. Homeostatic self-renewal has been shown in the digestive tract of multiple bee species. However, the molecular regulation of tissue regeneration in this phylogenetic group is incompletely understood. Using a thermal stress model coupled with RNAseq, we have identified broad remodeling of a number of key signaling pathways in the honey bee in response to tissue damage, including those pathways known to be involved in digestive tract regeneration in the fruit fly. We have also found that thermal stress affects many of these pathways in other tissues, suggesting a shared program of damage response. Current and future research is focused on understanding how the observed transcriptional changes are localized to different cells and regions of the digestive tract tissue, potentially enabling identification and molecular characterization of specialized cell populations in the midgut, including enteroendocrine cells and intestinal stem cells. Thus, this work stands to advance our understanding of digestive tract structure, function, and response to damage in this and other bee species more broadly.

DISSECTING THE GENETIC UNDERPINNINGS OF PATHOGEN LOADS IN THE HONEY BEE *APIS MELLIFERA*

Tanushree Tiwari, Clement Kent, Alivia Dey, Stephen Rose, Harshil Patel, Kathleen Dogantzis, Amro Zayed

York University, Biology, Toronto, Canada

The honey bee *Apis mellifera* is a model organism for sociogenomics and is one of the most important managed pollinators. As such, recent threats to honey bee health are particularly alarming. The social honey bees live in highly crowded nests providing favorable conditions for the spread of infectious diseases. But honey bees have several social and individuals mechanisms for protecting themselves against disease. The BeeOMICS consortium has sequenced the genomes of approximately 1,000 honey bee colonies in Canada, which were evaluated for a number of traits, including the abundance of several pathogens within each colony. I plan to carry out genome-wide association studies (GWAS) on colony pathogen loads to gain a deeper insight of the genetics of immunity in honey bees. This research will set the groundwork for breeding disease resistant honey bees using marker assisted selection.

A GENOMIC PERSPECTIVE ON EVO-DEVO OF ANT SUPERORGANISMS

Guojie Zhang[1,2,3]

[1]University of Copenhagen, Section for Ecology and Evolution, Department of Biology, Copenhagen, Denmark, [2]BGI-Shenzhen, China National Genebank, Shenzhen, Guangdong, China, [3]Kunming Institute of Zoology, Chinese Academy of Sciences, State Key Laboratory of Genetic Resources and Evolution, Kunming, China

The development of genomic technology has revolutionized research in many fields of biology and medicine, including the study of social insects. Most genomic studies of social insects have focused on particular species or lineages, while a phylogenomic approach for broader species sampling has rarely been established. My group has used such phylogenomic approach to investigate the genomic changes underlying the unique evolutionary transition to superorganismality in the ants and the subsequent lineage-specific adaptations that followed. The ants are a large monophyletic lineage with permanent caste differentiation that evolved from wasp-like ancestors ca. 140 million years ago, and their thousands of extant species have evolved a huge diversity of lifestyles and environmental adaptations. It therefore requests a series of normalization procedures to retrace the ancestral genetic regulatory networks that were likely involved in the origin of superorganismality, because they have invariably been modified in later adaptive radiations at the sub-family or genus level. On the other hand, permanent adult caste phenotypes are also the outcome of individual variation in larval development. This implies that a developmental time series of changes in gene regulatory networks is also essential for understanding the mechanisms of irreversible caste differentiation. By comparing the genomes and transcriptomes of specific organs across different taxa and developmental stages, we wish to reach a comprehensive understanding of the genetic basis for superorganismal evo-devo in the ants.

HYMENOPTERAMINE: DATA MINING WAREHOUSE OF THE HYMENOPTERA GENOME DATABASE

Christine G Elsik[1,2,3], Aditi Tayal[1], Deepak Unni[1], Deborah Triant[1]

[1]University of Missouri, Animal Sciences, Columbia, MO, [2]University of Missouri, Plant Sciences, Columbia, MO, [3]University of Missouri, MU Informatics Institute, Columbia, MO

HymenopteraMine (http://hymenopteragenome.org/hymenopteramine/), the data mining resource of the Hymenoptera Genome Database, accelerates genomic analysis by enabling researchers to create and export customized datasets for use in downstream analyses. HymenopteraMine uses the InterMine Data Warehousing platform to integrate reference genome assemblies and annotations of 16 hymenopteran species, 12 of which are eusocial. Data includes genes (RefSeq, Official Gene Set), proteins (UniProt), protein families and domains (InterPro), homologs (OrthoDB), pathways (KEGG), Gene Ontology (UniProt-GOA), variation (dbSNP) and publications (PubMed). By including fly homologs, HymenopteraMine allows users to leverage the Reactome pathways and BioGRID interactions that have been curated for Drosophila melanogaster. HymenopteraMine also includes pre-computed variant effects and RNAseq-based transcript expression levels for Western honey bee (*Apis mellifera*).

HymenopteraMine provides simple and sophisticated search tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries. The Genomic Region search tool executes queries based on lists of genome coordinates. The List tool allows users to upload identifiers to create custom lists, perform set operations such as unions and intersections, and execute template queries with lists. When used with gene identifiers, the List tool automatically provides gene set enrichment for GO and pathways, with a choice of statistical parameters and background gene sets. HymenopteraMine is particularly useful for tracking gene identifiers across gene sets to facilitate meta-analysis. Query results can be downloaded in several formats (tab delimited, GFF3, Fasta, BED, JSON, and XML). With HymenopteraMine, researchers without scripting skills can create and export customized annotation datasets merged with their own research data for use in downstream analyses.

GENERATION OF TRANSGENIC LINES USING PIGGYBAC TRANSPOSONS IN THE CLONAL RAIDER ANT

Thomas Hart, Amelia Ritger, Waring Trible, Leonora Olivos-Cisneros, Stephany Valdes Rodriguez, Daniel Kronauer

The Rockefeller University, Laboratory of Social Evolution and Behavior, New York, NY

Ants exhibit a fascinating range of developmental and behavioral traits, including the generation of distinct morphological castes from a single genome, complex chemical communication, and the flexible behavioral division of labor that underlies colony life. In recent years, new technologies such as whole genome sequencing, RNASeq, and CRISPR/Cas9 have advanced our ability to study these interesting phenomena on a molecular level. However, a complete genetic toolkit also requires the ability to create transgenic organisms by inserting custom-designed DNA sequences into the genome. To address this, we established an efficient piggyBac transposon-based gene integration protocol for the clonal raider ant (*Ooceraea biroi*) and used this protocol to generate the first transgenic ant lines. This protocol allows us to rapidly generate transgenic lines in clonal raider ants, which can then be propagated directly via asexual reproduction. We generated multiple independent lines that broadly express the fluorescent protein DsRed under the control of the baculovirus promoter *ie1*. Fluorescence from this transgene is visible in live animals from the larval stage through adulthood under epifluorescence. This protocol can now be applied for generating additional transgenic tools in ants, such as fluorescent reporters under tissue-specific promoters or genetically-encoded biosensors and modulators. Implementing these tools will open new opportunities for investigating the molecular mechanisms of physiology and behavior in clonal raider ants.

THE GENETICS AND NEUROPHYSIOLOGY OF VARIATION IN FORAGING ACTIVITY AMONG COLONIES OF RED HARVESTER ANTS (*POGONOMYRMEX BARBATUS*).

Daniel A Friedman[1], Anna Pilko[2], Dorota Skowronska-Krawczyk[3], Karolina M Krasinska[4], Jacqueline W Parker[5], Mimi Shin[6], Jay Hirsh[5], Barbara J Venton[6], Deborah M Gordon[1]

[1]Stanford University, Department of Biology, Stanford, CA, [2]University of California, Los Angeles, Department of Chemistry and Biochemistry and Institute for Quantitative and Computational Biosciences, Los Angeles, CA, [3]University of California, San Diego, Shiley Eye Institute, Richard C. Atkinson Lab for Regenerative Ophthalmology, Department of Ophthalmology, La Jolla, CA, [4]Stanford University, Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford, CA, [5]University of Virginia, Charlottesville, Department of Biology, Charlottesville, VA, [6]University of Virginia, Charlottesville, Department of Chemistry, Charlottesville, VA

Colonies of red harvester ant (*Pogonomyrmex barbatus*) persistently vary in how they regulate their collective foraging activity in response to changes in ambient conditions. Transcriptomic, pharmacological, and physiological experiments investigated the neuromolecular mechanisms underlying this ecologically important variation among colonies in foraging activity. RNA sequencing of forager brain tissue showed an association between colony foraging behavior and differential expression of genes related to neuropeptide signaling and biogenic amine metabolism. Pharmacological increases in forager brain dopamine titer were sufficient to increase foraging activity in field experiments over two years. Colonies most sensitive to experimental stimulation of foraging activity by exogenous dopamine showed the most drastic natural decrease in foraging activity in dry conditions. Colonies most sensitive to the stimulatory effect of dopamine on foraging activity were also found to have divergent forager brain biogenic amine titers. Variation among colonies in the biogenic amine metabolism and signaling of individual ants may lead to the observed persistent, and possibly heritable, variation among colonies in the collective regulation of foraging.

HARNESSING NATURAL VARIATION TO STUDY THE EVOLUTION OF SOCIAL BEHAVIOR IN BEES

Benjamin Rubin, Sarah Kocher

Princeton University, Lewis-Sigler Institute for Integrative Genomics, Princeton, NJ

Eusociality has been repeatedly gained and lost in halictid bees, generating substantial behavioral variation even among closely related species. This makes the group an ideal model for studying the proximate changes associated with the evolution of social behavior. To identify the common molecular mechanisms underlying sociality in this system, we have sequenced and annotated the genomes of eight solitary, six social, and three socially polymorphic halictid species. Comparing gene sets across species has uncovered clear signals of convergent selection on just a few hundred genes. Strikingly, many of these genes have been associated with other types of social behavior in both insects and vertebrates, including autism spectrum disorder in humans. These results suggest that similar molecular mechanisms may be involved in establishing and organizing social behavior even in distantly related species.

SELFISH GENE, SOCIAL OUTCOME: EXPLORING THE GENETIC BASIS OF A COMPLEX SOCIAL TRAIT

Jessica Purcell

University of California Riverside, Entomology, Riverside, CA

With the dramatic decrease in the costs of genomic sequencing, researchers are able to gain insight into the genetic mechanisms underlying a wide range of traits in non-model organisms. Theory developed decades ago predicted the function of genes that may underlie social organization, but we are only now able to investigate those predictions empirically. These genetic mechanisms may help us to address a long-standing evolutionary question: Why are there evolutionary transitions from simple family social groups to groups comprised of non-relatives? This transition is particularly evident in the ants, where there are multiple independent origins of polygyny (= colonies composed of multiple queens and their offspring). My collaborators and I previously discovered a large supergene (containing over 600 genes) associated with queen number in the socially polymorphic species *Formica selysi*. This presentation will explore current work investigating the evolutionary history of the *Formica* supergene and its contemporary function. Understanding the history and function of a social supergene provides novel perspectives on previous studies and points to exciting future directions at the interface between behavioral ecology and evolutionary genetics.

FORMICA ANTS REVEAL A GENE NETWORK THAT UNDERLIES SPECIATION

Jonna Kulmuni[1], Lucy Pluckrose[1], Kishor Dhaygude[1], Roger Butlin[2]

[1]University of Helsinki, Research Program for Organismal and Evolutionary Biology , Helsinki, Finland, [2]University of Sheffield, Department of Animal and Plant Sciences, Sheffield, United Kingdom

One of the holy grails of speciation research is to pin-point the genomic regions that drive species divergence and underlie differential adaptation. Many current approaches, however, rely on inferring the action of natural selection on specific genomic regions instead of directly measuring it. We make use of haplodiploidy to identify natural selection acting on candidate loci underlying speciation. Previously, we have shown that in a population of hybridizing Formica ants the haploid hybrid males suffer from inviability and hybrid breakdown. Yet diploid hybrid females with the same alleles persist in the population and seem to be favored over more parental-like individuals. This suggests negative epistatic interactions between the parental genomes of the hybrids are exposed to selection in the haploid males but masked in the diplopid females. Here we discover and annotate the candidate genomic regions underlying hybrid male breakdown using comparative genomics and find that they seem to be scattered across the genome. However, genes in these regions are enriched in GO term 'protein-binding' and have significantly more molecular interactions with each other, than what would be expected for a random set of proteins drawn from the genome. Thus, our data suggests a gene network underlying hybrid male breakdown and speciation in Formica ants. We then test if natural selection is acting on our candidate genomic regions in nature. Specifically, we genotype 184 candidate SNPs and 180 control SNPs to test if heterospecific combinations of candidate loci result in lower survival of males in nature. This allows for testing the role of genomic regions of differentiation in reproductive isolation within a natural population, which has not been possible in most genomic studies of speciation.

CONSERVATION GENOMICS OF *BOMBUS TERRICOLA*

Nadia Tsvetkov, Clement Kent, Amro Zayed

York University, Biology, Toronto, Canada

Bombus terricola is in decline and was recently placed on the IUCN Red List of Threatened Species. The reasons for the decline in not known, but pathogen spillover and pesticide use have been implicated. In order to investigate the potential reasons for this decline we first sequenced the individual genomes of 22 bumblebee gynes from Ontario and Québec. We found several immune-related genes that have signatures of recent positive selection, which is consistent with the pathogen-spillover hypothesis. We then sequenced the transcriptome of 30 *b. terricola* worker abdomens from agricultural and non-agricultural sites. We detected patterns of differential gene expression in several metabolic process. Our analyses suggest some clues regarding the causes of *B. terricola's* decline.

TRANSCRIPTOMIC IMPACTS OF DEVELOPMENTAL ENVIRONMENT AND “SUPERGENOTYPE” ON THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*

Samuel V Arsenault[1], Joan T King[1,2], Kip D Lacy[1], Kenneth G Ross[1], Brendan G Hunt[1]

[1]University of Georgia, Department of Entomology, Athens, GA, [2]Texas A&M University, Department of Entomology, College Station, TX

The red imported fire ant, *Solenopsis invicta* exists in two distinct colony social forms: monogyne colonies containing only one reproductive queen and polygyne colonies containing multiple queens. This polymorphism corresponds with changes in queen dispersal patterns, worker behaviors, and morphology, all of which have been shown to be regulated by an approximately 13Mb non-recombining supergene containing more than 400 protein coding genes. Here we explore the effect of the supergene and parental colony type on the gene expression patterns in virgin reproductive females as they depart on their mating flights. We performed RNA-sequencing on the brains and ovaries of individuals that differ according to supergene presence and parental colony type, and then assessed differential gene expression between the groups. Overall, our study gives insight into the molecular mechanisms underlying variation in complex social phenotypes in *Solenopsis invicta*.

INVASIVE ARGENTINE ANT POPULATIONS ARE CHARACTERISED BY DISTINCT TRANSCRIPTOMIC SIGNATURES ASSOCIATED WITH FUNCTIONAL ADAPTATIONS

Antoine Felden, Philip Lester, Monica Gruber

Victoria University of Wellington, School of Biological Sciences,
Wellington, New Zealand

Behavioural traits are increasingly being regarded as a major driver of biological invasions, especially in the context of ant invasions. It is crucial to understand what factors can contribute to the success of invasive species, and next-generation sequencing has increased our capacity to investigate the molecular basis of the functional changes associated with biological invasions. Here, we investigate if native and introduced Argentine ant populations are characterised by distinct transcriptomic signatures associated with range, focusing on the molecular basis of behaviour. We used RNA-sequencing of ant heads across the native range in Argentina, and the introduced range along an introduction pathway in California, Europe, Australia and New Zealand, respectively.

Our results indicate that native and introduced populations are characterised by distinct transcriptomic signatures. Interestingly, the largest differences in expression profiles were found between Argentina and New Zealand, both at the extremes of the introduction pathway studied here. Weighted Co-Expression Network Analysis showed a large number of gene modules correlated with invasion status. Differential gene expression analysis showed that specific genes associated with olfactory reception were differentially expressed across the ranges. Furthermore, we used the Argentine ant genome annotations to retrieve expression values for genes associated with specific functional groups. This analysis revealed that genes associated with certain neural pathways were overall downregulated in the introduced range, and genes associated with chemoreception displayed country-specific expression pattern regardless of the invasion status. Native and introduced populations also exhibited differences in the overall expression of genes associated with the TOLL and RNAi immune pathways.

Altogether, our results contribute to a better understanding of how the introduction of exotic species may shape their behaviour and biology. We show that native and introduced Argentine ant populations are characterised by specific differential expression pattern in key genes and functional groups, perhaps underlying the ant's invasion success.

POPULATION STRUCTURE AND COOPERATION IN *POLISTES* PAPER WASPS

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One of the key predictions arising from population-structured models of cooperation is that limited dispersal should facilitate the evolution of cooperative behavior. Here we analyze the population structure of *Polistes* paper wasp populations exhibiting a range of cooperative breeding behaviors. Using an identity by state clustering algorithm (PLINK), we are able to detect variation in population subdivision across sites. We couple our population structure analysis to observations of spring co-founding rates in each sampled location in order to explore the relationship between inferred dispersal and cooperation.

A TALE OF THREE BEES: HOW HONEY BEES MIX AND MATCH THE NURSE AND FORAGER TRANSCRIPTOMES TO GENERATE A UNIQUE WINTER PHENOTYPE

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Honey bees in temperate regions face a great challenge in winter when the forage is non-existent and the temperature is inhospitably low. In preparation for this harsh season, honey bee colonies hoard resources throughout the spring and summer seasons. By the end of summer, a physiologically distinct group of workers start to appear in the hives with increased vitellogenin and hemolymph protein content, lowered juvenile hormone titer, and small hypopharyngeal glands. These workers can survive up to four times longer than their sisters in summer and they form a thermoregulatory cluster in the hive to generate and trap heat throughout the winter. Although there is substantial literature about the molecular mechanisms by which honey bee workers produce distinct phenotypes such as nurses – which are the care givers to the brood – or foragers – which fly outside the hive to find and gather a variety of resources – there is little known about the production of winter bee phenotype.

While winter bees spend most of their time inside the hive and have biochemical similarities with nurses such as an increase in vitellogenin content, they also extensively use their flight muscles to generate heat throughout the winter which is more similar to foragers compared to the mostly flightless nurses of summer. Thus, we had hypothesized that winter bee transcriptome might be a mix and match between nurse and forager gene expressions in a tissue specific manner, where winter bees resemble nurses in their fat body gene expression yet they are similar to foragers when the flight muscle gene expression profiles are compared. To test this hypothesis, in a preliminary study, we first screened the literature to find genes that are differentially expressed between flight muscles (5 genes) or fat bodies (9 genes) of nurses and foragers. Comparing the expression levels of these select genes in the flight muscles and fat bodies of nurses, foragers, and winter bees collected from the same colonies, we found that the gene expression profile of the winter bees is more nurse-like for the fat body and more forager-like for the flight muscle (manuscript submitted).

In the current study, using the same samples as in the primary study, we used RNA sequencing to compare tissue specific gene expression patterns in nurses, foragers, and winter bees. The findings from this study will give us a new and broad understanding of the expression profile in winter bees and how their transcriptome compares to nurses and foragers. Investigating the specific biochemical pathways and genes involved in the production of the winter bee phenotype we aim to improve our insights to not only the physiology of the honey bees in winter but also how they use a single genome to create such distinctive phenotypes with different abilities, behaviors, and even longevity.

EVOLUTION OF DUPLICATE GENES THROUGH DNA METHYLATION IN EUSOCIAL INSECTS.

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Eusocial species are remarkable because members of different castes develop disparate phenotypes through differential gene expression. One way in which this expression profile may evolve is through gene duplication, which can resolve intralocus conflict and allow optimal expression patterns of paralogs across different phenotypic classes. However, it is not clear how duplicate genes and their functions are maintained. In this study, we examined the relationship between DNA methylation and gene duplication in the eusocial honeybee in order to investigate the influence of epigenetic information on duplicate genes. Our goal was to determine whether patterns of DNA methylation were associated with gene duplication events. Our preliminary results suggest that DNA methylation is linked to gene duplication. We found strongly dissimilar patterns of methylation between duplicated and non-duplicated genes, which could provide a mechanism for the evolution of diverging patterns of gene expression. Additionally, we uncovered more similar methylation patterns between gene pairs than expected, and found that methylation levels depended on the chromosomal location of the duplication event. Surprisingly our results also indicated a higher level of similarity in methylation level between older duplicate gene pairs than younger duplicate gene pairs. Overall, our results suggest DNA methylation may influence duplicate gene evolution and therefore may mediate the evolution of the genome function in eusocial insects.

DISPARATE SOCIAL STRUCTURES LEAD TO DISPARATE CHEMICAL COMMUNICATION SYSTEMS

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Reproductive division of labor is largely manifested by complex communication system between the fertile queen and her sterile workers. The prevailing hypothesis underlying this phenomenon is that workers forego their reproduction if they gain in doing so sufficient inclusive fitness. This kin-selection-based consideration largely depends on the social structure of the species. In polygyne systems the queen reproductive monopoly and worker sterility are maintained by active policing of reproductive workers. In monogyne systems, in contrast, workers gain from direct fitness may surpass their gain from inclusive fitness and therefore they should be selected to compete with the queen and among nestmate over reproduction.

Accordingly, in honeybee the queens advertise their dominance both by producing an idiosyncratic suit of esters as fertility signal in Dufour's gland. However, hopelessly queenless workers that have activated ovaries also produce these esters, corroborating their role as fertility signals. In bumblebees at the competition phase (when queenright workers initiate ovary activation), on the other hand, fertility signaling by the queen becomes irrelevant but worker-worker competition is high and aggressive. However, not all workers can successfully reproduce, creating two worker "castes", reproductive and non-reproductive. The latter, therefore, can gain at least inclusive fitness by caring to their sisters' brood. Analysis of Dufour's gland secretion revealed clear distinction between these two "worker castes". The sterile workers possess a suit of esters that disappears with ovary activation. Behavioral assays further showed that the secretion act as an appeasement, protecting sterile workers from aggressive attack by their reproductive nestmates. There is also a correlation between ester occurrence and foraging, strengthening the hypothesis that the esters signal "I am out of the competition and care for your brood".

The fact that closely related bees use similar (but not identical) suits of esters, but which have an opposite social, context-dependent, messages, emphasize the evolution parsimony of pheromones in social insects.

EPIGENETIC SIGNATURES OF DISCRETE INDIVIDUAL BEHAVIORS IN HONEYBEE BRAINS

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In honeybees, genetically similar sisters will assume a remarkable range of specialized behaviors based on the needs of the hive. Epigenetic modifications assist in maintaining phenotype differences between reproductive queens and sterile workers, and can provide flexible control in the case of switching back and forth between the nurse and forager subcastes of workers. While such large-scale differences have an epigenetic basis, there remains the open question whether other behaviors possess unique epigenetic signatures in the brain as well. To address this, we profiled DNA methylation in the brains of bees that exhibit aggressive behaviors towards an intruder and bees that scout for new food resources. For aggressive bees compared to controls, we identified only four genomic regions differentially methylated in the brain 5 minutes after intruder exposure, but found 50 regions differentially methylated 120 minutes following intruder exposure. This rapid increase in differences occurred at genes involved in neural plasticity, chromatin remodeling and hormone signaling. Additionally, there was a significant overlap of differential methylation with previously published epigenetic differences that distinguish aggressive Africanized and gentle European honey bees, suggesting an evolutionarily conserved use of brain DNA methylation in the regulation of aggression. Lastly, we identified individually statistically suggestive CpGs that as a group were significantly associated with differentially expressed genes underlying aggressive behavior and also co-localize with binding sites of transcription factors involved in neuroplasticity or neurodevelopment.

Comparing scout bees to recruit bees revealed 26 regional differences in DNA methylation in the brain. Epigenetic signatures of both scouting and aggression associate with neurological genes, however different sets of genes are affected. Both DNA methylation and transcript levels were profiled in the same individual scout brains, and a general positive relationship between gene body DNA methylation and gene expression was observed. Together, these results show that epigenetic signatures can both maintain persistent behavior (scouting) and also rapidly change in response to a stimulus (aggression), possibly altering future response to a second intruder.

CANDIDATE OLFACTORY RECEPTOR PROTEIN SUBFAMILIES INVOLVED IN THE SHIFT TO EUSOCIALITY : COMPUTATIONAL SURVEY OF ORs FROM TWO SOLITARY BEES

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Evidence for a connection between Olfactory Receptor (OR) evolution and evolution of eusociality is getting stronger. The identification of OR subfamilies responsible for specific eusocial behaviours still remains elusive. Previously we discovered that the dwarf Asian honey bee *Apis florea* possesses similar number of ORs as that of the Western honey bee *Apis mellifera* (1). The study also let us transfer annotations for major receptor for queen mandibular pheromone (QMP), 9-ODA, to the orthologous OR of the dwarf honey bee. We wondered, is the number of ORs in solitary bees also similar? As the genomes of solitary bees *Dufourea novaeangliae* and *Habropoda laboriosa* became available, we could employ our in-house semi-automated computational pipeline to discover the complete repertoires of ORs from the two species. We discovered 151 ORs in *Habropoda laboriosa*, and only 112 ORs in *Dufourea novaeangliae* genomes. Further analysis, including more species, showed that most of the hymenopteran species possess higher number of ORs compared to other insect groups irrespective of their degree of eusociality. Phylogenetic reconstruction of the ORs from the 2 solitary bee species, 2 honey bee, 1 ant and 1 wasp species identified 34 subfamilies. We predicted functions of few of them based on their homology with de-orphaned ORs from other species. Interestingly, the number of ORs belonging to the putative QMP component receptor subgroup from subfamily L showed marked difference between eusocial and solitary bees, with almost no ORs in solitary bees. In contrast, OR numbers in the 9-exon subfamily - putative cuticular hydrocarbon (CHC) receptors - did not show stark difference across solitary and eusocial species. Given the ubiquity of CHC on insects bodies, they got incorporated in diverse communications contexts in social and also solitary species. Finally, we identified ten conserved upstream motifs for ORs across the six hymenopteran species. The ten motifs displayed species-lineage specific and OR-subfamily specific patterns of distribution, likely involved in the regulation of the transcription of these ORs. From these, we identified a candidate master transcription factor binding site ([A/G]CGCAAGCG[C/T]) upstream of multiple olfaction related genes, which can be studied further for its role in eusocial and other behaviours. The results of this study were published recently (2).

1. Karpe,S.D., Jain,R., Brockmann,A. and Sowdhamini,R. (2016) Identification of complete repertoire of *Apis florea* odorant receptors reveals complex orthologous relationships with *Apis mellifera*. Genome Biology and Evolution, 8, 2879–2895.
2. Karpe,S.D., Dhingra,S., Brockmann,A. and Sowdhamini,R. (2017) Computational genome-wide survey of odorant receptors from two solitary bees *Dufourea novaeangliae* (Hymenoptera: Halictidae) and *Habropoda laboriosa* (Hymenoptera: Apidae). Scientific Reports, 7, 10823.

LINKING PHEROMONE INFORMATION BIAS AND OLFACTORY NEUROMORPHOLOGY IN DOLICHODERINAE (ODOROUS) ANTS

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Conspecific olfactory cues are a principal source of information in the organization of social insect behavior. Individuals must integrate experience and pheromone cues in decision-making and, while information gleaned from primary experience is theoretically more reliable, a bias for social information may produce rapid group-level responses that are advantageous in instances of colony defense or food exploitation. Olfactory circuits for sex pheromone processing are described for a number of insects, but analogous circuits for foraging and defense pheromone processing in social insects have not been easy to identify. In this study we compare species of Dolichoderinae ants and ask what parameters affect individual propensity choose social information over individual experience, including colony size, starvation, body condition, and experience. To assess whether patterns of social information bias are reflected in particular patterns of investment in olfactory processing, we compare the sensilla density and antennal lobe morphology of these species. We use a combination of neuromorphological and neuromodulatory markers and tract tracing to present a comparison of antennal lobe structure and projection neuron morphology across species.

INFLUENCE OF LANDMARKS ON HONEY BEE FORAGING AND WAGGLE DANCING.

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Animals use diverse sensory stimuli to navigate in their environment and to recognize rewarding food sources. Honey bees use the visual attributes of the food source, such as its color, shape, and size, in addition to its direction and distance from the hive to navigate on their foraging trips. They transfer the location information of the food source to other bees if the food source is highly rewarding. To investigate the relative importance of these different attributes, we tested if bees prefer to land on food sources that are placed at other nearby locations but have the same shape and color as a feeder they have been previously trained to, compared to a feeder that is located at the same exact location but is visually different. We also tested if changing the landmarks surrounding a feeder influenced how valuable it is assessed to be by bees. We find that bees rely heavily on the color and shape of the food source but not as much on its distance from the hive, within a short foraging range. Bees did not dance more vigorously for a food source that looks similar to or different from a learned feeder as long as its quality is constant.

PATHWAY PROFILES BASED ON GENE-SET ENRICHMENT ANALYSIS IN THE HONEY BEE *APIS MELLIFERA* UNDER BROOD REARING-SUPPRESSED CONDITIONS

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Perturbation of normal behaviors in honey bee colonies by any external factor can immediately reduce the colony's capacity for brood rearing, which can eventually lead to colony collapse. To investigate the effects of brood-rearing suppression on the biology of honey bee workers, gene-set enrichment analysis of the transcriptomes of worker bees with or without suppressed brood rearing was performed. When brood rearing was suppressed, pathways associated with both protein degradation and synthesis were simultaneously over-represented in both nurses and foragers, and their overall pathway representation profiles resembled those of normal foragers and nurses, respectively. Thus, obstruction of normal labor induced over-representation in pathways related with reshaping of worker bee physiology, suggesting that transition of labor is physiologically reversible. In addition, some genes associated with the regulation of neuronal excitability, cellular and nutritional stress and aggressiveness were over-expressed under brood rearing suppression perhaps to manage in-hive stress under unfavorable conditions.

ONE MECHANISM TO RULE THEM ALL? RESPONSE THRESHOLDS TO TASK-RELATED STIMULI MAY NOT DRIVE TASK ALLOCATION IN *TEMNOTHORAX RUGATULUS* ANTS.

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Division of labor among social insect workers is thought to be the key to their ecological and evolutionary success, however it remains unclear how exactly this division of labor is regulated. Here, we aim to test one of the leading hypotheses for division of labor, the response threshold mechanism. Specifically, we (1) measure individual responses of workers to isolated, potentially task-relevant stimuli across three tasks, and (2) assess whether these responses predict task performance in the colony context in the ant *Temnothorax rugatulus.* We expose ants to different amounts of hungry brood, fungal spores, or different sugar concentrations, stimuli that appear to drive brood care, grooming, and foraging behavior. Responses to different stimulus intensities (i.e. possible response thresholds) were not associated with worker task allocation in the colony. This seems to imply that when tested in isolation, worker sensitivity or response to different stimulus intensities are not driving division of labor.

SOCIOGENOMICS OF SOCIAL PARASITISM IN *DOLICHOVESPULA* WASPS (HYMENOPTERA, VESPIDAE)

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Social parasites usurp host colonies and subdue the resident queen to exploit social benefits provided by host workers. As a consequence, selection has favored the loss of the worker caste in socially parasitic species, diverting energy towards producing only reproductives. In obligate social parasites or inquilines the worker caste has been permanently lost, and therefore these species depend entirely on the workers of a host species to raise their offspring. Comparisons between social parasites and their hosts enable the discovery of novel molecular features associated with the loss of the worker caste and the gain of traits assumed to be associated with the socially parasitic lifestyle (e.g., recurved stings, large mandibles, chemical mimicry). Here, we conduct a sociogenomic analysis of the inquiline *Dolichovespula arctica* and its primary host, *D. arenaria*. Specifically, we test the hypothesis that worker-biased genes are no longer expressed in social parasites. We sequenced the genomes of the host-parasite species pair and 24 RNA-Seq libraries from brains of inquilines as well as foundresses, queens, and workers of the host species. We identified worker-biased genes in the host species and evaluated the expression patterns and rates of evolution of such genes in the parasite.

IMMUNITY IN BEES: COMPARATIVE ANALYSES AMONG BEE GENOMES, INCLUDING THE EUSOCIAL BEE *FRIESEOMELITTA VARIA* (APIDAE, MELIPONINI)

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Insects have a well-developed innate immune system that responds rapidly against foreign agents and pathogens. This immune system relies on both cellular and humoral responses that are generated by signaling pathways triggered upon recognition of patterns on the pathogen surface by receptors. Cellular responses include phagocytosis, nodulation and encapsulation mediated by hemocytes while humoral responses are based on the synthesis of antimicrobial peptides (AMPs). These AMPs are mainly produced by the fat body and secreted into the hemolymph. The pathogens that the insect may face are bacteria, fungi and viruses. These pathogens trigger four major signaling pathways: Toll, Imd, JAK/STAT and RNA interference (RNAi), involved in AMPs synthesis, phagocytosis/encapsulation/nodulation and antiviral immunity. In eusocial bees, besides this individual immune system, there is a social immunity that comprises hygienic behavior and nest protection with resins displaying antimicrobial properties (propolis). Although the eusocial honey bee has considerably fewer immune genes than many other insects, this feature is not exclusively due to social immunity, once solitary bees have also a similar low number of immune genes. Here, we compare 190 immune-related genes of the honey bee with their corresponding orthologs from genomes of 12 other bee species, including *Frieseomelitta varia*, a neotropical eusocial bee. From the 190 genes, we found 174 immune-related genes in *F. varia*. This gene number was similar to other solitary or social bees. Canonical genes from the Toll, Imd, JAK/STAT and RNAi pathways were also found in this genome, and phylogenetic analyses grouped *F. varia* genes with their corresponding homologs in other bees. We also validated the expression of four immune genes (cactus, relish, hymenoptaecin and defensin) in *F. varia* workers by qPCR using larva, pupa, newly-emerged and foragers, submitted or not to bacterial infection. Higher expression levels of the signaling genes cactus and relish were detected in non-inoculated foragers while the AMP gene hymenoptaecin was up-regulated in infected bees. These results resemble those previously reported for *A. mellifera*, where foragers exhibited activation of the immune system and infected bees a greater magnitude of AMP genes up-regulation compared to the signaling/recognition genes from Toll and Imd pathways.

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DIVISION OF LABOR, NEUROANATOMY, AND BRAIN GENE EXPRESSION IN FUNGUS-GROWING ANTS

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Complex social organization and division of labor among sterile workers are prominent features of fungus-growing ants, although attine species show broad variation in degree of worker polymorphism and behavioral differentiation. In derived genera such as *Atta*, workers size variation is striking, and subcastes differ in morphology and task performance in association with the agricultural life history of this species. Behavioral differences and specializations across subcastes likely relate to patterns of allometric scaling among functional regions of the brain. We found that higher-order processing regions (mushroom bodies) decrease and some sensory compartments (e.g. optic lobes and antennal lobes) increase in size disproportionately to total brain volume in larger workers. Differences in brain gene expression could underpin subcaste differences in tasks such as brood care and fungus tending performed by the smallest workers, leafcutting and leaf fragment transport performed by medium sized workers, and defensive behaviors performed by the largest workers. To identify patterns of gene expression in light of brain structure and attine social evolution, we sequenced and assembled transcriptomes from brains of *A. cephalotes* workers, which are highly polymorphic, and the basal monomorphic *Mycocepurus goeldii*. Initial observations suggest variation among *A. cephalotes* polymorphic workers and between the two species sampled. We are also currently measuring gene expression in isolated functionally specialized regions of the brain.

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 and ratios of observed to expected CpG dinucleotides (CpG O/E) 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 and highest mean CpG O/E compared to the other species. The distributions of CpG O/E were skewed to the left in all social hymenoptera, except for the simple eusocial bee, *Lasioglossum albipes*. 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.

EVOLUTIONARY CAUSES AND CONSEQUENCES OF HIGH RECOMBINATION RATE IN THE ANT *CARDIOCONDYLA OBSCURIOR*

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Eusocial insects exhibit the highest rates of homologous recombination reported so far for multi-cellular eukaryotes. The ultimate and proximate reasons for these high rates are not understood although they might represent some of the strongest evidence for adaptive evolution facilitated by homologous recombination. We present a linkage map based on 85 F2 recombinant males from a cross between two populations of the invasive ant Cardiocondyla obscurior. The map contains 3,130 SNPs and spans 1,793.9 cM. The estimate of overall recombination rate of 10.0 cM/Mb lies within the range of other eusocial hymenopteran species. Local recombination rate correlates negatively with transposable element content, and positively with single nucleotide polymorphisms, structural variants and simple sequence repeats suggesting that high recombination rates could counteract genetic homozygosity caused by low effective population size in social insects. Genes with biased expression among individual third instar larvae towards wingless males were located in regions with higher recombination rates compared to genes biased towards winged males, suggesting that selection for the evolutionary young innovation of wingless males is correlated with locally increased recombination rates, rather than facilitating female queen and worker phenotypic complexity. Finally, local recombination rates correlate positively with genes involved in insect innate immunity which are expected to be under diversity-enhancing selection especially in dense societies. Together this study highlights the role of recombination as a major driver of social insect genome evolution.

THE EFFECT OF UNCONTROLLABLE STRESS ON PLASTICITY AND STRESS RELATED GENE EXPRESSION IN THE HONEY BEE, *APIS MELLIFERA*

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Stress has been defined as any deviation from an organism’s baseline physiological levels. These deviations might cause alterations in the organism, which elicit cellular and neural adaptations as well as hormonal responses and proteomic shifts. For instance, stress could increase recall of highly relevant stimuli, and long-term exposure to high-risk situations decrease recall ability. The objective of this project was to explore if learning can cause physiological stress using honey bees (Apis mellifera) as a model. We focused on some of the genetic markers for stress in honeybees, and we also included genes associated with learning. Helpless learning and stress in honeybees has been modeled using a shuttle box protocol designed by Dinges, et al., 2017, where an apparatus may shock bees during learning assays. The box complex is divided in two independent lanes which are subdivided in two colored sections. This followed a discrimination task, in which we assigned a color that shocks one area in a lane, where one bee controls the shock for itself (“master” bee) in that lane, and another bee was subjected to shock events only when the “master” bee was shocked (“yoked” bee), independently of the area it was standing on. Results show that expression of stress related genes was not significantly altered by uncontrollable stress or helpless learning. When we looked at plastic genes, results show a significant increase in Dopamine receptor expression in “yoked” samples. This suggest that “yoked” bees, might be effectively losing learning potential since shock stimuli come from a random factor that they can’t respond to. It is still uncertain if learning can cause additional physiological stress or if its effect might be masked by the direct response to an environmental stressor.

THE GENETIC BASIS OF HYGIENIC BEHAVIOUR AND OVERWINTERING MORTALITY IN THE HONEYBEE

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The honeybee is an essential managed pollinator, making the recent high colony mortality concerning. Honey bee colony losses in Canada are often linked to harsh winters and disease. There is clear evidence that some aspects of the social immunity – the ability of honey bees to resist disease through social behaviors - are heritable, and there is tangential evidence suggesting that overwintering mortality is also heritable. Finding the genetic basis of both hygienic behavior and the abilities of colonies to survive the winter would immensely improve our abilities to breed disease resistant and winter hardy honeybees. The BeeOMICS consortium has sequenced the genomes of approximately 1,000 honey bee colonies in Canada, which were evaluated for a number of traits, including the hygienic behavior and overwintering survival. I will use the principles of Genome Wide Association Studies to identify genetic mutations that explain between colony variation for both of these traits; these markers can potentially be used to breed honeybees better equipped to handle disease and harsh winters.

GENE EXPRESSION PROFILES ASSOCIATED WITH COMMUNICATION SKILLS IN GOLDEN ANTS

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Many empirical and theoretical studies investigated the frequency at which group members make use of information transfer among them, that is, social information use. Although social information use is considered highly beneficial, in some highly social species some individuals engage exclusively in individual information acquisition. While there has been much empirical research on factors affecting the frequency of social information use, the evolutionary perspective has mainly been theoretically addressed. Here, we conducted behavioral experiments coupled with functional genomic analyses to identify genes underlying the observed variation in social information use in the ant Camponotus sericeus (Forel). C. sericeus workers recruit via tandem running. We predicted that the difference between ants participating in tandem runs and ants foraging alone is associated with differences in their gene expression. We used a tracking system to monitor individually-tagged workers of five colonies as they foraged in a 1.5 m long arena, and analyzed tandem running events. Forty individuals were selected based on their behavioral characteristics to represent three behavioral types: tandem leaders, tandem followers, and solitary foragers. RNA-seq libraries were constructed from total head mRNA extracts and sequenced to produce a total of 1083 million reads, an average of 27 million reads per sample. The reads were mapped to a de novo transcriptome assembly to quantify gene expression. Gene expression analysis identified genes differentially expressed between the behavioral types, as well as association between gene expression levels and behavioral characteristics of the tandem run, which are indicative of the strength of communication between the leader and follower. These results will provide first candidate genes for elucidation of the genetic and evolutionary basis of the sophisticated communication observed in ant societies.

EXAMINING THE DISTRIBUTION OF RECENTLY IDENTIFIED BEE-INFECTED VIRUSES IN MANAGED HONEY BEE (*APIS MELLIFERA*) POPULATIONS IN THE UNITED STATES

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A key component to the global deterioration of pollinator health is the widespread infections of bee populations by several distinct viruses. The US National Honey Bee Disease Survey annually examines the infection status of managed honey bee (*Apis mellifera*) colonies in apiaries across the United States, recording detection and prevalence of eight different viruses. These eight viruses are commonly observed in honey bee populations and have documented negative impacts on honey bees, but this suite of viruses is only a fraction of the viral diversity that has been identified in bee populations. We recently conducted a metagenomic survey of the viromes of bee communities across four continents and Oceania, identifying 29 viral contigs representing nine viral families, six of which were previously unidentified in bees. In addition to (+)ssRNA viruses, which are most commonly found in honey bees, this study also identified sequences corresponding to (-)ssRNA, dsRNA, and circular ssDNA viruses. Notably, many of these potential viruses were identified in both *Apis mellifera* honey bees and in co-foraging, non-*Apis* specimens, suggesting the potential for a wide distribution of these viruses across bee communities. To assess the prevalence and distribution of these newly identified pathogens in the United States, we screened previously collected samples from the US National Honey Bee Disease Survey. The results of this screen will determine if the viruses infecting the US bee population are more diverse than previously realized, potentially demonstrate associations between different types of viruses, and identify viruses that should be assessed for pathogenicity in future studies.

UNRAVELING CRYPTIC SPECIATION IN THE DESERT ANT *CATAGLYPHIS*

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Species are the fundamental units of which evolutionary research is based upon. In insects, due to high level of hybridization, the delimitation of these species could be challenging. The genus *Cataglyphis* comprises high level of diversification, making it a great model to study evolutionary paths. In Israel there seems to be a hot spot for recent speciation in this genus. Our study focuses on a seemingly species complex that is distinguishable by mitochondrial DNA but not through nuclear DNA. They also differ in their social structures. While generally the different species are allopatric, in our study site they all coexist. Using microsatellite markers analyses the three species seem to be all a part of a supercolony. In contrast, the three species are readily distinguished by their cuticular hydrocarbons (CHC). To resolve this apparent discrepancy, we conducted behavioral essays which showed that indeed only one of the suspected species creates a supercolony, consistent with the CHC's results. Possible explanation for this could be low discriminance by males of these species. These results support the assumption of cryptic speciation in this genus. We are currently attempting to delimit these species using RAD sequencing as well.

ASSOCIATION MAPPING TO STUDY THE GENETICS OF HONEY PRODUCTION AND AGGRESSIVE BEHAVIOUR IN HONEY BEES

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Most beekeepers prefer to work with gentle colonies that produce a lot of honey. There is strong evidence that both honey production and colony defense are heritable traits that can be improved by artificial selection. The BeeOMICS consortium has sequenced the genomes of approximately 1,000 honey bee colonies in Canada, which were evaluated for a number of traits, including the honey production and defensive behaviour. Here I applied modern computational methods to uncover associations between the frequency of specific genetic mutations within honey bee colonies and the colonies' phenotypes. This represents the first genome-wide association study of these traits in honey bees.

COMPARATIVE ANALYSIS OF HONEY BEE GENOMES REVEALS GENUS EVOLUTION

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The Western Honey Bee (*Apis mellifera*) is the best-studied species in its genus and shares numerous characteristics with its congeners, such as a perennial lifestyle in highly complex colonies with one multiply-mated queen and thousands of functionally sterile workers that construct wax combs for brood rearing and honey and pollen storage. The similarity extends to the molecular level with the evolution of major royal jelly proteins and exceptionally high recombination rates throughout the genus. However, the different honey bee species have independently evolved for millions of years, resulting in some interesting biological differences in anatomy, life history, and behavior. Here, we present the annotation of the dwarf (*A. florea*) and giant honey bee (*A. dorsata*) genomes that were completed to facilitate the study of molecular evolution of *Apis* at the genomic scale with all three subgenera. We present comparative data on gene inventories and diversification with special attention to select gene families. Using bumblebees as outgroup, these results are complemented with a global analysis of selection in all 1:1:1:1 orthologs. In addition to overall trends via GO-analysis of the genes under selection, we focus on potential explanations for body- and ovary-size differences among species and the genus-wide upregulation of recombination rates. Furthermore, we report genome-wide support for the basal taxonomic position of *A. florea*, confirming that cavity nesting in honey bees is a derived trait.

TRANSCRIPTOMIC ANALYSIS OF JUVENILE HORMONE SIGNALING IN BUMBLE BEE (*BOMBUS TERRESTRIS*) WORKERS: IMPLICATIONS FOR SOCIAL PHYSIOLOGY

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Juvenile hormone (JH) is a major endocrine factor controlling metamorphosis and reproduction in diverse insect species. In social insects, JH is involved in both the regulation of individual physiology and eusocial traits like caste determination and division of labor. In bumble bee workers, JH acts as a gonadotropin, controlling ovary development and reproductive physiology and behavior. However, little is known about the molecular mechanisms mediating these JH influences. We used JH-producing gland removal and replacement therapy to manipulate circulating JH titers followed by brain and fat body RNA sequencing to identify genes and molecular pathways regulated by JH in bumble bee workers. We identified hundreds of genes in the brain and fat body that are influenced by JH. Using KEGG pathway enrichment analysis, we found that JH affected the expression of ribosomal and proteasomal proteins, suggesting that this endocrine signal regulates protein turnover in both tissues, but cellular ribosomes appear to be downregulated in the brain while mitochondrial ribosomes are upregulated in the fat body. Another difference is that JH upregulated metabolic pathways like oxidative phosphorylation in the fat body but not in the brain. Comparing dominant and subordinate workers, we found that brain gene expression in dominant bees was more closely related to bees with high JH, suggesting that JH signaling is involved in social ranking and contributes to the formation of reproductive castes. This study begins to uncover the molecular mechanisms underlying JH control over reproductive physiology in order to better understand how social signals can affect this important pathway. Implications for the evolution of castes in bumblebees also will be presented.

THE GENOME OF THE EUSOCIAL *FRIESEOMELITTA VARIA* STINGLESS BEE: A MODEL SPECIES FOR REPRODUCTIVE DOMINANCE STUDIES

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Frieseomelitta varia is a stingless bee species characterized by a remarkable reproductive behavior. The queen holds the monopoly of reproduction, and there is no register of egg-laying workers, even in the absence of the queen, that favors worker oviposition in most bee species. The complete queen dominance makes *F. varia* a key species in the field of comparative genomics to understand caste systems evolution, division of reproductive labor, and eusociality.

We sequenced the genomic DNA of a single *F. varia* pharate-adult male using the Illumina protocol in the HiSeq2500 platform. The obtained reads were mapped against the reference genome of the phylogenetically closely related bee, *Apis mellifera*. Manual curation was used to assess the quality of genome annotation, and gene prediction was validated by comparison with a *F. varia* RNA-seq library and with protein data available for several bee species. A Gene Ontology enrichment analysis allowed us to assign functional characteristics to predicted genes.

The genome of *F. varia* comprises ~275 Mb with a total of 10,526 genes. A large portion of these genes (8,200) is orthologous with *A. mellifera* genes. Based on the chromosome position of *A. mellifera* orthologs, the *F. varia* scaffolds were joined in tentative linkage groups. Special attention was given to the genes related to reproduction and ovary activation, which were characterized in relation to other insect species. Genes of the innate immune system were used in a RT-qPCR procedure aiming at validating gene predictions. We identified sets of non-coding RNA genes including tRNAs, snRNAs, rRNAs, sno/scaRNAs, and micro-RNAs. The mitochondrial genome of *F. varia* was assembled by mapping the 9,518,616 reads against mitochondrial genomes of several members of the Apoidea superfamily. The mitochondrial genome is 15,144 bp in length, and encompasses 13 protein-encoding genes, 22 tRNAs, 2 rRNAs, and a non-coding region. Phylogenetic analysis using *F. varia* mitochondrial genes showed the closest relationship to *Melipona* species.

This study provides a foundation for post-genomic researches on bee systematics and evolution. In addition, the use of *F. varia* genome in comparative studies has the potential to elucidate queen dominance mechanisms leading to a complete or partial inhibition of ovary activation in the workers.

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OSIRIS GENE EVOLUTION - ESSENTIAL YET MYSTERIOUS

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Novel processes sometimes require novel genes, and these genes often originate from pre-existing genes via gene duplication events followed by neo-functionalization. The Osiris gene cluster is a group of ~20 genes with highly conserved synteny. The cluster appears to have originated from a single progenitor gene at the origin of the insects. While not all of the genes in the cluster seem to be essential, and some have little to no expression, some members of the cluster are the most highly expressed genes at some time points of development and the loss of these genes is lethal. When expressed, the genes in the cluster tend to be highly correlated, and recently duplicated genes show high levels of correlated expression, suggesting that there might be similarity or redundancy of function. Some genes in the cluster are essential for development, and some are also differentially expressed during development of alternative phenotypes. Further, these genes are often associated with immune and toxin-degrading responses. The precise function of the genes is unknown, but indirect evidence suggests that they are involved in cuticle function and possibly part of the endolytic pathway. Like the function, the origin and rapid expansion of this gene family are enigmatic. Current work suggests that the progenitor Osiris gene may be present in nearly all life and involved in detoxification - if true, the plant branch of this gene family has also greatly expanded and function in plant immune response and detoxification. The role these genes play in the social insects is still unclear, but they are differentially expressed between castes, and it is speculated that they function in regulating wing development. While much about these genes is still mysterious, they are emerging as major players in insect (including social insect) evolution.

GENOMIC IMPRINTING IN SOUTH AFRICAN HONEY BEES

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Colonies of haplodiploid Hymenopteran (ants, bees, and wasps) insects often served as model systems for examining how cooperative behaviour can evolve. More recently, insect colonies have emerged as important systems for understanding within-genome conflict.

In eusocial species such as honey bees, queens are polyandrous and mate early in life with an average of 12 males. The queen stores the spermatozoa of each drone in an organ known as the spermatheca, and utilises this stored sperm to fertilize queen- or worker-destined eggs. Honey bee colonies are therefore comprised of subfamilies of workers each of which share the same father. This generates a situation that is ripe with potential for conflict between males to increase the reproductive success of their female offspring. A father that can influence the expression of genes in offspring so that his daughters are more likely to develop as a queen or a reproductive worker has a much greater probability of reproductive success than another male that does not do so. In conrtast, the queen should be selected to reduce the effect of these paternal modifications that influence worker reproduction to ensure that she maintains her monopoly on reproduction. However, the queen must ensure that her alterations only decrease the fecundity of workers and not her future male and queen offspring. As a result, the imprinting of genes affecting reproduction are more likely to evolve in haploid drones (fathers) than in queens.

Recently, genes that influence worker reproduction have been found to have paternally-biased expression. That is the allele derived from the father is expressed whereas the maternal allele is switched off. This suggests reproductive conflict between the paternal and maternal genomes of females. The underlying mechanism that influences this maternal and paternal bias remains unknown, but DNA methylation has been proposed as the most likely mechanism.

To identify the genes that are modified by mothers and fathers and the mechanisms underlying differentially expressed genes, we generated replicate reciprocal crosses between two African honey bee subspecies, *Apis mellifera capensis* and *A. m. scutellata*. We then sequenced both parents (whole-genome) and offspring (transcriptome and methylome). The current findings of this work will be discussed.

SOLDIER-BIASED GENE EXPRESSION IN A TERMITE IMPLIES INDIRECT SELECTION FOR DEFENSIVENESS

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In a termite colony, reproduction is monopolized by a small number of sexuals that are supported by a large number of reproductively altruistic soldiers and workers. A recent RNA-Sequencing analysis of the Eastern subterranean termite *Reticulitermes flavipes* revealed, not unexpectedly, that castes are associated with clear-cut differences in gene expression. What Wu et al. (2018) did not predict, however, was that a majority (78%) of differentially expressed genes would show a soldier-specific pattern. This soldier-bias in genome-wide expression suggests that the defensively specialized caste is well-differentiated from both the reproductive (nymph) and the other non-reproductive caste (worker) of this species, despite a shared developmental program with workers.

The strong soldier bias suggests novel use of certain genes that are presumably related to this caste's defensive role. This pattern has led Behl et al. (in review) to propose that termite soldiers might in general be a source of genetic novelty. They used genomic phylostratigraphy to test this idea and showed that genes with soldier-biased expression are indeed more taxonomically restricted on the tree of life than are genes with no caste-biased expression. This restricted pattern suggests that caste-biased genes are relatively young and implies past selection for novel gene recruitment during termite caste evolution. Given that *Reticulitermes* and most termite soldiers are sterile, any past selection for genetic novelty in this caste must have been mediated indirectly through reproducing relatives.

The termite transcriptome data may therefore lend itself well to testing broader ideas on how indirect selection affects the rates of molecular evolution. On the one hand, genes indirectly selected for selfless defense and sterility may experience relaxed adaptive molecular evolution, relative to genes directly selected for reproduction. On the other hand, genes specifically associated with these traits may be less constrained and thus, free to evolve rapidly. These contrasting 'signatures of kin selection' remain unresolved (Linksvayer and Wade 2016). In this study, we exploit allelic diversity captured in the newly available RNA-Sequence data set of Wu et al. (2018) to test for differences in the rate of adaptive molecular evolution across soldier-biased and un-biased genes from the Eastern subterranean termite.

Wu T, Dhami GK, Thompson GJ (2018) Evol Dev 20:3-16
Behl S, Wu T, Thompson GJ (In review) Insect Soc
Linksvayer TA, Wade MJ (2016) Front Ecol Evol 4:65

WOLBACHIA SYMBIONTS IN LEAF-CUTTER ANTS DIFFER IN HORIZONTAL TRANSMISSION POTENTIAL AND FUNCTIONAL CAPABILITIES

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Strict vertical transmission and strategic host reproductive manipulation are commonplace for intracellular microbial symbionts. However, phylogenetic incongruences between host and symbiont suggest horizontal transmission events are critical to invasions of novel host lineages. *Wolbachia* is notorious for manipulating host reproduction to favor its transmission through the female germline. However, its natural potential for horizontal transmission remains poorly understood, with the expectation that such transfers require an intimate relationship between host species. We studied the relatedness of *Wolbachia* strains in the Panamanian leaf-cutting ant *Acromyrmex echinatior* and its inquiline social parasite *A. insinuator*. Sequencing of the *wsp* gene showed that each ant species harbored one dominant species-specific strain and one shared strain. The shared strain is commonly found in other neotropical ant species belonging to a wide range of ant subfamilies, but the two non-shared strains appear to be rare across other ant species. This suggests that obligate social cohabitation may not be a general facilitating factor for horizontal transfer and that *Wolbachia* strains associated with ants may have different potential for horizontal transmission. To examine whether these distribution patterns indicate that the species-specific *A. echinatior* strain is adapted to its host, we performed Illumina and PacBio sequencing to assemble and annotate the first ant-associated *Wolbachia* genome. Phylogenetic reconstruction using previously sequenced strains indicates ant-associated *Wolbachia* are a sister clade to *Wolbachia* supergroup A. Genome comparisons, however, reveal key differences exist in this strain relative to closely related strains from solitary insects. Among these differences is the presence of vitamin biosynthesis genes, which transcriptome data indicate is being expressed in brown pupae and worker life stages.

BRAIN GENE EXPRESSION RESPONSE TO PESTICIDE EXPOSURE INDICATES EFFECTS ON COGNITION.

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Insect pollinators including social bees are key to ecosystem stability as well as agricultural yields. Recent declines in social bees worldwide are thus concerning. A major factor implicated in these declines is the application of pesticides to crops. These are intended to control pest species, but can also negatively affect non-target wild pollinators. Behavioral and field studies have clearly demonstrated that exposure to neonicotinoid pesticides negatively impacts learning and memory abilities, foraging behavior and colony survival of social bees.

We know relatively little however about the molecular mechanisms by which pesticide exposure affects bee cognition. To address this, we exposed *Bombus terrestris* bumblebee colonies to commonly used pesticides. We find widespread effects of pesticide exposure on the gene expression in the brain, identifying candidate pathways involved in detoxification. We describe the signatures of selection on these genes in wild populations. Our work demonstrates a novel, straightforward manner of quantifying the effects of pesticide exposure.

HONEY BEE MALES PRODUCE SEMEN WITH UNIQUE DNA METHYLATION PROFILES

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Kin-selected conflicts are an inherent feature of social evolution. Honey bee queens are extremely polyandrous, so each colony is comprised of up to 30 worker patrilines, each sired by a different father, and possessing different reproductive traits. This high genetic diversity paves the way for genomic conflicts. Genes favouring worker reproduction are indeed predicted to be over-expressed when paternally inherited, while under-expressed when derived from the queen. Epigenetic modifications of the genome such as DNA methylation have the potential to regulate gene expression in a parent-of-origin manner. The existence of sperm-specific methylome profiles could lead to patriline-dependent reproductive traits. However, the individual specificity of epigenetic marks has never been investigated in social insects.

In this study, we compared genome-wide methylation profiles of the semen of three individual males. We found approximately 20,000 methylated sites in over 4,000 genes in each semen sample. While a large number of core methylated sites were common to all individuals, about one third were variable between each individual semen methylome. Average methylation levels at each site were over 95%, suggesting that methylation remains consistent in all individual spermatozoa within each male's semen. We saw between 200 to 300 differentially methylated genes (DMGs) in pairwise comparisons between semen methylomes. Further, we found a number of single nucleotide polymorphisms (SNPs) in regions with methylated sites. DMGs were twice as likely to contain SNPs compared to other genes, suggesting the importance of allele-specific methylation. Our results indicate that males harbour individual-specific DNA methylation patterns that are often associated with genotypic variation. This suggests that pooling samples of workers derived from different patrilines could introduce biases in methylome sequencing projects. Overall, our study shows that male honey bees differ widely in their methylomes, with epigenetic specificity being strongly linked with genotype. We discuss the evolutionary implications that this double layer of genetic and epigenetic variability could have for the expression of kin-selected conflicts in insect societies.

SOCIOGENOMICS AND THEORY - POTENTIAL AND PROBLEMS

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Social insect biology has traditionally been a field where empirical biology is interacting closely with theoretical biology. As social insect research is advancing faster and faster into modern genomics, there is a risk of losing this strength from mutual feedback with theory. In this talk, I will highlight questions, approaches and potential problems in bringing social insect genomics closer to theoretical work in the face of real-life complex nature of genomic and transcriptomic data.

MECHANISMS OF THE MAJOR EVOLUTIONARY TRANSITION TO SUPERORGANISMALITY

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Life on earth has been shaped by a series of rare but important events termed major evolutionary transitions; the evolution of multicellularity and superorganismality are perhaps two of the most striking and well-studied examples. Major transitions are predicted to develop in a series of stages under specific types of ecological conditions. There is also a growing body of empirical work on the molecular mechanisms underpinning major transitions in several model species. More work is needed, however, on reconciling predictions from social evolution theory about the ultimate evolutionary explanations for major transitions with predictions from sociogenomics about the underlying mechanisms of major transitions. Many empirical studies tend to focus on studying the characteristics of species that have already undergone a major transition, which is potentially problematic if one wants to understand the mechanisms that facilitate the occurrence of evolutionary change during the early or intermediate stages in a major transition. Here, we develop hypotheses about how different types of proximate mechanisms---phenotypic uncoupling, novelty, and plasticity---are likely to play distinct roles in the different stages of a major transition. We argue that empirical work on non-model organisms exhibiting the characteristics of species in the early and intermediate stages of a major transition will be needed to test these hypotheses and present results of our own tests from comparative sociogenomic analyses in a range of Vespid wasps.

FREQUENCY-DEPENDENT SELECTION OF A RECENTLY DERIVED SOCIAL PARASITE IN THE CLONAL RAIDER ANT

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Many ant species are host to inquiline social parasites, genetic lineages with constitutively queen-like morphology that exploit closely related wild-type colonies. Here we describe a recently derived socially parasitic lineage in the clonal raider ant, *Ooceraea biroi*. These parasites possess a host of heritable phenotypic differences from wild-types, including aberrant queen-like development of the ovaries and wings. The parasites lay eggs at a higher rate than wild-types, but this fitness boost is balanced by an elevated mortality during eclosion that increases as the parasites become more common within a colony. This parasitic lineage is therefore under negative frequency-dependent selection, allowing them to stably exist in wild-type colonies without going extinct or going to fixation. In contrast to the complexity of the mutant phenotype, microsatellites and whole-genome resequencing indicate that the parasites are derived from one of the previously described clone lines of *O. biroi*, and differ from wild-types at a small number of loci genome-wide. It is therefore likely that one or a few highly pleiotropic mutations gave rise to the parasitic phenotype. This conclusion, if true, would be relatively surprising in the context of classical evolutionary theory, in which pleiotropic mutations are expected to impede adaptive evolution because rare positive phenotypic effects are accompanied by more common negative effects. We propose that pleiotropic mutations may actually facilitate the evolution of certain kinds of adaptive phenotypes, such as the recurrent origin of inquiline social parasites in ants.

NESTMATE RECOGNITION CUES MATURE VIA SOCIALLY-REGULATED ONTOGENETIC PROCESSES IN THE HONEY BEE.

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Nestmate recognition, an important mechanism of colony defense in eusocial insects, depends on guarding individuals who use colony-specific cues on incoming conspecifics to determine if they are nestmates or non-nestmates. Robust nestmate recognition systems depend on the ability of large groups of animals to express common, colony-specific cues, which in the honey bee are comprised of pheromonal cuticular hydrocarbons (CHC). The widely accepted “Gestalt” model of nestmate recognition cue acquisition states that compounds used in recognition are shared by all members of the hive via passive transfer mechanisms, which lead to the emergence of a complex unique signal that is carried by all members of the colony. Although data support the gestalt model in some ant species, its applicability to other eusocial insect species remains unknown. Therefore, we tested this model in the honey bee, Apis mellifera by using a combination of colony demography manipulations, and behavioral, molecular, and chemo-analytical experimental approaches. We found that in contrast to predictions of the gestalt model, individual honey bee workers undergo quantitative and qualitative changes in their CHC profile as they mature, which are likely driven by intrinsic developmental regulation of the CHC biosynthetic pathway that is influenced by the individual’s social environment and is associated with the physiological processes that regulate the age-dependent division of labor in this species. Furthermore, we found that only forager honey bees seem to carry the colony-specific cue recognized by guard bees. Together, our data suggests that instead of following a gestalt model, nestmate recognition cues are acquired by individual honey bees via socially-regulated ontogenetic processes.

CONFLICT AND SHARING IN THE BACTERIAL GUT COMMUNITY OF SOCIAL BEES

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A distinctive bacterial community resides in the hindgut of each honey bee worker worldwide. About eight core species make up the bulk of this community, with each showing genomic and functional variation among strains. A closely related set of bacteria live in other social bees, including all bumble bees and many stingless bees (which are less studied).

These species can be grown in the laboratory, genetically manipulated, and introduced into symbiont-free hosts, enabling many kinds of experiments on how particular bacterial types or combinations affect their hosts. A lot has been discovered about the roles of bee gut symbionts using these approaches. It has become clear that the bee gut community affects disease resistance, nutrition and digestion of pollen, growth and appetite, endocrine signaling and gene expression. While many of the functions of the gut community appear to benefit their hosts, and to thereby extend the persistence of the community itself, these gut bacteria also devote considerable resources to competition and conflict. This talk summarizes some of the recent findings on bee gut communities from experimental and genomic studies. Many intriguing questions remain, including how social structure of the host affects the evolution of cooperative tendencies within the gut communities.

HOLOBEE 2.0: LEVERAGING METAGENOMICS FOR HONEY BEE HEALTH AND EVOLUTION

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Honey bees and other social insects interact with a variety of microbial organisms, some of which are beneficial while others attempt to kill them. For a much larger set of microbial associates, actual impacts on honey bee fitness have not been established. Honey bees also support several arthropod parasites, from mites to beetles and flies, leading to severe impacts on individual and colony health. As genetic and genomic resources expand, we hope to place these resources into a categorical public archive, Holobee. This archive will be curated by the community, and maintained as both an ‘all-inclusive’ sequence resource suitable for gleaning as many extra-bee organisms as possible (Holobee-Mop) and a curated single-copy resource better suited for quantifying the relative levels of species and strains (Holobee-Barcode). We will describe cases where this approach has given insights into honey bee health and the dynamics of their metagenomic communities. Current resources are at https://data.nal.usda.gov/dataset/holobee-database-v20161, and community participation in improving these resources is strongly desired.

STINGLESS BEES AND MICROBES: DIVERSITY AND DYNAMICS IN THE HIVE

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Social bees harbor distinct microbial communities in their intestines, usually consisting of few symbiotic resident species that constitute the bulk of the community and a small proportion of diverse transient microbes acquired from the environment. There is growing evidence that the microbiota of social bee species has co-evolved with the respective hosts, therefore every species-specific resident microbiota constitutes an individually evolved answer to the similar symbiotic host demands, such as degradation of recalcitrant food components, production of essential nutrients, and antagonism against pathogens and transient microbes. The honey bee *Apis mellifera* is established as model system for basic microbiota research. In this context, the >300 stingless bee species represent an underexplored reservoir of phylogenetically distant but functionally similar microbial communities. Since the food spectra (mostly nectar and pollen) are very similar, the metabolic and inhibitory properties of the symbiotic microorganisms can be directly compared and may thereby offer new insights into underlying mechanisms of microbial symbiosis. In this study we explore the intestinal bacterial communities of two sympatric, but distantly related species of Amazonian stingless bees, *Melipona seminigra* and *Duckeola ghilliani*. We study the microbial communities in the different gut compartments, as well as in fresh and stored pollen and in the derived larval food by 16S rRNA and metatranscriptome analysis. Thereby we obtain for the first time an comprehensive overview of the microbiota and the microbial succession from pollen to larval food in these stingless bees, providing novel insights into the diversity and dynamics of their microbial community. Our study will contribute to a better understanding of the interaction of social insects with their environment.

ANTIBIOTICS IN BEE HIVES INFLUENCE GUT MICROBEEOTA, METABOLISM, AND BEHAVIORAL DEVELOPMENT

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The gut microbiota, at times compared to a prokaryotic organ of eukaryotes, has been shown to be important for regulating behavior aside from other effects on the organism. Gut microbiota may alter endocrine regulation of brain gene expression. Honey bees show an intricate behavioral development, where adult bees emerge from cells and perform different jobs in the colony as they age. There is a typical lipid metabolism profile that follows the main job and age stages. Young bees have low adiposity, and the fat reserves increase with age, peaking in middle age nurse bees that tend to the brood. Adiposity decreases, as they shift from inside jobs to outside jobs such as foraging. However, role of microbiota in this gut-brain axis has not been studied. We hypothesize that bee microbiota ontogeny and host development are integrated and that antibiotics can alter this interaction, with large consequences for the organism. Here we studied the effect of antibiotics on the honey bee gut microbiota, metabolism, and behavior throughout honey bee individual development. The natural microbiota diversity, was similar during bee development but the composition varied, and clustered in two well defined groups, one of early stages, and another of late development. Exposure to beekeeping antibiotics, altered the developmental pattern of the bee microbiota, metabolism, and behavior. Antibiotic exposure reduced microbiota diversity and numbers and doubled bee adiposity. The timing of antibiotic, at adult or larval stages had distinct consequences for age at onset of behaviors such as cleaning, nursing and foraging. This behavioral development in honey bees is typically under endocrine regulation. These results support that microbeeota has an integral role in typical endocrine regulation of metabolism and behavioral development.

MOLECULAR UNDERPINNINGS OF COOPERATION AND CONFLICT IN *POLISTES* WASPS

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Polistes wasps form small colonies exhibiting an intriguing mix of cooperative, social behavior as well as aggressive conflict. Females in *Polistes* colonies show variation in their reproductive and nutritional physiology that correlate strongly with their social role. Here, I will describe a series of studies integrating genomics with experimental manipulations to address how the delicate balance of cooperation and conflict is orchestrated in *Polistes* colonies. To study these questions, we used experimental manipulations of environmental factors that influence social state in Polistes wasps (nutrition level, maternal behavior), measurements of physiological and molecular correlates of individual behavioral differences, and functional genomics approaches in natural populations of *Polistes* wasps. Results indicate highly nourished individuals are less likely to be cooperative, and invest in their own reproduction; whereas nutritionally depleted individuals are more likely to cooperate with others. These inter-individual differences in social behavior appear to be mediated by deeply conserved genes associated with reproduction and nutrient signaling. These results provide molecular evidence supporting the idea that resource limitation is an important factor in the evolution and maintenance of cooperation in social insects.

FROM SOLITARY TO EUSOCIAL: COMPARATIVE GENOMICS OF VERY EARLY STAGES OF INSECT SOCIAL EVOLUTION

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Understanding how eusociality evolves has always been a major and highly challenging goal of evolutionary biology. Genomic tools are now beginning to allow us to directly assess the roles of genes and genetic architecture in social evolution, though the approach is still in its infancy. One major problem so far has involved the focus on eusocial species instead of very primitively social species. Comparing the genetics of highly eusocial organisms like ants and honey bees to distantly-related solitary species can provide a ‘before and after’ comparison across an evolutionary chasm, though it will not tell us what kinds of intermediate changes were associated with this major transition. This study directly addresses this gap and is unique in its ability to utilize a monophyletic group of organisms that span the full range of the solitary-eusocial spectrum. In addition, phylogenetic relationships are already well resolved and dates of divergence are known with a high level of accuracy. By studying genes associated with the earliest steps into sociality as well as those in related but more advanced species, we can determine whether the transitions from solitary to simple to complex sociality represent incremental changes or genetic revolutions. Here I present data on the genomes and transcriptomes of six bee species to determine the molecular signatures of selection for social complexity. Using next-generation sequencing for the first de novo genome sequence of six carpenter bee species, RNA-sequencing to characterize transcriptomic signatures associated with behavioral castes, and phylogenetic analyses to identify novel genes and their role in social evolution. Genome sequencing revealed gene family expansions among carpenter bee species and lineages. Comparative transcriptomic analyses uncovered ancient conserved genes regulating reproductive and foraging behaviors across bee species, while novel genes were differentially expressed in each species, highlighting taxon specific genes underlying unique behavioral phenotypes. This project is enabling a deeper understanding of the relative roles of conserved and novel genes on the expression of social phenotypes as well as developing the carpenter bees, genus *Ceratina*, as emerging model species for the study of social evolution.

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 *Megalopta 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.

EVOLVING EUSOCIALITY: USING *DROSOPHILA* TO UNDERSTAND HOW QUEEN PHEROMONE INHIBITS REPRODUCTION IN *APIS MELLIFERA* WORKERS

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The evolution of eusociality has been an enduring puzzle in biology. One key aspect of eusociality is reproductive constraint. In may eusocial hymenoptera, reproductive constraint is mediated by queen mandibular pheromone (QMP). In honeybees, evidence implies that QMP acts through the highly conserved Notch cell signalling, yet how this constraint has evolved is still unknown. Interestingly, the non-social, 350 million year diverged *Drosophila melanogaster* also has their reproduction impaired by exposure to honeybee QMP, similar to the repression observed in worker bees. We are using the easily manipulable *Drosophila melanogaster* to investigate potential conserved mechanisms of reproductive repression.

Confirming previously published results; we show that QMP exposure causes a significant reduction in the number of mature oocytes in *Drosophila* ovaries. Extending this finding, we have demonstrated that this response is plastic and reversible by removing *Drosophila* from the QMP source and allowing ovarian development to proceed. Using RNA-seq on ovaries from QMP exposed, and non-exposed *Drosophila* across various time points, including those with recovered phenotypes we have identified genes that alter their expression due to removal of QMP.

That the non-social and diverged *Drosophila* responds to the eusocial honeybee QMP gives insight into potential mechanisms of QMP action. Our data implies that this response may include conserved mechanisms of responding reproductively to environmental cues. Such environmental response mechanisms may have been co-opted into reproductive constraint in eusocial species. *Drosophila* provide an excellent genetic tool to further understand both the mechanisms of QMP action, and their evolution.

COMPARATIVE GENOMICS PROVIDES INSIGHT INTO THE LOSS OF COOPERATIVE NESTING IN THE PAPER WASP *POLISTES METRICUS*

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Paper wasps in the genus *Polistes* are primitively eusocial species, forming nests with reproductively active dominants and subordinates. Nests can be founded by a single individual (non-cooperative) or by multiple foundress associations (cooperative). The frequency of cooperative nest founding, and the size of foundress associations varies within and among species. *Polistes metricus* has lost this flexibility in cooperative behavior. *P. metricus* nests are almost exclusively founded by a single foundress and foundresses can be highly aggressive towards each other. The other species in the fuscopolistes clade have retained cooperative behavior, as a result, genomics comparisons among these closely related species can give insight into the genomic regions or genes that are important for difference in cooperative behavioral strategies. We assembled de novo genomes for *P. metricus* and the closely related species *P. carolina* and *P. fuscatus*, using PacBio and 10X genomics data. In addition, we generated Illumina whole genome re-sequencing data for 10-30 individuals of each species. These species were all weakly differentiated (all pairwise $F_{ST} < 0.4$). Genome scans revealed that regions of high divergence were not evenly distributed throughout the genome. Instead, narrow regions of high differentiation were observed, similar to patterns of heterogenic genomic differentiation reported in genome-wide comparisons between other recently diverged species. Highly differentiated regions in *P. metricus* include genes involved in chemical communication and neural development. Additionally, we report preliminary evidence for structural differences between genomes, suggesting that inversions may help maintain adaptive differences among these species.

EPIGENETICS OF SOCIAL BEHAVIOR IN ANTS

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Ants live in sophisticated societies in which morphologically and behaviorally distinct types of individuals (castes) arise from a single genome, carry out different tasks, and respect the societal boundaries so that colonies can thrive. Female embryos become either reproductive queens or various types of workers, and, strikingly, these profound differences in developmental trajectory are independent of their genetic make-up. Hence, the molecular information that specifies the phenotypic differences among castes must be provided at an epigenetic level, that is, without changes in the DNA sequence.

We have sequenced the genome and obtained genome-wide DNA methylation and chromatin structure profiles for the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Camponotus* ants live in large colonies, where only the long-lived queen lays fertilized eggs. In contrast, *Harpegnathos* queens can be replaced by one or few workers, which acquire behavioral and physiological phenotypic traits typical of the queen.

The unique behavioral flexibility of *Harpegnathos* ants offers a natural experimental paradigm to interrogate the role of epigenetics pathways in regulating brain function and behavior. We obtained gene expression profiles for *Harpegnathos* brains before, during, and after their transition to queen status and identified key regulatory genes. In particular, we discovered that corazonin is a neuropeptide that promotes worker identity by stimulating foraging (hunting) behaviors while inhibiting ovary activation and therefore the transition to queen status. This is the first time that a neuropeptide, and transcriptional regulation in general, is shown to play a causal role in caste determination and caste-specific behavior in a social organism.

We also found that a role of corazonin in regulating the balance between aggression and reproduction exists in *Drosophila* suggesting that our research in social ants unveiled a deeply conserved pathway.

MOLECULAR EVOLUTION OF JUVENILE HORMONE ESTERASE-LIKE PROTEINS IN A SOCIALLY EXCHANGED FLUID

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Socially exchanged fluids, like seminal fluid and milk, present a direct and effective means through which an organism can influence conspecifics. Given the high stakes of behaviors wherein fluids are exchanged, the contents of these fluids can be subject to powerful selection pressures that can lead to novel functions. Our previous research indicated that when Carpenter ant workers (*Camponotus floridanus*) provide nutritive trophallactic fluid to developing offspring, they also transfer a key developmental regulator, juvenile hormone. To better understand the prevalence and origin of this exchange, we have explored the molecular evolution of a developmental regulatory enzyme family (juvenile hormone esterase, JHE) whose member-proteins constitute more than 18% of the total endogenous protein in this species' trophallactic fluid. We paired proteomic, behavioral, and small molecule measurements in a selection of species with phylogenetic and positive selection analyses of 30 species. Relative to other formicine ants (*Formica, Lasius, Cataglyphis*), *Camponotus* esterases have undergone positive selection, sustained multiple duplications, and have changed localization. To determine whether these esterases might function *in vivo*, we exposed the trophallactic fluid to a JHE-specific inhibitor by adding it to workers' food. The inhibitor altered the likelihood of pupation in the larvae reared by these workers in a manner similar to administering juvenile hormone. Together, these findings suggest JHEs may have undergone neofunctionalization in this genus, expanding from an intra- to inter-individual developmental regulator involved in the social control of larval development.

SOCIAL AND MOLECULAR REGULATION OF SIZE-RELATED DIVISION OF LABOR IN THE BUMBLEBEE *BOMBUS TERRESTIRS*

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Division of labor is an important organization principle of insect societies. It is therefore surprising that despite the scientific and economic importance of bumblebees, little is known on the molecular and social regulation of their division of labor. We combined sociobiological, physiological, and molecular approaches to study division of labor in the model bumblebee *Bombus terrestirs*. We found that by contrast to honeybees, division of labor in the bumblebee is not influenced by juvenile hormone (JH). Using RNA sequencing we found that many brain transcripts are differentially expressed in worker bees specializing in foraging or brood care activities. qPCR analyses further confirmed that some of these transcripts are differentially expressed in “nurses” and “foragers”, even when we controlled for genetic variation, age, and body size. There was very little overlap between the list of genes associated with task performance and those regulated by JH. This later finding lends credence to the hypothesis that JH does not regulate the division of labor in B. terrestris. Adenosine Deaminase Acting on RNA (ADAR) mediated RNA editing (“A-to-I RNA editing”) was ubiquitous in the bumblebee brain, including recoding of ion channels, transporters, and receptors that are predicted to affect brain function and behavior. Editing levels were influenced by task performance, but not by dominance or JH. Given that division of labor in bumblebees strongly relates to body size, we investigated the social factors influencing larva development and final size. We found that there is a critical period shortly after larval hatching during which direct contact with the queen shortens larval developmental duration. Ultimate body size is thus determined by interplay between the negative effect of contact with the queen and a positive effect of the total number of workers caring for the larvae. Taken together our results show that the social environment during larva development determines its ultimate body size and the propensity to perform brood care or foraging activities. Despite the flexibility in task performance, and the evidence that bees can forage and nurse brood during the same day, division of labor in *B. terrestris* is associated with differential gene expression and RNA editing. In my talk I will discuss specific genes and molecular processes that are associated with task performance.

THE GENETIC AND BEHAVIORAL MECHANISMS OF EUSOCIALITY IN THE ORCHID BEE, *EUGLOSSA DILEMMA*.

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Eusocial insects display striking adaptations of cooperative behavior that have allowed them to proliferate across terrestrial ecosystems. While much is known about the ultimate causes of eusociality, the proximate mechanisms enabling the initial transition from solitary to eusocial behavior remain poorly understood. Study of species at the edge of this transition, displaying primitively eusocial or facultatively eusocial life histories, can be particularly informative, as they allow comparisons across multiple solitary and social phenotypes. Orchid bees, many of which are facultatively eusocial, are the only such lineage among the otherwise obligately eusocial corbiculate bees. Consequently, orchid bees are a particularly compelling group from which to investigate the origins of corbiculate bee eusociality. Here, we explore the basis of eusocial behavior in the orchid bee, *Eugossa dilemma*, by examining aspects of its basic life history, as well as patterns of gene expression in the ovaries and brains across social phenotypes. We then relate these patterns to those observed across the corbiculate bees.

TO BEE OR NOT TO BEE A FEMALE - HETEROMERIC PROTEIN COMPLEXES INITIATE THE FEMALE SEX-DETERMINING CASCADE

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Sex in the honeybee (*Apis mellifera*) is determined by the homo- or heterozygosity at the *complementary sex determiner* (*csd*) gene, a locus at which more than 20 alleles are segregating in feral populations. Expression of two different Csd proteins activate the female pathway, whereas the expression of a single Csd protein results in male differentiation.
Up to the present, the mechanism how the activity of the *csd* gene is regulated by the more than 6 % amino acid differences between alleles is unknown. We found that heteromeric protein complexes are specifically formed in females that can provide sex-determining activity. We proposed that the amino acid differences between Csd proteins regulate binding abilities controlling female-specific splicing. We will present our results on the mapping of trimeric binding domains and their function in establishing female-determining activity that involve transgenic experiments. Finally, we will present a model how the mechanism is operating.

DEVELOPMENTAL PLASTICITY AS CAUSE AND CONSEQUENCE OF SOCIAL EVOLUTION

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The dawn of sociogenomics catalyzed the integration of mechanistic and functional explanations for eusociality. This has provided tremendous insight into the genes that regulate traits associated with social behavior, as well as how social behavior influences molecular evolution. Among the key insights to emerge from this research is the finding that many of the molecular pathways that regulate social behavior are highly conserved across insects and function in a variety of contexts. Additionally, the molecular evolution of these genes is not always predicted by their social function. These findings suggest additional genomic and evolutionary factors are missing from our analyses. Here, we provide evidence that developmental plasticity may fill this gap. Developmental processes both shape and respond to natural selection, and may therefore function as both cause and consequence of eusociality. We will present results from a population genomics study of a facultatively eusocial sweat bee (*Megalopta genalis*) aimed at testing the role of developmental plasticity in social evolution. We have identified causes and consequences of social plasticity related to pre-existing developmental plasticity. We use these results to identify the evolutionary processes by which different types of plasticity originate. Our results facilitate additional synthesis of proximate and ultimate explanations for eusociality by addressing a universal, but complex, component of evolution.

DO SLAVEMAKER ANTS LOSE PERCEPTION?: CONVERGENT LOSS OF OLFACTORY RECEPTORS ALONG INDEPENDENT ORIGINS OF SLAVERY.

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The evolution of an obligate parasitic lifestyle often leads to genomic reduction, in particular with the loss of functions associated with host-dependence. Reductive evolution has been intensely studied in micro-parasites, which are often very distantly related to their host. Contrastingly, socially parasitic ants, like slavemakers, exploit closely related ant species, on which they rely for brood care, foraging and all other colony maintenance tasks. Such recent common ancestry allows for direct comparisons of gene family size evolution between slavemakers and their hosts. We sequenced the genomes of three slavemaker ants and their closely related hosts, each representing an independent origin of slavery. Based on these genomes we investigate convergent evolutionary patterns in olfactory gene families in slavemakers and hosts. Gustatory receptors allow ants to perceive tastants and thus likely play an important role during foraging, a task slavemakers outsource to their enslaved host workers. In support of this, we find that the genome of slavemaker ants harbor only half of the number of gustatory receptors of that of their hosts. Likewise, slavemakers have a smaller repertoire of odorant receptors, which are often implicated in the detection of nestmate recognition cues. While accurate perception of these cues is pivotal for hosts that are targeted by slavemaker ants, a reciprocal rejection of heterospecific slaves is disadvantageous to slavemakers. This may explain why hosts have retained and even expanded their odorant receptor gene repertoire while fewer copies are found in slavemakers. Thus, gene loss in slavemakers suggests that the establishment of obligate parasitism triggers the relaxation of selective constraints on perception.

CASTE SPECIALIZATION: WHAT CAN WE LEARN FROM GENE CO-EXPRESSION NETWORKS?

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Reproductive division of labor in eusocial insects is a striking example of a shared genetic background giving rise to alternative phenotypes, namely queen and worker castes. Over the past decade, advances in molecular and genomic tools have begun to provide an overview of the genetic architecture behind phenotypic traits. Complex traits are typically controlled by multiple genes interacting with each other. Thus, traditional approaches such as differential expression, have limited powers to identify genes and molecular pathways involved in complex phenotypes. This network-centered view is changing the way we understand relationships between genes and traits, and provide the ultimate tool to find genetic pathways, represented by gene modules, associated with complex phenotypes.

Using a multi-species co-expression network, we previously identified conserved functionally important genomic units that likely serve as building blocks of phenotypic innovation. However, this approach averages species-specific differences and provides only broad patterns. In the present study, using queen and worker transcriptomic data to make species-specific co-expression networks from 5 different Formica ant species, we (re)tested the hypothesis that conserved sets of genes are involved in ant reproductive division of labor. We further hypothesized that such sets of co-expressed genes should be reproducible and conserved across all species.

We used weighted gene co-expression network analysis, which clusters co-expressed genes into modules. By applying this network analysis separately for each species for the first time, preliminary results show that most sets of genes correspond to female traits and that these modules are highly robust and reproducible between species. These cross-species module preservation analyses demonstrate that transcriptome organization is highly similar between closely related ant species, with a number of common core, functionally important transcriptional modules that serve as the foundation for species-specific elaboration of caste specialization.

GENOME EVOLUTION AND SPECIATION OF INQUILINE SOCIAL PARASITES

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The leaf-cutting ant genus *Acromyrmex* contains five known species of inquiline social parasites, each usurping different host species and with distinct geographical distributions in Latin America. All parasites show different degrees of convergent phenotypic modifications common to inquiline social parasites, such as the partial or complete loss of the worker caste and the reduction in body size. Extant *Acromyrmex* social parasites evolved between 1.5 to 4 MYA from their respective hosts and two social parasite species likely acquired reproductive isolation from their hosts in sympatry. Comparing eight reference genome sequences of host and parasite species, we find that gains and losses of genes are hallmarks of inquiline social parasite genome evolution. In particular, we detect convergent reductions of the olfactory receptor gene repertoire. Based on whole genome alignments of available ant genomes, we also identify rapidly evolving loci in the social parasites that are otherwise conserved in non-parasitic ant species. Furthermore, using genome-wide population genomic data for one host-parasite pair, we retrace the demographic and evolutionary history of the speciation event.

In conclusion, we demonstrate that the genome evolution of inquiline social parasites in *Acromyrmex* leaf-cutting ants involves extensive changes affecting traits associated with eusociality in ants and that speciation of social parasites can have complex demographic histories.

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.

COMMUNICATION AND SOCIAL BEHAVIOR IN THE CLONAL RAIDER ANT

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Ants and other social insects have evolved sophisticated societies with morphological castes and complex social behavior. The developmental trajectory and behavior of individual ants are highly contingent on the social environment, giving rise to division of labor, cooperation, and emergent group-level properties. Here I will discuss our recent efforts to develop the clonal raider ant *Ooceraea biroi* as a novel model system to study the molecular basis of phenotypic plasticity and social behavior, as well as the mechanisms underlying communication and social organization.

DIFFERENT GENETIC MECHANISMS DRIVE PARALLEL COLOR VARIATION IN MIMETIC BUMBLE BEES

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Bumble bees exhibit an exceptional diversity of segmental color patterns as a result of convergence and divergence onto numerous global mimicry patterns. We are taking a comparative and integrative approach towards studying the genetic and developmental mechanisms underlying this adaptive diversification. Using advanced bioinformatics tools such as GWAS, we have identified a narrow locus driving a red/black abdominal hair color polymorphism in the mimetic bumble bee species Bombus melanopygus. This is located in a regulatory region of the abdominal Hox genes, abd-A and Abd-B, major developmental genes that are highly evolutionarily conserved across arthropods. Results from quantitative PCR suggest that abd-A is changing its expression pattern in pupal epidermal tissues to impart this color variation. Examination of the same locus in comimicking species revealed that none of the genetic changes associated with color variation in B.melanopygus are correlated with the red/black color switch in other species, suggesting that they acquired the same color patterns as B. melanopygus convergently. Using independent GWAS data, we identified alternative genes that drive parallel color variation in comimicking species, B. bifarius. These data suggest that varied mutations across different parts of the molecular pathway as opposed to sorting of ancestral polymorphisms may be driving this variation. This research provides new insights into how the genome is modified under adaptive diversification, while also revealing processes involved in pigment and Hox gene regulation.

EXPLORING THE ROLE OF ODORANT RECEPTOR SIGNALING IN DISTINGUISHING FRIEND FROM FOE IN *CAMPONOTUS FLORIDANUS*

Stephen T Ferguson, Kyu Y Park, Alexandra A Ruff, Laurence J Zwiebel

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Aggression is a facet of many animal societies which, in various instances, may be partially modulated by chemosensory signals. In ants and other eusocial insects, aggressive behaviors require a sophisticated ability to discriminate chemical signatures that distinguish friend from foe. Indeed, the chemosensory requirements of maintaining a colonial lifestyle are likely to have driven the significant expansion of odorant receptor (*OR*) gene repertoires in the genomes of ants and other eusocial insects. Despite the observation that ants display robust behavioral and pheromonal responses as well as possess the largest number of *OR* genes among insect taxa, the precise role of this class of receptors and, more broadly, olfactory signal transduction in regulating social behaviors remains an intriguing question. I have developed an aggression-based nestmate recognition bioassay and have employed a suite of selective pharmacological tools to explore the role of OR-based signaling in these social behaviors. These data support the role of olfaction as well as the critical importance of expanded OR repertoires in the discrimination of foes from friends and the evolution of eusociality in ants.

GENETIC MAPPING IN THE GENOMICS ERA - THE UNUSUAL GENETIC ARCHITECTURE OF THELYTOKOUS REPRODUCTION IN HONEYBEES

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Large scale sequencing approaches facilitated mour understanding of evolutionary processes within social insects in general, and have helped to unravel the genetic architectures underlying phenotypic novelties in particular. However, broad (population) sampling and variable genetic backgrounds often result in added complexity, covariation and lower power to resolve a precise genetic mechanism. On the other hand, the application of controlled genetic crosses/mapping approaches in combination with genomics offers powerful possibilities, but are often challenging due to experimental accessibility of the particular species or the required large sample sizes. However, ever dropping sequencing costs increasingly make such approaches feasible and clear experimental frameworks combined with sufficient sequencing resolution can be extremely beneficial to resolve long standing questions about precise genetic architectures of phenotypic traits for good.

Here, we use phenotyped offspring of a naturally (multiple) mated Cape honeybee queen to study the mendelian trait of thelytokous reproduction, which is characteristic for this subspecies exclusively. Employing high depth whole genome sequencing on >70 indiviuals we find strong signatures of genetic differentiation between arrhenotokous offspring and thelytokous offspring at a novel single locus with an unexpected genetic architecture. Our results show that a single gene, unusually shaped by both, a strong selective sweep, as well as a partial increase in heterozygosity, and a specific single nucleotide variant is putatively underlying the thelytoky phenotype. We corroborate our results using RNAseq data and propose a model by which both reproductive modi are maintained in the population.

DEGENERATIVE EXPANSION OF A YOUNG SOCIAL CHROMOSOME

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Suppressed recombination is thought to reduce the efficacy of selection due to the Hill-Robertson effect. The long-term consequences of this effect have been widely studied in sex chromosome systems: loss of chromosomal segments and accumulation of repetitive elements lead to a dramatic reduction in size, gene number and gene density. In contrast, few empirical studies have examined the effects of suppressed recombination over shorter timescales, or in other “supergene” chromosomal regions that are inherited as a single block and determine complex phenotypes

The fire ant Solenopsis invicta carries a supergene region that controls a key social dimorphism: whether a colony accepts one or multiple queens. This supergene can be used to study the effect of suppressed recombination on chromosome evolution because the homozygous state of one of its variants (Sb) has low fitness, meaning that this variant rarely recombines. Here, we test whether Sb is affected by the accumulation of structural mutations.

Using long-molecule optical mapping of haploid individuals carrying each of the two variants of this supergene (SB and Sb), we show that the variant with restricted recombination (Sb) has undergone a ~15% increase in length during the last ~400 000 years, and that this has arisen via an accumulation of large additional sequences in the supergene. We found this pattern in two additional fire ant species expected to carry a supergene region similar to that of S. invicta.

Our results support the hypothesis that regions with recently restricted recombination should undergo degenerative expansion. Degenerative expansion has been previously shown to occur in the young sex chromosome systems of several plant species, and is thought to occur when selection is less effective for insertions, which are generally mildly deleterious, than for deletions, which are generally more strongly deleterious. Our study represents the first empirical evidence for degenerative expansion in an animal supergene.

HOW INSTINCTS EVOLVE

Andrew B Barron

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The honey bee dance is considered perhaps the most complex form of insect communication, and yet bees can perform dances perfectly even if they have never previously experienced one. There are many other examples of animal behaviour that occur with little or no obvious learning or relevant experience. Such behavioural responses are commonly described as innate or instinctive. They are assumed to be heritable or somehow ‘hardwired’ rather than learned. While we have a good general model of how behaviour can be learned, it is far less clear how behaviour might be hardwired. Here I argue that neuroplasticity is basal and that specific instincts evolved by an evolutionary process of canalisation of a plastic developmental program to a determinate end point. The development of both instinctive and learned behaviour is therefore supported by a common set of neurogenomic and cellular mechanisms. If a plastic behavioural adaptation increases fitness then selection should favour adaptations that cause the trait to develop faster, earlier and with less practice. Selection acting to adjust the timing and extent of plasticity can thus produce an instinct. This process need not result in the programming of every single detail of behaviour. An initial behavioral bias followed by an autoshaping process driven by predictable patterns of environmental reinforcement can result in complex but highly sterotyped and invariant behaviour. New findings from neuroepigenetics lend support to this model. Epigenetic mechanisms that operate on developmental timescales in the process of structuring and wiring the nervous system, also support learning by operating on physiological timescales to stabilise experience-dependent changes in neuron connection strength. It is possible to envisage evolutionary changes in the timing and regulation of epigenetic mechanisms that sculpt a learned behavior into an instinct by decreasing dependence on external stimuli in favor of an internally regulated program of neural development.

SPATIAL PATTERNS OF GENE EXPRESSION IN ANT BRAINS BASED ON *IN SITU* SEQUENCING

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The discrimination of functional components of the brain and linking the cellular activity to specific behavioral outcomes are among the biggest challenges in neuroscience. In social insects, genetic studies focused mostly on genes that are involved in cellular mechanisms underlying caste determination and social behavior. Most sequencing methods assessing gene expression only produce limited or no spatial information of expressed genes and requests additional efforts to reassemble the cellular composition. Yet, spatial information of gene expression is crucial to understand the functional composition of cells and tissues and the spatial organization of regulatory networks.

In this project, we used *in situ* sequencing (ISS) to address fundamental neurobiological questions about the neural constitutes of the complex brain tissue and the spatial gene expression regulation in social brains. The transcriptome data produced by ISS on cryo-sections of *Acromyrmex echinatior* brains offers unprecedented spatial resolution on single cell levels and the possibility to correlate transcriptomics with brain structure. With this, we aim to identify the subtle differences in brain anatomy and associated genes across castes. Our data offer closer insights into molecular and cellular processes collectively cooperating to produce high-order brain functions.

COOPERATIVE DEFENSE OPERATES BY SOCIAL MODULATION OF BIOGENIC AMINE LEVELS IN THE HONEYBEE BRAIN

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The defense of a society often requires that some specialized members coordinate to repel a threat at personal risk. This is especially true for honey bee guards, which defend the hive and may sacrifice their lives upon stinging. Central to this cooperative defensive response is the sting alarm pheromone, which has isoamyl acetate (IAA) as its main component. Although this defensive behavior has been well described, the neural mechanisms triggered by IAA to coordinate stinging have long remained unknown, in particular with respect to neurotransmitter release upon alarm pheromone perception. Here we show that IAA upregulates brain levels of serotonin and dopamine, thereby increasing the likelihood of an individual bee to attack and sting. Individuals from gentle and fierce colonies differ in their brain contents of these amines. Similarly, individuals differing in their propensity to attack in a laboratory defensive assay exhibit clear differences in the contents of these amines in specific brain regions. Pharmacological enhancement of the levels of both amines induces higher defensive responsiveness, while decreasing them via antagonists decreases stinging. Our results thus uncover the neural mechanism by which an alarm pheromone recruits individuals to attack and repel a threat, and suggest that the alarm pheromone of honey bees acts on their response threshold rather than as a direct trigger.

EGR-1, A CANDIDATE MOLECULAR PLAYER INVOLVED IN TIME-RELATED LEARNING AND MEMORY PROCESSES IN HONEY BEES ?

Aridni Shah, Axel Brockmann

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Honey bee foraging is one of the best studied and most fruitful behavioral paradigms in understanding the sensory and cognitive capabilities of insects and animals in general. During the last years, our lab has developed and used different molecular techniques to identify neuromodulators and genes involved in foraging-related learning processes.

We demonstrated that continuous foraging in honey bees is accompanied by a sustained upregulation of the transcription factor *Egr-1* (early growth response protein-1) and candidate downstream genes involved in learning and memory (*EcR, DopEcR, Ddc, Dop2*).

Since *Egr-1* expression changes were strongly associated with onset of foraging, we asked if the time of foraging affects *Egr-1* expression. We found that *Egr-1* expression was upregulated at the time of training in morning (8-10 am) and evening (4-6 pm) feeder trained foragers even in the absence of food reward. At all the other tested time points, expression levels were low. In addition, training bees to 2 feeders,that were separated in space and time,we found that *Egr-1* expression was highly dependent on the individual foraging activity of the bee. Bees that foraged only in the morning or the evening feeder showed higher expression at the respective timepoints only whereas bees that visited both the feeders showed higher expression at both the timepoints. When we prevented time trained foragers from flying out using an "artificial rain" set-up, foragers showed slight but significant increases in *Egr-1* expression around the time of feeder training. In-situ hybridization demonstrated that foraging- and feeder time-training particularly lead to an upregulation of *Egr-1* in the small-type Kenyon cells of the mushroom bodies.

Based on our results, we propose that *Egr-1* is a potential molecular link between the output of the circadian clock and the learning and memory systems involved in foraging.

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. However, 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.

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Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

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