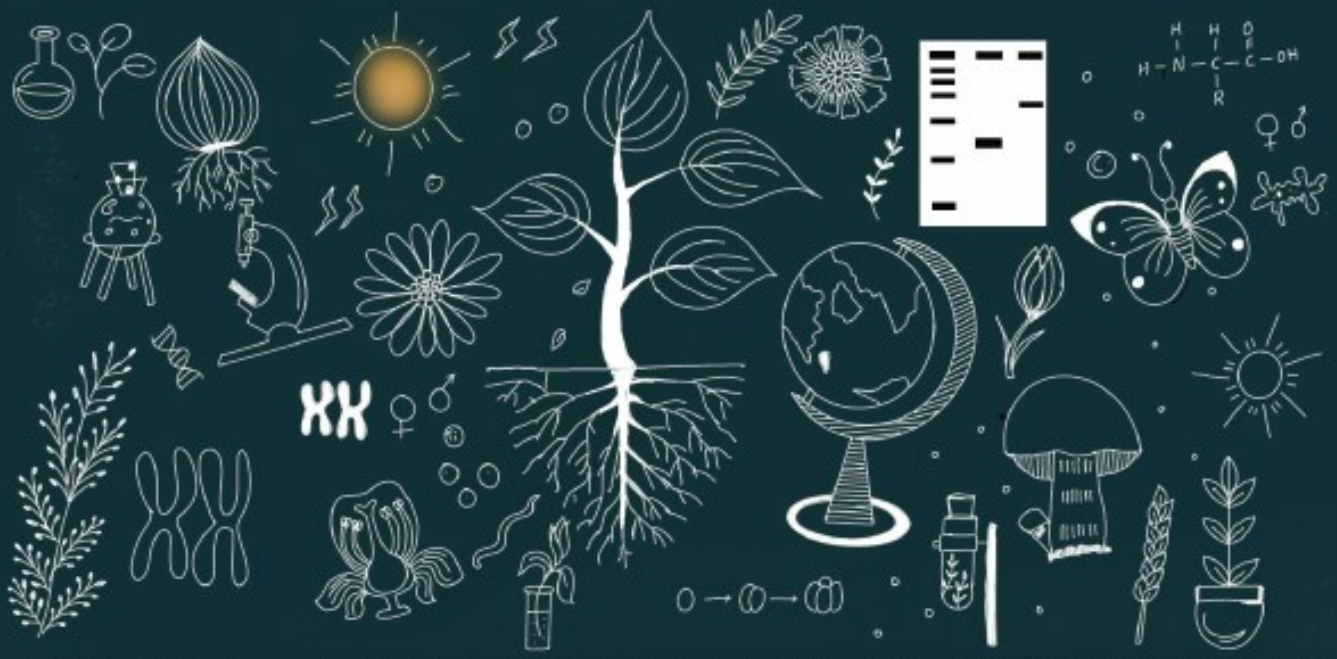
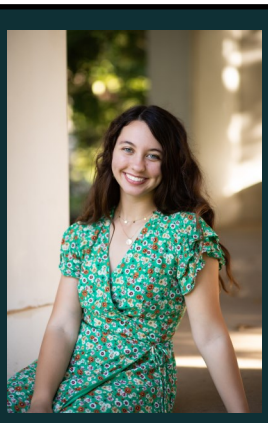


INTERDISCIPLINARY BIOLOGICAL SCIENCES
SYMPOSIUM

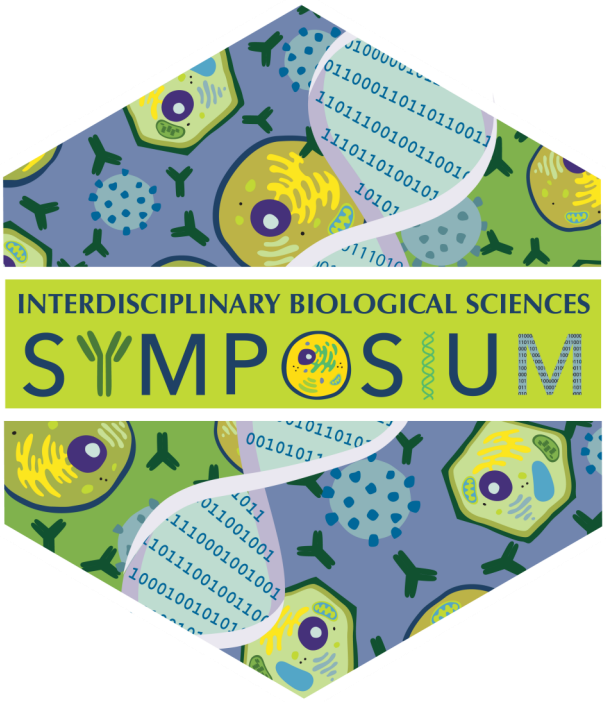
Innovation Through Collaboration in the Biological Sciences Symposium

April 26 & 27, 2023



Thank you to Amelia Case for the IBSS LOGO!

Amelia Case is a freshman at Iowa State University studying Biological and Pre-medical Illustration, a field that puts art and science together. Along with an interest in the natural sciences, she has been passionate about improving her artistic skills since her childhood. In the future, she plans to attend one of the four graduate programs in North America and become a certified medical illustrator. She is excited to continue learning more about new areas of science, as well as improving her skills as an artist.



Welcome to the 1st Annual Interdisciplinary Biological Sciences Symposium

April 26—27, 2023

Innovation Through Collaboration in the Biological Sciences

SCHEDULE
Wednesday, April 26, 2023
Memorial Union—South Ballroom and Sun Room

8:00 - 9:00 Breakfast, Networking, and Registration

8:50 - 9:00 Introduction and announcements - **Vishesh Bhatia** and **Sage Becker** - Co-directors of the IBS Symposium
Opening Remarks **- Dr. Carolyn Lawrence-Dill,** CALS Associate Dean for Research and Discovery

9:00 - 10:00 **KEYNOTE SPEAKER - Dr. Trista North** - Associate Professor of Pediatrics—Boston Children's Hospital
"*Extrinsic regulation of vertebrate hematopoietic stem cell development*"
- Introduction by Anthony Sillman

10:00 - 10:15 Break

10:15-11:30 Student oral talks - Group 1

10:15 **Vaishali Todi** (MCDB, GDCB) Advisor: Dr. Mohan Gupta
"Understanding chromosome segregation by drug-mediated tension responses"

10:30 **Thea Gessler** (IGG, EEOB) Advisor: Dr. Nicole Valenzuela
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11:15 **Dalena Rogers** (ENSCI, NREM) Advisors: Dr. Gail Nonnecke and Dr. Emily Zimmerman
"*Determining Germination Practices of U.S. Virgin Islands Native Species-Sophora tomentosa*"

11:30 - 1:00 Lunch - ISU Catering - Baked Potato Buffet

1:00 - 2:00 **KEYNOTE SPEAKER - Dr. Reinhard Laubenbacher** - Professor and Director of Systems Medicine-University of Florida
"*Digital twins in medicine and the life sciences*"
- Introduction by Sabrena Rutledge

2:00 - 2:15 Break

2:15-3:30 Student oral talks - Group 2

2:15 **Ashley Paulsen** (IGG, PPEM) Advisor: Dr. Larry Halverson
"From DNA extraction to genome assembly: Building a genome-enabled synthetic bacterial community"

2:30 **Marissa Roghair Stroud** (MICRO, PPEM) Advisor: Dr. Larry Halverson
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"Single-cell genomics data incorporation into agricultural G2P research by building a FAIR data ecosystem"

3:00 **An Phan** (BCB, MATH, V MPM) Advisors: Dr. Claus Kadelka and Dr. Iddo Friedberg
"Gene Ontology and protein-protein interaction analyses reveal under-annotated proteins in the human proteome"

3:15 **Renee Alexander** (Kinesiology) Advisor: Dr. Tim Derrick
"*Inclusion of distal fibular translations or rotations in multi-segmented foot modeling for lateral ankle ligament strains*"

3:30-5:30 Poster session - Sun Room, Memorial Union

SCHEDULE
Thursday, April 27, 2023
Hach Hall—Thiel Atrium

8:00 - 9:00 Breakfast, Networking, and Registration

8:50– 9:00 Announcements—**Vishesh Bhatia** and **Sage Becker**—co-directors of IBS Symposium

9:00 - 10:00 **KEYNOTE SPEAKER - Dr. Ryan Rapp -** Operating Partner at Flagship
"*Different models for startups and careers in industry*"
- Introduction by Vishesh Bhatia

10:00 - 10:15 Break

10:15-11:30 – Student oral talks - Group 3

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"*Genotype by environment interaction in switchgrass"*

11:15 **Xinglin Jia** (BCB, MATH) Advisor: Dr. Claus Kadelka and Dr. Gregory Phillips
"*Concise functional enrichment of ranked gene lists"*

11:30-1:00 Lunch - Panera Lunch Boxes

1:00-2:00 **KEYNOTE SPEAKER - Dr. Amanda Ramer-Tait** - Associate Professor, University of Nebraska—Lincoln
"***Modulating microbiomes to limit metabolic inflammation"***
- Introduction by Sage Becker

2:00-2:15 Break

2:15-3:30 – Student Oral Talks - Group 4

2:15 **Suyeon Hong** (ImBio, V MPM) Advisor: Dr. Jodi McGill
"Altered cytokine and gene expression in vitamin A deficient mice after RSV infection and vitamin A supple mentation"

2:30 **Jaspreet Rishi** (IGG, ANS) Advisor: Dr. Aileen Keating
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3:15 **Molly Kroeger** (ImBio, VDPAM) Advisor: Dr. Pablo Pineyro
"Humoral Responses to Porcine Circovirus Type 3 Infection"

3:30-3:45 Break

3:45-3:55 Closing Remarks - **Dr. Surya K. Mallapragada**, Associate Vice President for Research

3:55-5:00 Award Ceremony
- Poster Award Presentation
- Oral Presentation Award Presentation
- Lauter Awarded for Graduate Student Creativity and Research Excellence presented by **Dr. Erik Vollbrecht** - DOGE of Genetics and Genomics

Keynote Speakers—April 26, 2023

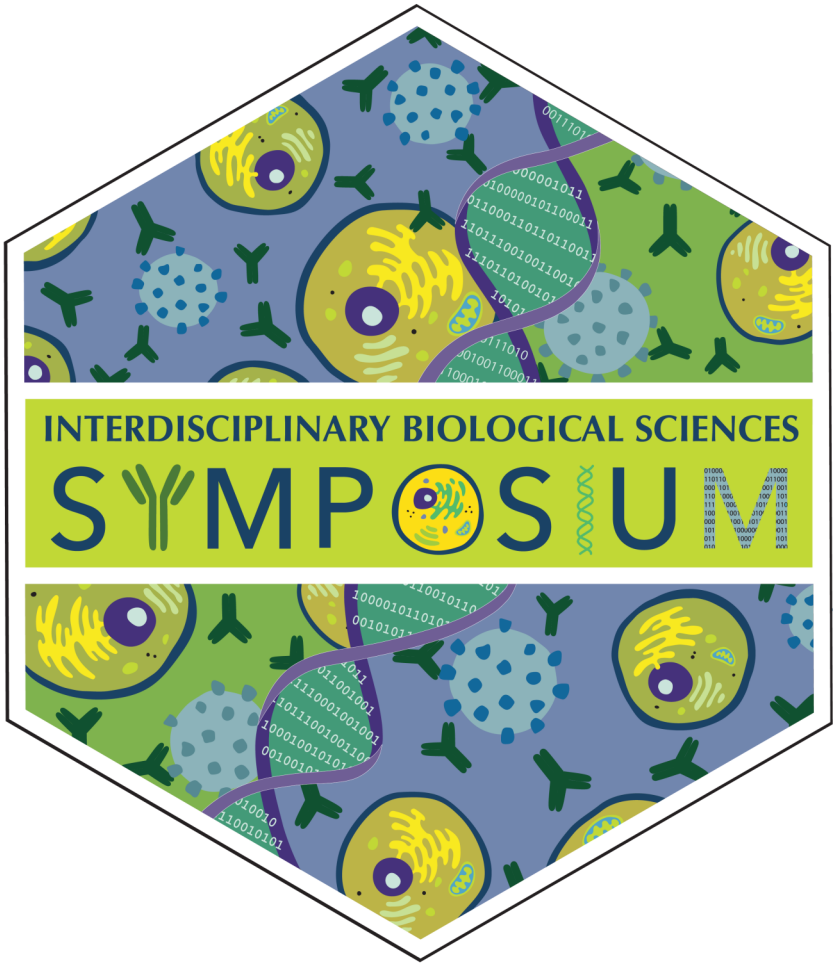
Dr. Reinhard Laubenbacher is the Director of Laboratory for Systems Medicine and a professor at the University of Florida in the Department of Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine. He also holds the titles of fellow of AAAS, the Society for Mathematical Biology, and the American Mathematical Society and editor-in-chief of the Bulletin of Mathematical Biology, the flagship journal of the Society for Mathematical Biology. His research interests include multi-scale modeling and control of disease processes, systems biology, and computational immunology. His lab utilizes mathematical and computational technology to study disease modeling.

Dr. Trista E. North is an Associate Professor of Pediatrics at Boston Children's Hospital (BCH) and Harvard Medical School HMS) in Boston, Massachusetts, USA. She is Co-Director of the Developmental and Regenerative Biology Program and Faculty of the Biological and Biomedical Sciences Graduate Program at HMS. She is also Principal Faculty at the Harvard Stem Cell Institute (HSCI), a member of the Dana-Farber/Harvard Cancer Center (DF/HCC) Leukemia Program and affiliated faculty in the Harvard University Department of Stem Cell and Regenerative Biology. Dr. North received her BA from Bowdoin College in 1996, and her PhD from Dartmouth College in 2002. She conducted her graduate work with Dr. Nancy A. Speck (University of Pennsylvania) and her postdoctoral research with Dr. Leonard I. Zon at Boston Children's Hospital. Dr. North started her own laboratory 2008, rejoining the Stem Cell Program and Department of Hematology and Oncology Boston Children's Hospital in 2017. Dr. North currently serves as Treasurer for the Zebrafish Disease Models Society (ZDMS), the Scientific Committee on Stem Cells for the American Society of Hematology (ASH) and as a member of the Nominating Committee of the International Society of Experimental Hematology (ISEH).

Keynote Speakers—April 27, 2023

Dr. Ryan Rapp is an ISU alumnus who now works as an Operating Partner at Flagship Pioneering in Cambridge, MA. Since his graduation from the Interdepartmental Genetics and Genomics program at ISU he has worked with several companies including Pairwise, Illumina, and Monsanto. His work centers bringing new companies with new agricultural technology to fruition. Dr Rapp brings an interesting perspective with his experience in industry and the skill sets needed to succeed in an industry position. He plans to discuss the experience of working for a a large corporation vs working in a startup and the Flagship model (which has brought companies like Moderna, Indigo, and Inari to life).

Dr. Amanda Ramer-Tait is an ISU alumna of the immunobiology program and currently a Maxcy Professor of Agriculture and National Resources Associate Professor, Department of Food Science and Technology at the University of Nebraska-Lincoln and the director of the Nebraska Gnotobiotic Mouse Program. Her work focuses on host-microbiota interactions including understanding the causative relationship between the microbiota of the gut and chronic inflammatory disease and manipulating the microbiome to prevent or treat disease.



Student Oral Presentation Abstracts

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"Humoral Responses to Porcine Circovirus Type 3 Infection"

Student Oral Presentations

Understanding chromosome segregation by drug-mediated tension responses

Vaishali Todi[1,2], Mohan Gupta[2]

[1]Interdepartmental Molecular, Cellular, and Developmental Biology, Iowa State University, Ames, Iowa

[2]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa

Improper chromosome segregation during cell division can be deleterious by causing birth defects and aneuploidy, or even resulting in the development of tumors. Chromosome biorientation is crucial to ensure faithful segregation, wherein kinetochore-microtubule attachment as well as microtubule-generated tension across the attachments of sister chromatids is required to satisfy the Spindle Activation Checkpoint (SAC) and progress into anaphase. It is therefore, essential to understand the pathways leading to activation of the SAC and associated mechanisms that promote chromosome biorientation prior to anaphase. Toward this goal, we developed a novel Taxol-sensitive yeast strain with which we isolated the tension-specific and attachment-mediated responses at kinetochores, which both induce delay in anaphase onset. This revealed discrete functions for SAC proteins; whereas Bub1, Bub3, Mad1, Mad2 and Mad3 are all required for SAC signaling due to unattached kinetochores, Bub1 and Bub3 specifically facilitate a transient delay in response to low tension at attached kinetochores. When microtubule-kinetochore attachments lack sufficient tension, Aurora B kinase (Ipl1 in yeast) phosphorylates kinetochore-associated proteins to promote detachment and subsequent SAC activation. Our Taxol-sensitive yeast model offers a unique opportunity to investigate the functions of microtubule-generated tension and associated proteins in tension-mediated error correction and anaphase onset delay mechanisms which help promote the high-fidelity of chromosome segregation. It will also help elucidate the mode of highly successful cancer therapeutics. Our overall goal is to understand how microtubule-generated tension at kinetochores regulates key aspects of bipolar attachment and chromosome separation. The inclusive impact of the tension- and attachment-based responses will significantly advance our knowledge of how healthy cells ensure genome stability and support the development of therapeutic strategies for states such as cancer and developmental disabilities.

Keywords – chromosome segregation, microtubules, anti-cancer drugs, mitosis, yeast

Characterization of the First Turtle Organoids: RNA-seq Profiling

Christopher Zdyrski[1,2], Vojtech Gabriel[1], **Thea B. Gessler**[3], Abigail Ralston[2], Itzel Sifuentes-Romero[3], Debosmita Kundu[4], Sydney Honold[1], Hannah Wickham[1], Nicholas E. Topping[3], Dipak Kumar Sahoo[5], Basanta Bista[3], Jeffrey Tamplin[6], Oscar Ospina[7], Jacob Galan[8], Pablo Piñeyro[9], David K. Meyerholz[10], Karin Allenspach[1,2,5], Jonathan P. Mochel[1,2], Nicole Valenzuela[3]

[1]SMART Pharmacology, Department of Biomedical Sciences, Iowa State University, Ames, Iowa
[2]3D Health Solutions Inc., Ames, Iowa
[3]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa
[4]Department of Statistics, Iowa State University, Ames, Iowa
[5]Department of Veterinary Clinical Sciences, Iowa State University, Ames, Iowa
[6]Department of Biology, University of Northern Iowa, Cedar Falls, Iowa
[7]Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, Florida
[8]Department of Human Genetics, University of Texas Rio Grande Valley, Edinburg, Texas
[9]Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa
[10]Department of Pathology, University of Iowa, Iowa City, Iowa

Painted turtles are remarkable for their well-developed freeze tolerance and associated resilience to hypoxia/anoxia, oxidative stress, and ability to supercool. They are, therefore, an ideal model for biomedical research on hypoxia-induced injuries (such as strokes), tissue cooling during extensive surgeries, and organ cryopreservation. Yet, the seasonal reproduction and slow maturation of turtles hinder basic and applied biomedical research. To overcome these limitations, we have developed for the first time, adult stem cell-derived turtle hepatic organoids, which provide 3D self-assembled structures that closely mimic their original tissue and allow for in vitro testing and experimentation without constantly harvesting donor tissue and screening offspring. Our pioneering work with turtles represents the first for this vertebrate Order and complements the only other organoid lines from non-avian reptiles, which were produced from snake venom glands. Here we present the results of triplicate stranded RNA-seq for Chrysemys picta liver tissues and their derived organoids, examining both hatchling and adult life stages. We find that tissue gene expression patterns are unsurprisingly more complex given their diverse cell types. Additionally, organoids largely overlap with tissues in both gene expression and functional profiling, however they also display characteristics unique to organoids.

Keywords – organoids, RNA-seq, ecology and evolution, turtles

bds1 and bds2 function redundantly to regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis

Brian Zebrosi[1,2], Erica Unger-Wallace[1], Kokulapalan Wimalanathan[1], Norman B. Best[3,4], Erik Vollbrecht[1]

[1]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa

[2]Interdepartmental Genetics and Genomics, Iowa State University, Ames, Iowa

[3]Bond Life Sciences Center, University of Missouri, Colombia, Missouri

[4]USDA-ARS, Colombia, Missouri

Shoot architecture is a key determinant of grain yield in maize. Among the major plant growth regulators, brassinosteroids (BRs) affect multiple developmental processes and plant architecture traits, including organ size, sex determination, and leaf angle. However, the genetic mechanisms by which BRs regulate plant architecture traits in maize remain poorly understood. We recently generated, identified, and characterized a recessive, EMS-induced maize mutant, which we tentatively named brassinosteroid deficient semi-dwarf mutant1 (bds1). Mutants have a semi dwarf stature due to compressed internodes and are partially rescued by brassinolide. bds1-ref mutants also have short leaf sheaths and twisted leaf blades and display a partial tassel-seed phenotype in the Mo17 background and reduced tassel branch number in B73. We localized bds1 to a small genomic region containing a point-nonsense mutation in a gene involved in brassinosteroid biosynthesis using map-based cloning and whole-genome sequencing. Non-complementation of bds1-ref and a bds1-Mu allele confirmed that bds1 encodes an enzyme likely involved in brassinosteroid biosynthesis. Using phylogenetic and blast analysis, we identified that bds1 has a close homolog. We named bds2 and generated several mutant alleles by remobilizing a nearby Ds transposable element.
Contrary to bds1 mutants, the bds2 single mutants are indistinguishable from wild-type plants, while the bds1-ref;bds2-Ds double mutants are severely dwarfed with other defects similar to those observed with BR-deficient mutants nana1 and nana2. To understand the genetic interaction between brassinosteroids and Jasmonic Acid, generated double-mutants between bds1-R with tassel-seed 1 (ts1) mutant. We observed synergistic interaction between bds1-R and ts2, the bds1-R;ts2 double mutants have dramatically increased tassel feminization and reduced plant height and tassel branches. Suggesting that both BR and JA biosynthesis are required for sex determination and plant architecture regulation.

Influence of immune cells in dengue and Zika dissemination in the mosquito host

David R. Hall[1,2], Hyeogsun Kwon[2], Rebecca Johnson[3], Zannatul Ferdous[3], Viridiana Laredo-Tiscareño[4], Bradley J. Blitvich[4], Doug E. Brackney[3], Ryan C. Smith[2]

[1]Interdepartmental Genetics and Genomics, Iowa State University, Ames, Iowa

[2]Department of Plant Pathology, Entomology, and Microbiology, Iowa State University, Ames, Iowa

[3]Center for Vector-Borne and Zoonotic Diseases, Department of Environmental Sciences, The Connecticut Agricultural Experiment Station, New Haven, Connecticut

[4]Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, Iowa

Mosquito-borne viruses are a rapidly increasing threat to public health, causing over 400 million infections annually and putting over half of the world's population at risk. A mosquito acquires a viral infection by taking a blood meal from an infected host, and the virus must then traverse physical barriers and evade immune defenses within the mosquito until it reaches the salivary glands. Once a salivary gland infection has been established, the virus can then be transmitted to a new host by saliva excreted during another blood meal. The mechanisms by which arboviruses elude these defenses and spread throughout the mosquito are a critical component of vector competence,but are not well understood. In this study, we provide evidence for the dissemination of virus by immune cells, known as hemocytes, which circulate throughout the hemolymph. By infecting Aedes aegypti mosquitoes with dengue virus (DENV) and Zika virus (ZIKV), we demonstrate that the depletion of phagocytic hemocytes results in delayed virus dissemination in the mosquito host. Using immunofluorescence assays of virus-infected hemocytes, we demonstrate that phagocytic hemocytes are a focal point of infection in the hemolymph,and are capable of attaching to the ovaries and salivary glands of mosquitoes, two tissues that are integral to virus transmission. To further dissect this mechanism, hemocytes from virus-infected mosquitoes were able to transfer infection to non-infected mosquitoes, demonstrating that hemocytes are an essential tropism for virus replication and dissemination. Taken together, the results of this study support a model for virus dissemination whereby hemocytes acquire a viral infection and transport that infection through the hemolymph to uninfected tissues. This study significantly advances our understanding of the dynamics of virus infection in mosquitoes and the role of hemocytes in mosquito vector competence for DENV and ZIKV.

Keywords – arbovirus, hemocyte, dissemination, mosquito, dengue

Determining Germination Practices of U.S. Virgin Islands Native Species-*Sophora tomentosa*

Gail Nonnecke[1], Emily Zimmerman[1,2], Susana Goggi[3,4], **Dalena Rogers[1,2]**

[1]Department of Horticulture, Iowa State University, Ames, Iowa

[2]Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa

[3]Department of Agronomy, Iowa State University, Ames, Iowa

[4]Seed Science Center, Iowa State University, Ames, Iowa

Native plant species conservation in St. John, U.S. Virgin Islands is important for the continuity of key ecological services, particularly after the island's vegetation was severely degraded during the 2017 hurricane season. A lack of industry methods for seed propagation, nursery production, and high capital resources on-island presents a barrier to the ex-situ production of native plants in St. John. Two low-technology-germination practices of seed coat puncture and Gibberellic acid (GA_3) application were evaluated for germination effectiveness on one valuable native shoreline species, *Sophora tomentosa*. A randomized experimental design with four treatments was executed using a total of 4,000 seeds collected from mature *S. tomentosa* plants. Seeds were placed on wet germination towels with one of the following four treatments applied to the towel (GA_3) and/or to the seed (punctured seed coat): (1) Control (water only), (2) Water + puncture, (3) Water + GA_3, and (4) Water + GA_3 + puncture. The towels were then rolled, placed in a plastic cup, and sealed with a plastic bag to maintain moisture. Germination success was recorded every other day for two weeks from Nov. 22nd, 2022, to Dec. 2nd, 2022. The study took place in shaded, ambient environmental conditions in St. John. The combination treatment of GA_3 application and seed coat puncture (Treatment 3) resulted in the highest average germination of 38%, while the puncture-only treatment resulted in 33% average germination. The control and GA_3-only treatments each had an average germination rate of less than 1%, implying that the seed coat must first be damaged for germination to occur. The germination differences between the control, GA_3-only, and puncture treatments provide evidence of physical dormancy in *S. tomentosa* seeds. Seed coat puncturing can be recommended as a low-capital germination practice to restore *S. tomentosa* in St. John's native landscapes with the addition of GA_3 to further increase germination, if desired.

Keywords – germination, native species, dormancy, *Sophora tomentosa*

From DNA extraction to genome assembly: Building a genome-enabled synthetic bacterial community

Ashley A. Paulsen[1,2], Marissa Roghair Stroud[2,3], Larry Halverson[3]

[1]Interdepartmental Genetics and Genomics, Iowa State University, Ames, Iowa

[2]Department of Plant Pathology, Entomology, and Microbiology, Iowa State University, Ames, Iowa

[3]Interdepartmental Microbiology, Iowa State University, Ames, Iowa

The maize rhizosphere microbiome is a complex and dynamic system that can significantly affect plant growth and health. The inherent complexity of these microbial communities has hindered our ability to understand how microbes interact with each other and plants. One approach to address this is to use synthetic communities of reduced complexity to identify rules for these rhizosphere interactions. Here we describe the construction of the Maize Rhizosphere Synthetic community (MARSc), which is modeled after the maize rhizosphere microbiome observed in Iowa soils. After screening >250 bacterial isolates we selected 31 species, representing 4 Phyla and 20 families, to comprise our MARSc. To functionally validate MARSc, we examined the influence of MARSc on maize growth. Plants treated with MARSc then planted in soil from low- N fertilizer plots produce more biomass and finer roots than un-inoculated treatments, indicating that one or more MARSc members can promote plant growth. As a first step towards understanding the potential metabolic functions of MARSc we chose to generate de novo genome assemblies for each member. This process began with Illumina sequencing, but early assembly attempts showed the difficulties inherent in short read only assembly. We used high-molecular weight DNA extraction, size selective DNA precipitation, and Nanopore sequencing to generate quality sequences averaging over 20 kb in length. These long reads were combined with the Illumina short reads in a hybrid consensus assembly pipeline to generate complete de novo assemblies for all members of MARSc. The assemblies are now being annotated and mined for known plant growth promoting genes.

Keywords – sequencing, genome assembly, microbiome, maize

CRISPRi to identify Pseudomonas putida gene networks essential for rhizosphere microbiome assembly

Marissa N. Roghair Stroud[1,2,4], Dua X. Vang[1,2,4], Ashley A. Paulsen[1,3,4], Hannah Burkhart[1], Lily M. Thompson[4], Larry J. Halverson[1,2,3,4]

[1]Department of Plant Pathology, Entomology, and Microbiology, Iowa State University, Ames, Iowa

[2]Interdepartmental Microbiology Graduate Program, Iowa State University, Ames, Iowa

[3]Interdepartmental Genetics and Genomics, Iowa State University, Ames, Iowa

[4]Ames National Laboratory, Ames, Iowa

Our goal is to understand the processes contributing to microbiome assembly and maintenance in a dynamic environment, the maize rhizosphere. Our approach is to use CRISPR interference (CRISPRi) to identify genes that are essential to fitness of *Pseudomonas putida* KT2442 in the rhizosphere in the presence/absence of one or more cohabitating bacteria (a microbiome). We created a synthetic maize rhizosphere community (SynCom) that reflects maize rhizosphere bacterial communities in Iowa soils. This SynCom was used to develop an interaction network to identify bacteria whose growth or behavior is stimulated by *P. putida* or vice versa. A CRISPRi system was developed with constitutive promoters of varying strength controlling expression of sgRNAs and an inducible nuclease-deactivated variant of *Streptococcus pyogenes* Cas9 (dCas9) that is incorporated into a neutral site on the chromosome. We have experimentally validated our CRISPRi system using an arabinose-inducible dCas9 system, which was tested with an essential gene (*ftsZ*, used in cell division) and a non-essential gene (*pvdH*, required for pyoverdine expression). Importantly, we show that CRISPRi is functional in the maize rhizosphere both when dCas9 is expressed during the onset of *P. putida* root colonization and when dCas9 is expressed after *P. putida* has colonized the root. Additionally, CRISPRi of *pvdH* alters interaction patterns between *P. putida* and other members of this community. These results provide the foundation on which to use CRISPRi-seq to identify *P. putida* genes that contribute to microbiome assembly and maintenance in the maize rhizosphere by iteratively increasing community complexity.

Keywords – microbiology, CRISPR interference, synthetic microbiome, *Pseudomonas putida*, rhizosphere

Single cell genomics data incorporation into agricultural G2P research by building a FAIR data ecosystem

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The agriculture genomics community has numerous data submission standards but limited knowledge describing and storing single-cell data. Other scgenomics infrastructure efforts, such as the Human Cell Atlas Data Coordination Platform, have resources that could benefit our community. For example, the HCA DCP is integrated with Terra, a cloud-native workbench for computational biology developed by Broad, Verily, and Microsoft that houses tools for scGenomics analysis. We describe a pilot-scale project to determine if our current metadata standards for livestock and crops can be used to ingest scRNAseq datasets in a manner consistent with HCA DCP standards and if established resources can be used to analyze the ingested data. Currently, the most comprehensive data ingestion portal for high throughput sequencing datasets from plants, fungi, protists, and animals at the EMBL-European Bioinformatics Institute, Annotare, ensures that sufficient metadata are collected to enable re-analysis and dissemination via the Single Cell Expression Atlas(SCEA). Annotare supports an auto-complete function that allows the users to search for and use the appropriate terms from many ontologies and can readily be used to process and search single cell plant data via the SCEA and transferred to the Galaxy analysis space. Bioinformaticians manually curate all experiments submitted to ArrayExpress via Annotare. Another EMBL-EBI portal limited to animal datasets, the FAANG portal, provides bulk and scRNAseq data access. scRNAseq data can be submitted to the FAANG portal using a semi-automated process where files are validated using the HCA DCP metadata and data validation service. These files are also incorporated using EMBL-EBI's HCA DCP ingestion service and then transferred to Terra for further analysis. We intend to build upon these existing tools to construct a scientist-friendly data resource and analytical ecosystem to facilitate single cell-level genomic analysis through data ingestion, storage, retrieval, visualization, and comparative annotation across agricultural species.

Keywords – single cell, bioinformatics, data ingestion, crop and livestock

Gene Ontology and protein-protein interaction analyses reveal under-annotated proteins in the human proteome

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Gene Ontology (GO) annotation databases contain the total knowledge of protein function, but they are biased towards the well-studied proteins that are clinically meaningful. Current -omics experiments annotating protein functions focus on these well-studied proteins, leaving many others under-annotated. To better understand these biases, we focused on GO annotations of human proteins with an emphasis on annotations with experimental evidence. We examined the information content of annotations experimentally assigned to each protein, and categorized these proteins into highly annotated, moderately annotated, and less annotated groups. By analyzing protein-protein interactions from the STRING database, we characterized the less annotated proteins that associate strongly with highly annotated proteins, but they have been ignored in function annotation experiments due to the lack of previous information. These proteins, collectively termed the "ignorome", merit additional study because of their interactions with highly annotated proteins. We present a protocol to prioritize the ignorome proteins (based on confidence of interaction and information content) for use by experimentalists to target functional studies. We hope our work shall stimulate future functional studies on the prioritized under-annotated proteins to fill the gaps in knowledge of the ignorome.

Keywords – functional annotation, data mining, annotation assessment metrics, human

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Inclusion of distal fibular translations or rotations in multi-segmented foot modeling for lateral ankle ligament strains

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Over 80% of sports injuries are ankle sprains. Damage to the ATFL, PTFL, or CFL define a lateral ankle sprain. Musculoskeletal models can be used to assess ligament strains if bone kinematics are known. The distal fibula translates and rotates relative to the tibia during plantar flexion and dorsiflexion. It is unknown how these translations or rotations will affect ligament strains. Fibular movement may impact ligament strains and the potential for a sprain. Our purpose was to use a model to estimate the effects of fibular motion on lateral ankle ligament strain during walking. A musculoskeletal model of the foot was created in MATLAB. Anteroposterior, vertical, and mediolateral axis rotations plus vertical and mediolateral translations of the distal fibula relative to the distal tibia were estimated from Huber 2012 for a walking range of motion of -20° plantar flexion to 20° dorsiflexion. Anatomical position strains were predicted to be 3, 8, and 7% for the ATFL, PTFL, and CFL ligaments respectively (reference). Three models were developed: with translations, with rotations, and with neither. As plantar flexion increased, strain increased in the ATFL, decreased in the PTFL and remained relatively constant in the CFL. Fibular translations and rotations increased ATFL and CFL strain in plantar flexion. PTFL strains decreased. Dorsiflexion increased PTFL and CFL strains with translations; rotations increased PTFL strain in dorsiflexion but not CFL strains. Inclusion of fibular translations and rotations increased the ATFL and PTFL strain with tibiotalar motion, and thus increased their maximum strain and laxity for the tested range of motion in both models. CFL strains were relatively constant between the models. Fibular rotations or translations were added into a model to estimate their effect on ankle ligament strains during walking. Ligament laxity and strains increased based on the ankle position. Musculoskeletal models can become more accurate by including fibular translations or rotations; however, it is unclear if these small values can be estimated in vivo.

Keywords – biomechanics, ankle sprain, musculoskeletal modeling, kinesiology

Mdr1 demethylase and the intersection between the epigenome, genomic imprinting, and transposable elements in maize endosperm

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In 1970 Jerry Kermicle published the first evidence for a phenomenon now known as genomic imprinting. Genomic imprinting is the expression of an allele dependent on the parent of origin. The first imprinted gene discovered was in maize. Since then, theories about the evolutionary underpinnings, and the search for the molecular mechanisms causing imprinting have intrigued scientists. Here we present new findings that provide insights into the role of maternal de-repression of R1(Mdr1), a regulator of R1, in maize endosperm. The mdr1 mutant was first discovered through its role in imprinting. In WT plants, Mdr1 demethylates the maternal allele of R1, setting up maternal expression in the endosperm and causing a solid purple color on maize ears. While in the mutant, R1 remains methylated and loses expression in the endosperm, resulting in mottled color on maize ears. To test the genome-wide effects of mdr1 on expression, we performed RNA-seq on mdr1 mutant and wild-type endosperm at 14 days after pollination. This revealed 97 genes, and 89 transposable elements, that are differentially expressed in the mutant. All but one gene and three TEs are down-regulated in the mutant, as was previously seen with R1. These down-regulated genes have many shared associations with other genomic datasets, including an enrichment for overlap with differentially methylated regions (DMRs) between mutant and wild type, overlap with a helitron family with several members which are differentially expressed in the mutant, and genes that are maternally expressed (imprinted) in the endosperm. To further explore the role mdr1 plays in the endosperm epigenome we assessed the histone landscape using CUT&Tag, and found an association between H3K56ac, DMRs, and differentially expressed genes. Together, these data suggest Mdr1-dependent demethylation contributes to the distinct transcriptomic environment in endosperm, and to epigenome changes associated with maternally expressed genes and transposable elements.

Keywords – epigenomics, genomics, transposons, maize

Unsupervised Contrastive Peak Caller for ATAC-seq

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The assay for transposase-accessible chromatin with sequencing (ATAC-seq) is a common assay to identify chromatin accessible regions by using a Tn5 transposase that can access, cut, and ligate adapters to DNA fragments for subsequent amplification and sequencing. These sequenced regions are quantified and tested for enrichment in a process referred to as “peak calling”. Most unsupervised peak calling methods are based on simple statistical models and suffer from elevated false positive rates. Newly developed supervised deep learning methods can be successful, but they rely on high quality labeled data for training, which can be difficult to obtain. Moreover, though biological replicates are recognized to be important, there are no established approaches for using replicates in the deep learning tools, and the approaches available for traditional methods either cannot be applied to ATAC-seq, where control samples may be unavailable, or are post-hoc and do not capitalize on potentially complex, but reproducible signal in the read enrichment data. Here, we propose a novel peak caller that uses unsupervised contrastive learning to extract shared signals from multiple replicates. Raw coverage data are encoded to obtain low-dimensional embeddings and optimized to minimize a contrastive loss over biological replicates. These embeddings are passed to another contrastive loss for learning and predicting peaks and decoded to denoised data under an autoencoder loss. We compared our Replicative Contrastive Learner (RCL) method with other existing methods on ATAC-seq data, using annotations from ChromHMM genome and transcription factor ChIP-seq as noisy truth. RCL consistently achieved the best performance.

Keywords – peak calling, ATACseq, contrastive learning, deep learning, chromatin accessibility

Trained immunity in bovine innate cell populations following subcutaneous BCG administration

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Trained immunity is characterized by an altered epigenetic status of innate immune cells after an initial challenge, resulting in an enhanced immune response to an unrelated second challenge. The Bacillus Calmette Guerin(BCG)vaccine, administered to prevent tuberculosis, is a well-studied inducer of trained immunity in human and mouse monocytes. We characterized the trained immune phenotype exhibited by monocytes and gamma delta T cells in pre-weaned calves after BCG administration. In two studies, pre-weaned Holstein calves were subcutaneously administered 10^6 or 10^7 CFU BCG Danish strain. Control calves received PBS. Gamma delta T cells and CD14+ monocytes were isolated from peripheral blood at various times after treatment. Cells were stimulated in-vitro with Lipopolysaccharide (1µg/mL), Pam3CSK4 (10µg/mL), or Poly IC (50ug/ml) for 48 hours, and IL-1b, IL-6, and TNFa production were measured by ELISA. Gamma delta T cells from control and BCG-treated calves were also subjected to ATAC-seq to evaluate chromatin accessibility in trained and non-trained cells. The PBMCs and CD14+ monocytes from BCG calves showed increased IL-1b and IL-6 secretion compared to control calves, consistent with the trained immune phenotype observed in other species. Interestingly, Gamma delta T cells from BCG calves also showed enhanced IL-6 and TNFa production upon in-vitro stimulation with LPS, Pam3CSK4, and Poly IC. Analysis of the ATAC-seq data from gamma delta T cells is currently underway. BCG vaccine administered subcutaneously can induce trained immunity in bovine innate cell populations. While there are previous reports of innate training being induced in monocytes, we report for the first time that gamma delta T cells, a nonconventional T cell population, have the potential to be trained in vivo following BCG administration. This memory-like phenotype is exhibited by increased cytokine production in response to TLR stimulation. ATAC sequencing will provide novel information regarding the altered epigenetic status of trained innate immune cells in cattle.

Keywords – trained immunity, innate cells, BCG, bovine

Genotype by environment interaction in switchgrass

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Switchgrass (Panicum virgatum) is a perennial grass that was a dominant species in the tallgrass prairie of North America and is grown today as a biomass crop for bioenergy. Notably, flowering time strongly influences biomass yield in switchgrass, as later flowering enables a longer vegetative growth stage and therefore greater biomass accumulation. These important traits are plastic, controlled by genes, the environment, and their interaction. In this project, we systematically dissect major genetic and environmental determinants to explain and predict switchgrass performance trends using a multi-environment trial from a four-grandparent mapping population and a diverse population grown in locations spanning almost 2,000 kilometers. We used the CERIS (Critical Environmental Regressor through Informed Search) algorithm to identify environmental factors that most strongly influence agronomic traits including flowering time and biomass as well as adaptive traits such as survival. Regressing phenotypes onto identified environmental indices enabled estimation of each genotype's intercept (trait mean) and slope (plasticity) with respect to the index, which were then used to predict performance under future climate models. These intercept and slope values were also used as phenotypes for QTL mapping, revealing a pleiotropic gene with high frequency structural variations that impacts the plasticity of biomass, flowering time, plant height, and adaptation.

Keywords – GxE, plasticity, climate, switchgrass

Concise functional enrichment of ranked gene lists

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The next-generation sequencing technology has heralded an era of high-throughput biological data. RNA-seq, as one of many sequencing techniques, allows quantifying and comparing the expression levels of thousands of genes and experiment samples. The output of, e.g., a case-control study is a ranked list of differentially expressed genes. However, interpreting and analyzing this long list of perturbed genes is challenging. Functional enrichment methods take a list of genes as input and return a set of functional categories linked to more perturbed genes than expected. Most functional enrichment methods work in a binary mode, i.e., they consider a gene either expressed or not. These approaches may disregard useful information provided by the full ranking. The few enrichment methods that do operate on ranked lists output highly redundant or non-specific functional categories.
Here we present a new functional enrichment method, Concise Ranked Functional Enrichment (CRFE), that effectively utilizes the ranking in gene expression data sets and computes a small set of non-redundant functional categories that are primarily linked to highly ranked genes. We apply CRFE and three widely-used functional enrichment methods (GSEA, clusterProfiler, MGSA) to 50 treatment-control gene expression data sets and compare their performance based on their emphasis on top perturbed genes, conciseness, and redundancy. CRFE performs well in all criteria, whereas every other method performs poorly in at least one.

Keywords – computational biology, functional enrichment, Bayesian network, Markov chain Monte Carlo

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Altered cytokine and gene expression in vitamin A deficient mice after RSV infection and vitamin A supplementation

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Respiratory syncytial virus (RSV) is a primary cause of lower respiratory tract disease in young infants and children. Vitamin A (VA) deficiency (VAD) increases susceptibility to severe respiratory infections. However, little is known regarding how VA impacts lung immune function. Our primary objectives were to investigate how VAD impacts innate immune function during viral infection and if local delivery of VA by polyanhydride-nanoparticles (NPs) restores the immune response in the lung. Vitamin A sufficient (VAS) and VAD BALB/c mice were infected intranasally with RSV. Groups of VAD mice were treated intranasally with VA-encapsulated NPs 24 hours after infection. Lung tissues were collected on day 3 post-infection, and innate immune populations were analyzed by multicolor flow cytometry. Luminex bead-based multiplex assays and RT-qPCR were used to analyze cytokine production and gene expression in lung tissue lysates following RSV infection and NP supplementation. Cytokine and chemokine production was altered in the lungs of VAD animals after RSV infection. VAD mice had more neutrophils recruited to the lungs and changes in the accumulation of CD27+ and CD27neg γδ T cell populations in the lung, suggestive of an altered the balance by Th1 and Th17 type immunity. Intranasal VA supplementation after RSV infection induced cytokine and chemokine production and upregulation in the expression of retinoic acid-producing enzymes and tight junction proteins. VAD alters the inflammatory environment in the lung, which may contribute to disease susceptibility. Intranasal supplementation with VA-encapsulated NPs alters chemokine expression in the lungs and may impact lung repair mechanisms by altering tight junction proteins.

Keywords – immunology, virus, mice

Manipulation of ATM alters the ovarian proteome response in cultured mouse ovaries during DMBA exposure

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Dimethylbenz[a]anthracene (DMBA) is a chemical carcinogen released during the combustion of organic matter, including wildfires, car exhaust, and cigarette smoke. Genotoxic damage initiates the ovarian DNA damage repair (DDR) response, coordinated by Ataxia Mutated Telangiectasia (ATM). Inhibition of ATM previously prevented toxicant-induced follicle loss; thus, this study investigated the ovarian proteomic response after manipulating ATM. PND4 mouse ovaries were cultured for 4d containing: 1) vehicle control (1% DMSO), 2) DMBA (1 μM), 3) ATM inhibitor (ATMi; 10 nM), 4) DMBA (1 μM) + ATMi (10 nM), 5) ATM activator (ATMa) - AVEN (1 μM) or 6) DMBA (1 μM) + ATMa (10 nM). Ovarian proteome was analyzed via LC-MS/MS. DMBA altered 167 proteins (P < 0.05; 91 proteins increased and 76 decreased), ATMa affected 60 proteins (P < 0.05; 21 proteins increased and 39 decreased) compared with control. DMBA with ATMi altered 23 proteins (P < 0.05; 17 proteins increased and six decreased), while during ATM activation, DMBA exposure altered 137 proteins (P < 0.05; 91 proteins increased and 76 decreased). Exposure to DMBA altered ovarian DDR proteins; HR proteins: KH domain-containing protein 3 and Actin-related protein 2/3 complex were increased (P < 0.05) by 1.3- and 0.34-fold, respectively, whereas an NHEJ regulator, 26S proteasome non-ATPase regulatory subunit 14, was decreased by 0.35-fold (P < 0.05). There was an absence of a DDR protein effect during DMBA exposure with ATMi. Conversely, several DDR-related and chromatin regulation-related proteins were increased in ATMa ovaries with DMBA. Host cell factor 1was increased by 0.50-fold; actin-related protein 2/3 complex subunit 1B, which promotes HR, was increased by 0.95-fold; nucleolin which induces chromatin decondensation, was increased by 1.0-fold; whereas, FACT complex subunit, a histone chaperone was increased by 2.7-fold (P < 0.05). Together the alterations in the abundance of these proteins suggest that ATM plays a role in the coordination of the ovarian DDR response during DMBA-induced ovotoxicity.

Keywords – ovary, DMBA, Ataxia Mutated Telangiectasia, mouse, LC-MS/MS

Assessing interactions among Beauveria bassiana, western corn rootworm larvae and maize

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Western corn rootworm, Diabrotica virgifera virgifera (LeConte) (Coleoptera: Chrysomelidae), is a serious pest of maize. Western corn rootworm larvae reside in the soil where they feed on maize roots, which can substantially reduce yield. Because resistance by western corn rootworm to management tactics such as crop rotation, insecticides and Bt maize has been documented, additional management tools including the use of biological insecticides, should be assessed. Entomopathogenic fungi (EPF), such as Beauveria bassiana, are naturally occurring in the soil and have, in some cases, been shown to grow endophytically, within plant tissue. Due to its persistence in the soil and endophytic competence, B. bassiana may be a beneficial tool in the management of western corn rootworm. We studied the effects of two isolates of B. bassiana on the mortality of western corn rootworm larvae in bioassay environments that contained and lacked soil. Additionally, maize seeds were inoculated with conidia of B. bassiana and plants were tested to determine whether B. bassiana was able to establish endophytically and whether it was able to persist in the rhizosphere. Results from this research will provide a better understanding of interactions among EPF, crop plants and insect pests, and the potential uses of EPF in pest management.

Keywords – Entomopathogen, bio-pesticide, Western corn rootworm

Genetic control of gene expression and protein abundance in the blood of young healthy pigs

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Blood is a composite tissue that comprises proteins produced by classical blood cells, and those that leak from other tissues. Its different components like antibodies, cell composition, and other biophysical properties are good indicators of the animal's body state. mRNA levels in blood cells can only partly explain the abundance of proteins in the blood. This study aimed at exploring the existence of a common genetic control for mRNA and protein levels in the blood of young healthy pigs. Blood samples from 912 young healthy F1 Yorkshire X Landrace barrows were assayed to quantify the mRNA and protein levels. A total of 16,546 genes were found to be expressed in blood plasma cells and 377 proteins were found to be abundant. Of these, 127 proteins corresponded to transcripts expressed in blood cells. Variance components for the levels of mRNA and proteins were estimated using univariate animal models and 66 protein-gene pairs were found to be heritable above 0.05. Results from the bivariate analysis indicated that the phenotypic correlation between protein-gene pairs in blood was generally low (-0.14 to 0.28). However, the genetic correlation between any protein-gene transcript pair was as high as 0.80 (-0.82 to 0.96), suggesting the presence of a common genetic control for these two molecular phenotypes. A bivariate genome-wide association study (GWAS) potentially identifies regions of the genome that drive the genetic covariance between gene expression and protein levels in the blood of young healthy pigs. These results support our hypothesis that gene expression and protein abundance in the blood of young healthy pigs could have a common genetic control, and hence subsequent analyses are aimed at establishing the commonality amongst the regions that drive the genetic covariance through gene ontology and pathway analyses. Findings from this study will enhance the possibility of using this common genetic control to understand the effectiveness of trait inheritance across generations and response to genomic selection.

Keywords – genetic control, correlation, heritability, pigs, GWAS

Humoral Responses to Porcine Circovirus Type 3 Infection

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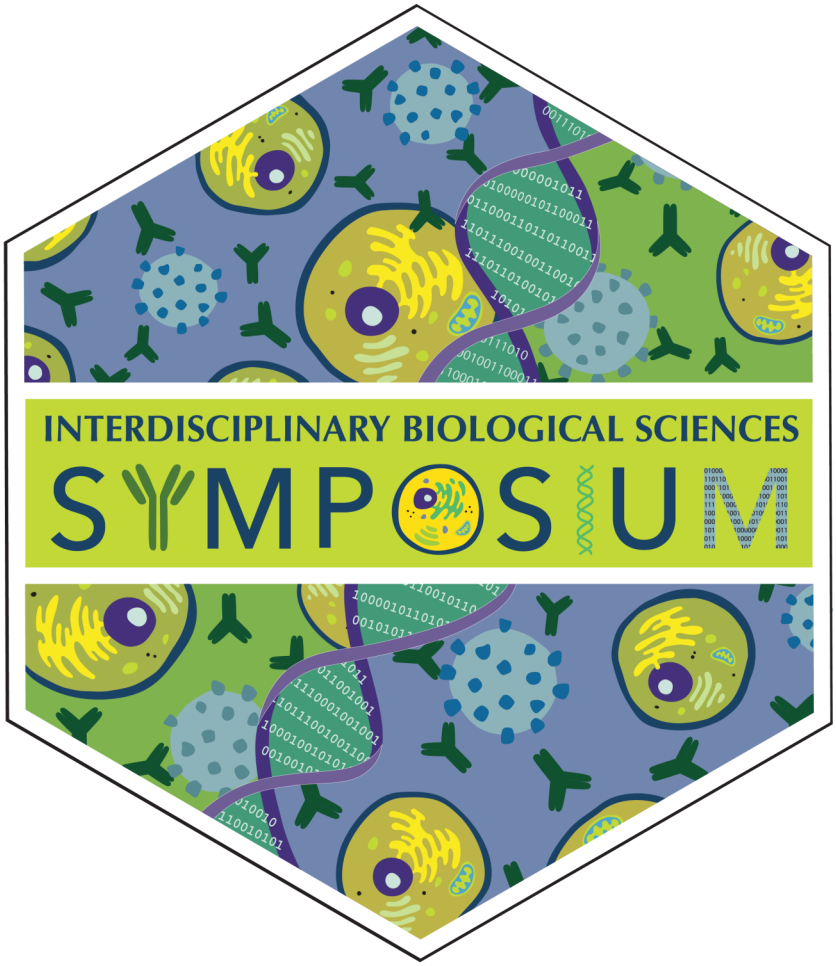
Porcine circovirus type 3 (PCV3) was first identified in the United States in 2016 and has been associated with reproductive failure and multisystemic inflammation. PCV3 is a circular, single-stranded DNA virus of 2 kb with two major structural proteins, the capsid (Cap) and replicase (Rep). Data related to the PCV3 humoral response is scarce. The objective of this study is to evaluate the Cap and Rep humoral response dynamic following PCV3 experimental infection. Eighteen 5-week-old CD/CD pigs were randomly assigned to four treatment groups: PCV3, PCV3+KLH, Negative Control, and KLH control. PCV3 and PCV3 + KLH groups were intramuscularly and intranasally inoculated with 2mL of PCV3 tissue homogenate. PCV3 + KLH and KLH control groups were administered KLH. Serum was collected weekly until day 42. For the IFA, PCV3 Cap and Rep genes were cloned into pcDNA3.1+N-6HIS and transfected into HEK293T cells. After 48 hours, 2-fold serial dilutions of swine serum were added, followed by FITC conjugated anti-swine IgG or IgM secondary diluted to 1:100. The IFA titer was expressed as the highest dilution where fluorescent signal was detected. For the Cap IgG antibody response, a single pig (PCV3+ KLH group) had a detectable antibody level on 14 days post infection (dpi). All pigs in both PCV3 and PCV3 + KLH inoculated groups had detectable antibodies by 35 dpi and reached and average maximum titer of 1:4,096 by 42 dpi. For the Rep IgG antibody response, a single pig (PCV3 group) had detectable antibodies at day 28 dpi. All pigs in both PCV3 and PCV3 + KLH groups had detectable antibodies by 35 dpi and reached and average maximum titer of 1:64 by 35 dpi. No Cap or Rep IgM was detected at any time points in all groups. Transfected Cap and Rep HEK293T cells were able to detect Cap and Rep specific antibodies in swine serum. PCV3 induces a delayed humoral response detectable several weeks post-infection with a much higher magnitude against the Cap compared to the Rep. Future investigations should evaluate the biological relevance of these antibodies on viral clearance.

Keywords – PCV3, antibodies, experimental infection, swine

Notes

Notes



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23055	***Becker, Sage***	*"Butyrate modulation of inflammatory responses to lipopolysaccharide in porcine monocytes"*
23056	***Bhusal, Bandana***	*"Evaluation of selected Bacillus spp. as biological control agents for Phytophthora Blight disease (Phytophthora capsici) in pepper"*
23002	***Cheng, Chelsea***	*"Nod1-dependent NF-$_k$B activation initiates hematopoietic stem cell specification in response to small Rho GTPases"*
23019	***Chung, Henri***	*"Prediction of Functional Linkages with Fusion based Phylogenetic Profiles"*
23021	***Cook, Tanner M.***	*"Establishing a System for High-Throughput Screening of Factors Affecting Meiosis"*
23012	***Das, Anurag***	*"The Role of Peroxisome mediated glial-neuronal communication and its impact on neuronal health during aging in the adult Drosophila"*
23016	***DeTemple, Joseph***	*"CERIS-JGRA analysis of flowering time in CML277 and Tzi8"*
23052	***Entriken, Seth***	*"Exploring the Role of Connexins in the Establishment of Left-Right Asymmetry during Embryonic Development"*
23007	***Fattel, Leila***	*"GO-Based Comparative Functional Genomics in Maize NAM Founder Lines"*
23020	***Fonder, Catherine***	*"Magnetic Stimulation Influences Differentiation of Rat Adult Hippocampal Pro genitor Cells In vitro"*
23043	***Franco, Carlos E.***	*"Dietary zinc and BRD effects on labile zinc levels and zinc transporter gene expressions in bovine peripheral immune cells"*
23058	***Guo, Yuxiang***	*"Maize orphan genes and their potential association with cuticle synthesis"*
23038	***Jia, Kejue***	*"Predicting RNA Structures from Triplet Sequence Correlations"*
23036	***Joshi, Parnal***	*"Identifying protein function annotation differences between mouse and human orthologous proteins"*
23050	***Kapoor, Mehak***	*"Modularity in Gene Regulatory Networks"*
23032	***Kilinc, Mesih***	*"A Static Website Generator for Bioinformatics Research"*
23039	***Kinney, Shelly***	*"Single kernel NIR spectrometer calibration to distinguish between waxy and wild type maize"*
23028	***Krull, Abigail***	*"Creation of Anxa2 Deletion Mutants in Zebrafish Using CRISPR/Cas9 Gene Editing"*
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23001 – Poster

Mechanism of Fyn kinase-mediated microglia activation and inflammatory associated neurodegeneration

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Cellular kinases play a central role in protein aggregation in dementia and neurodegeneration, and their involvement in inflammatory signaling in Alzheimer's Disease (AD) and related neurodegenerative diseases (ADRD) is now emerging. The Src family member Fyn kinase mediates AD amyloid b and tau protein pathophysiology. More recently, Fyn was identified as a Parkinson's Disease (PD) risk locus and in vitro has been shown to regulate microglia PKC-d/NF-kb inflammatory signaling in response to a-synuclein aggregate uptake. While protein aggregation and microglia activation reinforce neuroinflammation, the cellular mechanism at the intersection of these pathways is incompletely understood. An in vivo cell type-specific model of Fyn signaling is needed to reveal new insights into mechanisms driving microglia activation and to provide an effective genetic tool to identify novel Fyn effectors of neurodegeneration. In my talk, I will discuss two projects to examine in vivo the mechanism of Fyn signaling in microglia activation and neurodegeneration. First, using confocal live imaging and gene expression analysis, I demonstrated our neuronal Gal4, UAS: Fyn zebrafish PD model drives dopaminergic neuron loss in the 3-5 day larval brain diencephalon. This loss correlates with microglia activation and elevated expression of inflammatory cytokines IL-1b, IL12b, and TNFa. Chemical inhibition studies showed Fyn-driven neurodegeneration and cytokine expression were mediated by the canonical PKC-d/NF-kb/caspase-1 pathway. This work also identified the PKA pathway as a novel downstream effector in the mechanism of Fyn signaling. In a second project, I used our GeneWeld CRISPR precision knockin strategy to isolate loss of function and Cre-regulated conditional alleles in the zebrafish caspase-1 homolog caspa. Together with our CRISPR-generated mpeg1.1-2A-KalTA4 knockin, these novel caspa genetic tools will be used to examine the cell-autonomous requirement of canonical PKC-d/NF-kb/caspase-1 signaling in Fyn-driven microglia activation.

Keywords - Fyn Kinase, Neurodegeneration, Zebrafish, CRISPR, Neuronal model

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23002 – Poster

Nod1-dependent NF-kB activation initiates hematopoietic stem cell specification in response to small Rho GTPases

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The possibility of specifying functional hematopoietic stem and progenitor cells (HSPCs) from human pluripotent stem cells (hPSCs) would overcome current limitations related to HSPC transplantation. However, generating hPSC-derived HSPCs has been elusive, necessitating a better understanding of the native developmental mechanisms that trigger HSPC specification. Here, we revealed *in vivo* an intrinsic inflammatory mechanism triggered by Nod1 that drives early hemogenic endothelium (HE) patterning to specify HSPCs. Our genetic and chemical experiments showed that HSPCs failed to specify in the absence of Nod1 and its downstream kinase Ripk2. Rescue experiments demonstrated that Nod1 and Ripk2 acted through NF-kB, and that small Rho GTPases are at the apex of this mechanism. Manipulation of NOD1 in a human system of hPSCs differentiation towards the definitive hematopoietic lineage indicated functional conservation. This work establishes the RAC1-NOD1-RIPK2-NFkB axis as the earliest inflammatory inductor that intrinsically primes the HE for proper HSPC specification. Manipulation of this pathway could help derive a competent HE amenable to specify functional patient specific HSPCs for the treatment of blood disorders.

Keywords – Hematopoiesis, stem cells, inflammation, zebrafish

23003 – Poster

A model of Grna-Jak-Stat signaling in myeloid development

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In research, an interesting phenotype always deserves an elucidating molecular mechanism. Previous research demonstrated that Grna was required for the development of neutrophil and macrophage populations but also played an inhibitory role in the expression of the erythroid master transcription factor, gata1 (Campbell et al., Blood Adv. 2021). We now aim to develop a molecular mechanism to explain the role of Grna in myeloid differentiation. Bulk RNA-sequencing of zebrafish hematopoietic tissue (kidney marrow) was utilized to identify candidate pathways. For targeted knockdown of jak1 and stat3 gene expression, we used translation-blocking morpholinos and a constitutively active Stat3 construct(Bromberg et al., 1999) for rescue experiments. The recently published Cut&Run protocol (Barakat et al.; Zebrafish, 2022) was utilized to test potential Stat3 binding sites within the untranslated regions of grna. Transcriptomic comparison of grna-/- and grna+/+ kidneys revealed downregulation of the Jak1-Stat3 pathway in grna-/- tissue. It also showed an upregulation of jak2a in grna-/-s. Previously published work with Jak2 and its phosphorylation target, Stat5, demonstrated that both are important for erythropoiesis (Ha et al., Blood., 2007; Grebien et al., Blood., 2008). Knockdown of jak1 and stat3 decreased the number of neutrophils and macrophages, similar to the grna-/- embryos phenotype. Conversely, the introduction of constitutively active Stat3 was able to rescue these populations within grna-/- embryos. Furthermore, analysis of predicted Stat3 binding sites on grna indicated several valid sites. Based on current data, we hypothesize that Grna acts on myeloid differentiation in two ways: 1) promoting Jak1-Stat3 signaling with a feedback loop between Grna and Stat3 and 2) inhibiting Jak2-Stat5 signaling. There is still room for expansion of this model, i.e., with mediators between Grna and Jak1/Jak2a to clarify how Grna interacts with these two kinases. This model provides the potential for future targets that can be used in myeloid-based disease therapeutics.

Keywords – myeloid development, Jak-Stat signaling, granulin, zebrafish

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23007 – Poster

GO-Based Comparative Functional Genomics in Maize NAM Founder Lines

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Better gene function predictions enable better hypotheses for testing gene functions via laboratory and field experiments, which saves time and resources. As more genomic sequences become available and the quality has become comparable, scientist are using genomic analyses to answer increasingly complex biological questions. Biologists have been using the genomic differences observed with comparative genomics to better understand the relationship between genes and phenotypes within and across species. We are interested in creating a proof of concept analytics software to help researchers access and use large-scale datasets to generate hypotheses and prioritize candidate genes for phenotypes of interest. Using GOMAP, we annotated gene function for several crop genomes. We recovered known evolutionary histories across plant species based on their functional annotation datasets. This indicates that the datasets across species retain sufficient biological signal to carry out and make use of GO for comparative functional genomics. As a next step, we are investigating the potential use of GO for comparative functional genomics within species to bridge the gap between gene function and phenotype for making biologically meaningful inferences and hypotheses. Here we focus on potential methods and ideas on how to analyze associations between genes and phenotypes using the NAM founder lines.

Keywords – functional genomics, gene ontology, GOMAP, maize, crop plants

23008 – Poster

Proof of Concept for Spoken Natural Language Descriptions of Phenotypes for Association Genetics Applications

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Imagine walking through a field and saying aloud what you see, then using that audio file to conduct an association study. Advancements in Natural Language Processing (NLP) have allowed for processing large volumes of descriptive data. To make these advancements applicable for biologists interested in better understanding phenotypes and traits, we are developing methods to collect and process in-field descriptions of maize using recordings of spoken phenotype descriptions. We planted the Wisconsin Diversity panel in Boone, Iowa (summer of 2021). Nine undergraduate student workers recorded spoken descriptions of the Wisconsin Diversity panel lines in the field. We instructed the students to describe certain plant parts and other miscellaneous attributes using their own words. For comparative purposes, we also collected numerically scored phenotypes using traditional data collection techniques and images of each line. Our pipeline for processing the hundreds of hours of spoken descriptive data starts with the Amazon Web Services (AWS) cost-effective transcription service Transcribe. We can compute on the text descriptions of plants produced by Transcribe using NLP tools, which enables us to generate the input for tools that are commonly used by the maize research community to perform Genome-Wide Association Studies (GWAS). The results of the association studies completed using spoken data are then compared to published associations. Generating spoken descriptive datasets and protocols for demonstrating biologically relevant approaches for these data are anticipated to enable the maize research community to use innovations in language processing for in-field phenotyping methods.

Keywords – phenotyping, natural language processing, Genome Wide Association Studies, Maize

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23010 – Poster

Characterization of candidate genes related to cuticular wax deposition on maize silks

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The hydrophobic cuticle, which covers aerial portions of plants, is the first line of defense against environmental stresses, including drought, UV radiation, temperature, and insects and pathogens. This cuticle is comprised of a cutin polyester matrix that is infused with and laid atop by cuticular waxes, comprised of differing combinations of very long chain fatty acids (VLCFAs), hydrocarbons, aldehydes, alcohols, esters, and ketones. The cuticle on maize silks is rich in hydrocarbons, with minor amounts of VLCFAs and trace aldehydes and alcohols that together provide important protection for this tissue during the pollination period. Cuticle biosynthesis within the epidermal cells is tightly regulated at both the transcriptional and post-transcriptional levels. Many of the transcription factors known to regulate cuticle biosynthesis and deposition in Arabidopsis and in maize are not expressed in silks, suggesting different transcriptional regulation in this important organ. We have identified two transcription factors through multi-omics approaches that are putatively related to cuticle composition in either silks or seedlings (i.e. FDL1 and a bZIP TF). In addition, we have identified genes involved in the fatty acid elongation pathway (e.g. a Ketoacyl-CoA Synthetase) and in lipid transport (e.g. lipid transfer proteins that may facilitate wax transport through the cell wall) that are putatively associated with cuticular wax composition on maize silks. The potential functions of these candidate genes in regulation of cuticle biosynthesis and deposition on silks have been assessed by profiling cuticular waxes on silks from UniformMu mutants of these candidate genes. Characterization of these genes will allow a better understanding of how the plant cuticle is formed and deposited, and lays the foundation for future applied breeding approaches to generate “designer” protective cuticles.

Keywords – reverse genetics, cuticle regulation, maize

23012 – Poster

The Role of Peroxisome mediated glial-neuronal communication and its impact on neuronal health during aging in the adult Drosophila

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Peroxisomes are ubiquitous organelles which are responsible for various metabolic activities through the regulation of antioxidant enzymes and oxidases. One such process is highlighted during the process of cellular aging when increased oxidative stress leads to a reduced import efficiency of peroxisomal matrix proteins containing a C-terminal peroxisomal targeting signal type 1 (PTS1). One of the key import factors that is predicted to enable cargo proteins holding PTS1 and its delivery to the peroxisomal matrix is the PEX5 (peroxin-5) protein (Huang et al., 2020). The peroxins are a group of peroxisomal proteins which regulate the assembly and import of peroxisomal import proteins. There is an emerging evidence of peroxisomal distribution in the central nervous system and its dependency on glia cells that has been reported recently (Chung et al., 2020). Our preliminary results comparing peroxisomal distribution matches established knowledge that the adult Drosophila brain has a higher prevalence of abundant glial subpopulation in adult brain (Kremer et al., 2017). Thus, we aim to establish a closely intertwined connection between peroxisome biology and glial of the adult Drosophila during the process of cellular aging. The goal of my project is to see the dynamic changes in transport of the peroxisomal import machinery from the cytosol to the peroxisome marked with eYFP carrying the peroxisomal targeting signal - PTS1. Utilizing Drosophila genetics, we are using a combination of anti-GFP and anti-PMP70 as reporter genes to identify how impaired peroxisomal import contributes to neuronal health defects in flies. We demonstrate that eYFP.PTS1 reporter driven by Repo-GAL4, a pan-glial marker, can be used to understand the decline of peroxisomal transport in the glial cells of the adult female Drosophila brain. We have further identified the antennal lobe as a region of interest in the adult Drosophila brain with high abundance of peroxisomes. Our preliminary data suggest that peroxisome import declines in aged glial cells within the antenna lobe region.

Keywords – peroxisome, cellular aging, glia, Drosophila melanogaster, inter-organ communication

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23016 – Poster

CERIS-JGRA analysis of flowering time in CML277 and Tzi8

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Genotype by Environment (GxE) effects are important for understanding plasticity and genotypic means in diverse genetic lines and different environments. The Maize Nested Association Mapping (NAM) families provide a GxE context within which individual traits, such as Flowering Time (FT), can be studied and compared. For this study, we looked at Recombinant Inbred Lines (RILs) from the CML277 and Tzi8 families, two tropical NAM founders with potential for genetic transformation, and compared the genotypic mean and plasticity of Flowering Time across environments. This comparison was done using the CERIS-JGRA approach, where environmental factors are replaced with an environmental index—the combination of a factor over a specified time window. By identifying temporal windows of high correlation with phenotypes, the selected environmental indices are relevant to early-season development as well as end-of-season phenotypes. QTL analysis was performed on the slope and intercept of these lines to find genetic loci associated with the identified environmental indices.

Keywords – quantification genetics, breeding, genotype x environment, Zea mays

23017 – Poster

Structure-function insights and detection of plant root chitinases for rhizosphere studies

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Chitinases are glycoside-hydrolytic (GH) enzymes that degrade chitin, a linear homopolymer of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc) residues found in the cell wall of fungi and exoskeleton of arthropods. Chitinases are secreted into the rhizosphere, a complex and dynamic environment where intense nutrient exchange occurs between plants and microbes. Although studies have been conducted on plant chitinases, no studies have been done on chitinases secreted by plant roots. Here we modeled, expressed, purified, and characterized *Zea mays* and *Oryza sativa* root chitinases, and that of a symbiotic bacterium, *Chitinophaga sp*. to determine their role in the rhizosphere and to further understand the molecular mechanisms underlying plant-microbe interactions. Results showed that maize BEA, and rice OsCh belong to the GH19 protein family of chitinases having an N-terminal chitin-binding domain (CBD), an α-helix-rich catalytic domain characterized by a deep cleft, and a flexible C-terminal arm. The bacteria CspCh belongs to the GH18 family with a triosephosphate-isomerase (TIM) barrel (β/α) catalytic region. BEA hydrolyzes 4-MU-GlcNAc3 with apparent K_m, V_m and K_{cat} values of 41μM, 3.4μMmin^{-1} and 32min^{-1} respectively. OsCh maintains activity at pH5–7 while BEA and CspCh maintains activity at pH7-9 and at high temperatures of 40–60°C. Mutations of BEA shows the active residues to be the negatively charge residues E147 and E169 strategically positioned at ~8.6Å in the catalytic cleft. Studies of the truncated protein also showed that the CBD and flexible C-terminal arm were vital for inhibiting fungal growth using *Aspergillus niger*. The results indicate the possible involvement of these chitinases in plant defense which is promising in further investigations of their application as antifungal agents and in rhizosphere microbiome studies. Plants are essential in human lives, required for atmospheric oxygen, food, and medicine, therefore understanding their cellular processes and how they defend themselves against harmful pathogens is critical for plant and human survival.

Keywords – Chitinases, rhizosphere, chitin-binding domain (CBD), *Zea mays*, C-terminal arm

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23018 – Poster

The effect of "fear" on two species competition

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Non-consumptive effects such as fear of depredation can strongly influence predator-prey dynamics. There are several ecological and social motivations for these effects in competitive systems as well. In this work, we consider the classic two species ODE and PDE Lokta-Volterra competition models, where one of the competitors is "fearful" of the other. We find that the presence of fear can have several interesting dynamical effects on the classical competitive scenarios. Notably, for fear levels in certain regimes, we show novel bi-stability dynamics. Furthermore, in the spatially explicit setting, the effects of several spatially heterogeneous fear functions are investigated. In particular, we show that under certain integral restrictions on the fear function, a weak competition type situation can change to competitive exclusion. Applications of these results to ecological as well as sociopolitical settings are discussed, that connect to the "landscape of fear" (LOF) concept in ecology.

Keywords – Lokta-Volterra competition model, fear effect, landscape of fear, stability, bifurcation

23019 – Poster

Prediction of Functional Linkages with Fusion based Phylogenetic Profiles

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Despite extensive research into bacterial functions, the vast majority of protein interactions remain unknown and unannotated. In this work, we present a novel improvement to phylogenetic profile-based methods to identify functional linkages with Fusion, a database of 15 million functionally similar proteins., Phylogenetic profiles can indicate the functional relatedness of proteins by comparing the co-presence and abundance of these proteins across related genomes. Linked protein functions, e.g. members of the same pathway, are likely to have been preserved or eliminated in new species throughout their evolutionary history. A reference set of linked protein functions was collected from KEGG, the Kyoto Encyclopedia of Genes and Genomes, based on shared participation proteins in the same metabolic pathway. We used Fusion with phylogenetic profiles to identify linked proteins across 1,402 unique bacterial species. Fusion clusters proteins into groups of identical predicted protein function based on sequence similarity and length. We show Fusion groups more accurately identify functional linkages against other measures of orthology, such as PFAM domains. We further show groups of proteins identified with this method are proximal on a given organism's chromosome, another indicator of their functional relatedness. We demonstrate the number and taxonomy of species used for profile selection significantly affects prediction results, affirming conclusions found in previous studies, and expand upon their work by comparing predictions between different prokaryotic phyla. With the rapid expanse in genomic sequence information, expanding and improving fundamental prediction methods will be invaluable for efficiently analyzing new genomes.

Keywords – bacteria, function, proteins

23020 – Poster

Magnetic Stimulation Influences Differentiation of Rat Adult Hippocampal Progenitor Cells In vitro

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Transcranial magnetic stimulation (TMS) is a clinical therapy for various central nervous system diseases; however, the cellular mechanism of its effects on adult neural stem cells is not well understood. Our in vitro study investigates the effects of low-frequency, pulsed magnetic stimulation on adult rat hippocampal progenitor cell (AHPCs) neurospheres. AHPCs are capable of self-renewal and multilineage differentiation including neurons, oligodendrocytes, and astrocytes. When cultured as non-adherent neurospheres, the AHPCs are maintained within a three-dimensional microenvironment with increased cell-cell interactions allowing for more accurate modeling of the natural in vivo environment of these cells compared to two-dimensional cell cultures. Daily sessions of magnetic stimulation consisting of 100 pulses at 0.1 Hz were performed for up to 10 days in vitro (DIV). At 2 DIV and 7 DIV, AHPCs were stained with propidium iodide (PI) to evaluate cell viability following stimulation, where it was found that the neurospheres maintained high cell viability even after 7 days of treatment. At 7 DIV and 10 DIV, AHPCs were fixed and immunolabeled for proliferation (Ki-67) and cell-type specific markers for immature and maturing neurons (TuJ1 and MAP2ab, respectively), astrocytes (GFAP), and oligodendrocytes (RIP) to assess differentiation. No significant differences in proliferation were observed between stimulated and unstimulated AHPCs. Magnetically stimulated AHPCs had increased oligodendrocyte differentiation at 7 and 10 DIV, while MAP2ab and TuJ1 differentiation increased at 7 and 10 DIV, respectively. qPCR gene expression analysis supported our differentiation analysis with increased neuronal and oligodendrocyte markers seen at both 7 and 10 DIV. These results show potential stimulus-dependent cellular effects of TMS, which can aid in the clinical advancement of the therapy and allow for tailored TMS therapy for specific diseases.

Keywords – magnetic stimulation, TMS, neurosphere, adult hippocampal progenitor cells, differentiation

23021 – Poster

Establishing a System for High-Throughput Screening of Factors Affecting Meiosis

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While recent advances in plant biology have shed light on meiotic recombination and cycle progression, knowledge regarding the transition between meiosis induction and mitosis in plants is still very limited. Research exploring this issue has involved mostly aberrant gene analysis to see effects on meiosis, but induction research is rare. By producing a tool to screen for meiosis induction at high throughput in A. thaliana, we enable the testing of thousands of suspected meiosis inducers in a controlled and efficient manner. Quantifiable meiosis induction will provide a solid foundation to explore the intricate underpinnings in the development of germ cells. By building such a tool we hope to establish fluorescent markers to successfully differentiate gametes and somatic cells in high-throughput. With this, we aim to inform methods to accelerate crop breeding cycles to increase genetic gain.

Keywords – plant biology, *in vitro* breeding, meiosis

23022 – Poster

Key developmental windows and environmental parameters that influence cuticular wax composition on maize silks

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Desiccation of maize silks hinders fertilization; thus, silk drought tolerance is a potential breeding goal to maintain or enhance maize productivity in increasingly dry environments. Maize silks, and other aerial organs, are coated with a hydrophobic cuticle that limits water loss and protects against other abiotic and biotic stresses. Prior work has found silk cuticular wax accumulation increases dramatically in growing seasons that experience low precipitation. Moreover, cuticular wax composition can vary among genotypes and accumulate to higher concentrations in portions of the silks that are emerged from encasing husk leaves into the external environment. While genotype-by-environment effects have been implicated in cuticular wax accumulation, a true multivariate analysis of cuticular wax phenotypes is still needed. To parse the effects of genotype and environment on cuticular waxes, 468 Wisconsin Diversity Panel inbreds were grown in both Iowa and Minnesota in 2016 to 2017, providing silk cuticular wax data for 45 metabolites across three environments, with high resolution weather data at each site. A similar experiment was performed in 2020 wherein 11 strategically selected inbreds from this panel were grown across 6 planting dates in both Iowa and Minnesota. Linear modeling and canonical correlation analysis were used to find putative associations between silk cuticular waxes and weather throughout plant development. Low precipitation and high solar radiation immediately prior to silking were correlated with increased cuticular wax accumulation, though intensity of solar radiation weeks prior to silking may also influence the silk cuticle. This analysis provides a foundation for controlled environment studies that assess the impact of specific weather parameters at specific stages in plant development on the silk cuticle. Ultimately, this research will lead to a better understanding of abiotic factors that impact cuticle composition and whether these modified cuticles protect against specific stresses.

Keywords – cuticle, drought tolerance, fatty acids, maize

23023 – Poster

Influence of the microbiota on *Drosophila* immune system development

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The gut microbiome is a complex ecosystem of microorganisms that plays an essential role in the health of host organisms. Evidence suggests that many physiological processes, including immune function, are influenced by the presence or absence of different microbial species. As a result, there is great interest to better understand these host-microbe interactions in the context of health and disease across animal systems. Here, we use *Drosophila melanogaster* as a model to investigate the interactions between the microbiota and the fly immune system. By rearing flies under germ-free (axenic) conditions, we demonstrate defects in immune cell populations by measuring the expression of known crystal cell and plasmatocyte markers, supporting that the microbiota contribute to fly hematopoiesis. In addition, axenic flies display reduced antimicrobial peptide (AMP) expression and fared worse against bacterial infections, suggesting that humoral immunity is similarly compromised. These results strongly suggest that the absence of the microbiota has significant effects on fly immune function. With *Drosophila* serving as an important model system with relevance for other insect and mammalian systems, our findings will have broad utility to increase our collective understanding of the mechanisms by which the microbiota influence hematopoiesis and maturation of the innate immune system.

Keywords – axenic, immune development, microbiota, *Drosphilia*

23024 – Poster

Cre/*lox* regulated conditional rescue and inactivation with zebrafish UFlip alleles generated by CRISPR-Cas9 targeted integration

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The ability to regulate gene activity spatially and temporally is essential to investigate cell type-specific gene function during development and in postembryonic processes and disease models. The Cre/*lox* system has been widely used for performing cell and tissue-specific conditional analysis of gene function in zebrafish. However, simple and efficient methods for isolation of stable, Cre/*lox* regulated zebrafish alleles are lacking. Here we applied our GeneWeld CRISPR-Cas9 targeted integration strategy to generate floxed alleles that provide robust conditional inactivation and rescue. A universal targeting vector, UFlip, with sites for cloning short homology arms flanking a floxed 2A-mRFP gene trap, was integrated into an intron in *rbbp4* and *rb1*. *rbbp4*^{off} and *rb1*^{off} integration alleles resulted in strong mRFP expression, >99% reduction of endogenous gene expression, and recapitulated known indel loss of function phenotypes. Introduction of Cre led to stable inversion of the floxed cassette, loss of mRFP expression, and phenotypic rescue. *rbbp4*^{on} and *rb1*^{on} integration alleles did not cause phenotypes in combination with a loss of function mutation. Addition of Cre led to conditional inactivation by stable inversion of the cassette, gene trapping and mRFP expression, and the expected mutant phenotype. Neural progenitor Cre drivers were used for conditional inactivation and phenotypic rescue to showcase how this approach can be used in specific cell populations. Together these results validate a simplified approach for efficient isolation of Cre/*lox* responsive conditional alleles in zebrafish. Our strategy provides a new toolkit for generating genetic mosaics and represents a significant advance in zebrafish genetics.

Keywords – Cre/*lox*, conditional rescue, conditional inactivation, zebrafish, GeneWeld

23025 – Poster

Effect of adjusting for neutrophil proportion in whole blood transcriptomic analysis of influenza-infected pigs

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Advantages of whole blood (WB) in transcriptomic studies include simplicity of collecting and frequent sampling of the same individual, and whole blood transcriptomics has been used to study response to infection and vaccination in pigs. Interpretation of this type of data, however, can result in complexities due to variations in the proportions of cell types among blood samples versus in immune responses within specific cell types, both of which can cause differences in observed gene expression patterns. We utilized RNA from WB samples collected immediately before and six days after influenza challenge of 4 pigs, to determine the differentially expressed genes (DEGs) and their functions through 3'mRNA-seq analysis using DESeq2. Comparison of gene expression six days post-influenza infection compared to day 0 identified 884 significant DEGs. Adjustment of gene expression levels for the proportion of neutrophils in each blood sample, using a means-reference model for the effect of influenza, resulted in the detection of no significant DEGs, although a t-test found no significant difference in neutrophil proportion pre- vs. post-influenza challenge. We propose this indicates that the loss of DEGs after adjustment likely is not caused by neutrophil changes due to infection; rather, DE genes may stem from random variation in blood cell composition. In conclusion, adjustment for cell composition is important for confidence in results when investigating gene expression in a complex mixture. Additionally, these results show that six days post-influenza may not be the ideal sampling time to maximize the collection of variable transcriptomes. Further, these results provide insight for planning future experiments in our deconvolution project, including using inexpensive 3'RNAseq assays to identify the most informative time points for maximizing transcriptomic diversity for future deconvolution of whole blood.

Keywords – 3' mRNA-Seq, whole blood, influenza, pigs, porcine

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23026 – Poster

Evaluation of selected Bacillus spp. as biological control agents for Phytophthora Blight disease (Phytophthora capsici) in pepper

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Phytophthora blight (Phytophthora capsici) is one of the most important diseases in horticultural crops including pepper (Capsicum annuum). Pepper growers rely heavily on pesticides and pesticide resistance is problematic. In addition, toxicity hazards to growers, consumers, non-target organisms and environmental contamination is of great concern. The objective of this study was to evaluate five isolates of Bacillus spp. (IMC8, PRT, PS, PSL, YNP5) as biological control agents for P. capsici disease management. While Bacillus isolates promotes plant growth by increasing plant height, shoot weight, root length, root weight and chlorophyll content, three isolates IMC8, PS, PSL inhibited mycelial growth of P. capsici in in-vitro studies. The three isolates were evaluated further individually and in mixed populations in greenhouse environment using Phytophthora-infested soil. Mixtures of IMC8+PS, IMC8+PSL and IMC8+PS+PSL were similar to fungicide metalaxyl, in reducing P. capsici disease severity and in promoting plant growth. However, Bacillus isolate PSL displayed best potential in P. capsici disease management and plant growth promotion in Phytophthora-infested soil.

Keywords – biological control agents, disease management, Bacillus spp., Phytophthora capsici

23027 – Poster

Harnessing the Helitron

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Transposable elements are the DNA sequences that can move from one place to another in the genome. They are found in both prokaryotes and eukaryotes. There are two classes of transposable elements: Class I Retrotransposon and Class II DNA transposon. The maize genome contains a large number of LTR retrotransposons, TIR DNA transposons, and Helitron DNA transposons. Helitrons were first identified in 2001 by computational analysis of the genome of Arabidopsis, Rice, and Caenorhabditis elegans. In maize, helitrons contribute to genomic diversity by capturing fragments of genes and moving these fragments to new positions in the genome. Helitrons are unusual in that they lack the structural features of TIR transposons and LTR retrotransposons, including both direct or inverted repeats at the TE ends and target site duplications flanking insertions. This makes helitrons especially tricky to annotate, which had led to inaccuracies in annotation of large numbers of putative genes. Accurate annotation of helitrons is critical to understanding the origin of gene and pseudo-gene sequences found interspersed throughout the genome. Here, we propose a gene-centric method for identifying helitron insertions where we first identify trans duplicated gene fragments genome-wide, then implement filtering and characterization steps to identify TE ends and family structure. The vast majority of gene fragments contained within helitrons are likely to have lost their original functions due to fragmentation and epigenetic silencing. However, the presence of a vast pool of transduplicated genic DNA is likely to have impacted evolution of the host gene function in surprising ways. With this work, we aim to better characterize the structure and distribution of helitrons to better understand the functional impact that these enigmatic transposable elements have had on the evolution of the maize genome as a whole.

Keywords – transposable elements, Helitrons, transduplicate, maize, annotation

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23028 – Poster

Creation of Anxa2 Deletion Mutants in Zebrafish Using CRISPR/Cas9 Gene Editing

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Annexin A2 is a calcium-dependent phospholipid-binding protein that facilitates angiogenesis via endothelial barrier maintenance and sprout formation. However, the functions of Annexin A2 are not well established in endothelial cells in vivo. The Annexin A2 gene in humans corresponds to a duplicated locus in zebrafish, called *anxa2a* and *anxa2b*. We are using CRISPR-Cas9 to make mutations of the Annexin A2 homologs in zebrafish to take advantage of the optical clarity of the early embryo to follow endothelial morphogenesis. Due to the possible overlapping functions of these homologs, we designed gRNAs that, when complexed with Cas9, targeted the 5' and 3' UTRs of both *anxa2a* and *anxa2b*. This was done to induce whole gene deletions instead of creating loss-of-function mutants through indel formation. These whole gene deletions allow us to avoid genetic compensation possibly induced by mutant transcripts. We also designed a tag-RFP construct flanked by short homology arms for integration into the deleted locus to visually track *anxa2a* and *anxa2b* expression during development. Here, we describe a whole gene deletion of *anxa2a* (14,172 base pairs) via CRISPR-Cas9 injection in *fli1-egfp* embryos. We have recovered a stable germline deletion in anax2a. Our next steps are to induce a whole gene deletion in *anxa2b* for further functional analysis and create mutants homozygous for both deletions. This will allow for loss-of-function analysis of the Annexin A2 duplicated locus in zebrafish for a more complete phenotypic analysis and characterization of the genetic requirements of *anxa2a* and *b* during endothelial morphogenesis.

Keywords – gene editing, CRISPR-Cas9, functional genomics, zebrafish, whole gene deletion

23029 – Poster

NRF2 agonists modulate the pro-inflammatory response and viral replication during RSV infection

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Bovine respiratory disease (BRD) causes significant morbidity and mortality within the cattle industry worldwide. The abundant use of antimicrobials has led to drug-resistant pathogens, leading to the introduction of immunomodulation as an alternate strategy to reduce the severity and prevalence of BRD. Here, we recognize the NRF2 pathway for its crucial role in the downregulation of inflammatory response in respiratory viral infection, highlighting its potential as an immunomodulatory target. In this work, we evaluated the effects of NRF2 agonists 4-octyl itaconate (4-OI) and Dimethyl fumarate (DMF) on viral replication and innate inflammatory responses to Respiratory Syncytial Virus (RSV) infection using in-vivo and in-vitro models. Bovine Turbinate cells (BTs) and human lung epithelial cells (BEAS-2b) were stimulated with 4-OI, DMF, or vehicle, then infected with bRSV or hRSV, respectively. RNA was isolated post-infection, and RT-PCR was used to evaluate pro-inflammatory cytokines, chemokines, and antiviral mediators. Mice received i.n. 4-OI or DMF or vehicle, then infected with hRSV, and monitored. Lung lysates and RNA were obtained at three DPI to evaluate transcription of key inflammatory cytokines through RT-PCR and ELISA and viral replication targeting N-hRSV gene copies. Four-OI and DMF treatment inhibited transcription of pro-inflammatory cytokines and chemokines and type I IFN responses during RSV infection in BT and BEAS-2b cells. Similarly, we observed a decrease in transcripts of several pro-inflammatory mediators, IL-6 levels, and viral copies in lungs of hRSV-infected mice compared to untreated animals. Moreover, we observed less weight reduction in mice treated with 4-OI and DMF. These results suggest the use of NRF2 agonists 4-OI and DMF reduce the inflammatory response to RSV in-vitro and in-vivo and reduces viral replication. Further studies are planned to explore effects of NRF2 agonists on restoring the antioxidant capacity of RSV-infected tissue and their potential as an immunomodulation strategy in the prevention of BRD and RSV infection.

Keywords – immunology, epithelial cells, respiratory infection, cattle

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23032 – Poster

A Static Website Generator for Bioinformatics Research

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Motivation: Presenting the results of bioinformatics research can be challenging and requires sophisticated visualization components, which can be time-consuming to develop. This presents a barrier to effectively communicating research findings.
Results: To address this challenge, we have developed a static website generator, JSONWP, specifically designed for bioinformatics research. Utilizing React, we have integrated publicly available bioinformatics visualization components to provide standardized access to these components. JSON is used as the input source due to its ability to represent nearly all types of data using key and value pairs. This allows researchers to use their preferred programming language to create a JSON representation, which can then be converted into a website by JSONWP. No server or domain is required to host the website, as only the publicly accessible JSON file needs to be available.
Conclusions: Overall, JSONWP provides a valuable tool for bioinformatics researchers to effectively communicate their findings. The open-source implementation can be found at https://github.com/MesihK/react-json-wpbuilder, and the tool can be used at www.jsonwp.onrender.com.
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Keywords – bioinformatics website, protein visualization, MSA

23035 – Poster

Characterization of novel butyrate-producing bacteria isolated from swine feces

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The ability to identify putative members of the porcine gut microbiome and their metabolites has potential applications that extend to maintained animal health. Representative species that produce the short-chain fatty acid butyrate in particular are valuable points of study given the compound's important role in promoting colonic homeostasis and conveying increased resistance to enteric pathogens. Two rod-shaped, obligately anaerobic, butyrate-producing species previously cultured from the feces of a sow and post-weaning piglet were designated Roseburia sp. 499 and 831b based on phylogenetic analysis of conserved genes and fragmented genomes. Here, we describe efforts to expand upon the evolutionary relationship and physiology of these isolates. The addition of long-read Oxford Nanopore sequences resulted in complete, circular genomes for 499 (3.38 Mbp; 38.2% GC) and 831b (3.13 Mbp; 40.7% GC) with evidence of a large 243 kbp circular plasmid in 831b. The isolates share low average nucleotide identity (<85%) and alignment fraction with other members of their respective genera. The substrate utilization and carbon metabolism of 499 diverged from other species classified as Petralouisia in GTDB, including the presence of unique carbohydrate-binding modules for starch molecules, pyruvate formate-lyase activating enzyme, citrate (Re)-synthase, certain glycoside hydrolases, polysaccharide lyases targeting pectin (PL9). The metabolic profile of 831b displayed an array of carbohydrate-active enzymes shared across Roseburia. Further, genomic predictions for substrate metabolism were largely congruent with in vitro testing. The unique major cellular fatty acids of these strains were C14:0, C15:0, C17:0 (499), C14:0, C17:0, and C18:0 (831b). The range of pH and temperatures supporting growth of the isolates in turn reflects an ecological niche within the large intestine. Collectively, this data provides evidence for the establishment of isolates 499 and 831b as novel species within the genera Petralouisia and Roseburia, respectively.

Keywords – microbial ecology, gut microbiome

23036 – Poster

Identifying protein function annotation differences between mouse and human orthologous proteins

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The accurate annotation of biological functions of proteins is an open problem in life science. While improvements in sequencing techniques have facilitated novel data generation at a massive scale, there is still a major lag in the development of methods that can translate the sequence data into biologically insightful function information. Accurate knowledge of protein function is crucial for our understanding of disease phenotypes as well as to precisely select targets for drug design. While experimental assays are the best means of characterizing the function of a protein, they are expensive, time-consuming, and limited to certain species. As there are constraints to the experiments that can be carried out in humans, accurate annotation transfer from model organisms to humans is a centerpiece of biomedical research. The inference of protein function in humans relies heavily on experimental observations in model organisms. In this study, we analyzed experimental protein function annotations of mouse and human from Uniprot-GOA, a database of curated protein function annotations. Our assessment shows a strong imbalance, where annotations derived from the same scientific papers are more similar than annotations derived from different papers. These results indicate species- and experiment design-associated biases in protein function annotation databases. However, the exact description of the experiment that generated an annotation in a given species is not available in databases, and there exists no manually curated database that contains this information. Therefore, using Named Entity Recognition (NER), and ontologies, we demonstrate the ability to identify experimental assays from literature as a first step towards investigating biases due to the choice and limitations of assays, and choice of species.

Keywords – model organisms, protein function annotations, named entity recognition

23037 – Poster

Ribosome profiling, a deep sequencing technique to explore global effects of plant and human virus infection on host translation.

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Viruses are parasites of the host's translational machinery, yet translation is an underappreciated step in gene expression regulation. Therefore, we are investigating the global effect of virus infection on translation (the translatome) in plant and human hosts, especially in infections of Arabidopsis thaliana by red clover mosaic necrotic virus (RCNMV), and human placental cell line JEG3 by Zika virus (ZIKV). These RNA viruses rely on a similar mechanism in which noncoding (nc) RNAs are generated from the 3' untranslated region (UTR) of the viral genome via host exonucleolytic degradation of coding regions. To understand the effect of the ncRNAs on the host translatome, either for tombus- (RCNMV) or flaviviruses (ZIKV), we use ribosome profiling (ribo-seq) which is a powerful deep sequencing technique in which a translation-arrested cell lysate is digested with RNase to degrade mRNA not protected by a translating ribosome. The resulting ribosome protected fragments (RPFs) of mRNA are sequenced. Their abundance reflects the translational efficiency (TE) of the mRNA from which they arose. Ribo-seq of RCNMV-infected plants revealed that (i) the early translational response to RCNMV infection is enriched in plant immune response genes, (ii) at later infection stage, unfolded protein response was elicited, (iii) the coat protein open reading frame (ORF) is translated ~100-fold more efficiently than the RNA-dependent RNA polymerase ORF, and (iv) a strong ribosomal pause site in the movement protein ORF of RCNMV RNA2. Currently, we are working to determine the effect of ZIKV non-coding RNAs, that are called sfRNAs on host cell, since sfRNAs have been found to be responsible for pathogenicity, suppressing host defenses, binding a variety of host proteins and many other functions crucial for flavivirus infection. This work provides a framework for future research on translational control of and by viral RNA, and the under-explored translational regulation of plant and animal genes in response to viral infection.

Keywords – ribosome profiling, translation regulation, ribosome protected fragment, RCNMV, ZIKV

23038 – Poster

Predicting RNA Structures from Triplet Sequence Correlations

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The existing measures for describing coevolution correlations have primarily focused on pairwise coherences. However, the extension of such measures to higher-order contexts, where the coevolution involves multiple bodies simultaneously, has lagged behind. Biological molecules, such as RNA, have multiple interactions that can reveal important information about their function and structure mechanisms. While some work has been done with the consideration of multi-body correlations, existing approaches often employ pairwise measures to identify the higher-order dependencies. The direct measures of higher-order dependence have not been fully explored. In this study, we introduce an information-theoretic measure to quantify the higher-order dependencies within an RNA protein multiple sequence alignment. Our results demonstrate a significant accuracy in identifying triplet nucleotide interactions within an RNA molecule. We applied our method to 40 RNA families and obtained an average Area Under Curve (AUC) of 0.8 in predicting intra-molecule nucleotide interactions. Our findings indicate the existence of higher-order evolutionary dependencies in RNA molecules that can be captured by our computational approach. These higher-order correlations identified by our computational approach provide important information on RNA structure and evolution, as well as insights into the formation mechanism of complex tertiary structures, such as pseudoknots and G-quadruplexes.

Keywords – RNA structure prediction, higher-order dependence measures, coevolution correlations

23039 – Poster

Single kernel NIR spectrometer calibration to distinguish between waxy and wild type maize

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Waxy maize is high in amylopectin, making it important in agriculture for animal feed, in food products for its higher stability, and in adhesives for its cohesiveness. Waxy maize is characterized by recessive alleles at the wx1 locus. The current method of detection is by staining the endosperm with an iodine solution; this is time-consuming and potentially damaging to the kernel. A more efficient selection method is to use single kernel near infrared (NIR) spectroscopy to scan each kernel and use a calibration equation to predict whether kernels are waxy or wild type. The objective of this project is to create a calibration that differentiates waxy and wild type maize kernels for faster and simpler selection in breeding programs. For this project, maize kernels were scanned using a single kernel NIR spectrometer to collect spectra. Kernels were then identified as either waxy or wild type using an iodine staining laboratory procedure. A principal components analysis was used to identify group separations between waxy and wild type, and a discriminant analysis was used to develop a prediction model. To validate the model, maize kernels that were not included in the calibration set were scanned, the waxy status was predicted, and the kernels were tested in the lab for the waxy trait. Out of 416 scanned kernels for the validation set, 16 were misclassified. In addition, kernels with different genetic backgrounds could be distinguished with a principal components analysis. In conclusion, the model shows promising prediction with accuracy up to 96% for waxy and wild type maize kernels.

Keywords – NIR spectrometry, calibration, genetics, maize (Zea mays)

23043 – Poster

Dietary zinc and BRD effects on labile zinc levels and zinc transporter gene expressions in bovine peripheral immune cells

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Zinc (Zn) is the second most abundant transition metal in the body and is critical for innate and adaptive immune functions. Zn transporters regulate intracellular Zn homeostasis. In humans and mice, Zn deficiency may lead to increased disease susceptibility. However, trace mineral requirements, evidence of Zn dysregulation, and transporter expression in immune cells are poorly defined in cattle. We evaluated intracellular Zn concentrations, relative expression, and profiles of Zn transporters in circulating immune cells from healthy growing steers, calves, and animals experiencing a respiratory infection. Immune cells were collected during two separate animal trials. In trial 1, 18 Angus crossbred steers were stratified by body weight and randomly assigned to two dietary groups: control, receiving no supplemental Zn or HiZn, supplemented with 150 mg Zn/kg DM. Circulating immune cell populations were sorted with FACS and MACS methods and analyzed for intracellular Zn concentration using FluoZin-3 by flow cytometry and Zn transporters gene expression via RT-PCR on day 33. In trial 2, 10 Holstein calves were infected with BRSV, followed by 6.88x108 CFU of Pasteurella multocida. Whole blood was collected and analyzed for Zn transporters pre-bacterial infection and 48 hours post-infection. In trial 1, labile Zn levels based on FluoZin-3 MFI in monocytes, granulocytes, B cells, CD4, CD8, and gd T cells were influenced by dietary Zn. Relative expression of ZIP11 and ZnT1 in CD4 T cells, ZIP6 in CD8 T cells, and ZnT7 in B cells were modulated in steers fed a high Zn diet. In trial 2, ZIP 3, 7, 13, 14, and ZnT4 – 7 and 9 were downregulated, and ZIP2 was upregulated in calves 48 hours post bacterial challenge. These results suggest that dietary Zn intake can influence labile Zn levels and Zn transporters in circulating immune cells. BRD can also modulate the expression of Zn transporters in calves. In addition, Zn transporters were profiled in the circulating immune cells under physiological conditions, which might help us understand their unique role in Zn homeostasis.

Keywords – immunology, bovine respiratory disease, zinc, bovine

23045 – Poster

Characterization of Lamp1

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The endolysosomal system plays a crucial role in maintaining material and energy homeostasis by regulating the degradation and recycling of all macromolecules as per the cell's needs. Any lysosomal dysfunction can lead to serious human health risks. Lysosome-associated membrane proteins, LAMP1 and LAMP2, have been very well-characterized for their role in autophagy and cholesterol transport in vertebrates. In our work, we have shown that Drosophila Lamp1 is the ortholog of mammalian LAMP1 and LAMP2 and is not required for viability or autophagy, unlike in the mammalian LAMP1/LAMP2 double mutants. Further, Lamp1 deficiency also results in lipid metabolism defects, a previously unknown role for Lamp1, as the 3rd instar larvae presented with increased levels of sterols and medium-chain diacylglycerols (DAGs). Since the latter are exclusively used for transporting lipids in the insect's hemolymph, we hypothesize that the defect in Lamp1 causes aberration of the lipid transport in our mutants. Therefore, we have analyzed the gene expression profiles of prominent lipid metabolism moderators and genes in fat-body and gut tissues of Drosophila larvae to localize the accumulation of DAGs and gain better understanding of Lamp1's function. Our results overall suggest that Drosophila is a suitable model organism for investigating the function of LAMP proteins in lipid absorption, without complicating the investigation with the influence of lipid storage and without disrupting autophagic mechanisms.

Keywords – autophagy, LAMP proteins, lipid transport, *Drosophila*, lysosome

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23049 – Poster

Functional characterization of STRUBBELIG-receptor family 3 in regulating plasmodesmal function and plant immunity

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Plants rely on effective cell-to-cell communication to grow and respond to external stimuli such as pathogen infection. Plasmodesmata (PD) provide the plasma membrane, endoplasmic reticulum, and cytoplasmic continuity between adjoining plant cells, allowing direct cell-to-cell communication. Given their fundamental roles, PD are essential for plant survival. Recent studies highlighted the important role of PD-located proteins (PDLPs) in regulating PD function through an unknown mechanism. Using enzyme-catalyzed proximity labeling, we identified STRUBBELIG-receptor family 3 (SRF3), which is a leucine-rich repeat receptor-like kinase, as a functional partner of PDLP5. SRF3 was previously reported to be associated with PD and involved in iron homeostasis, root growth, and sensing bacterial flagellin (flg22). We confirmed the PD association of SRF3-sfGFP in Nicotiana benthamiana. Using co-immunoprecipitation, we demonstrated the physical interaction between PDLP5 and SRF3. We showed that srf3 mutants are compromised in the flg22-induced activation of mitogen-activated protein kinases. In addition, srf3 mutants are compromised in flg22-induced resistance to Pseudomonas syringae pv. tomato DC3000. In addition to PD association, SRF3-sfGFP is also detected in membrane-bound vesicles formed at PD. The formation of PD-associated vesicles can be induced by bacterial infection. The vesicles accumulate reactive oxygen species (ROS). The findings suggest the role of SRF3-containing vesicles in maintaining ROS homeostasis during bacterial infection. In line with the hypothesis, genes involved in ROS responses are misregulated in srf3 mutants. Together, our findings suggest that SRF3 might function at PD to regulate ROS homeostasis during bacterial infection.

Keywords – cell biology, plasmodesmata, PTI ETI, Arabidopsis, SRF3

23050 – Poster

Modularity in Gene Regulatory Networks

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Gene regulatory networks are models that illustrate how interactions between regulating components and their targets (such as transcription factors and target genes) determine a cell's decision-making. Numerous publications describe biological networks as modular without a rigorous definition of network modularity. Loosely speaking, a modular network can be divided into subgraphs that are weakly connected to one another yet densely connected within themselves. However, what constitutes weak and dense connections is undefined. Recently, we introduced strongly connected components of a directed graph as good candidates for modules. This structure-based definition of modularity implies a dynamic modularity, enabling an understanding of the dynamics of the whole network from the dynamics of the modules. The intent of the study is to find evidence for this type of modularity in published gene regulatory network models. We used Boolean and ordinary differential equation (ODE) models from established model repositories to construct the wiring diagram of each model, from which one can infer the strongly connected components. Among 132 Boolean models, which range in size from 3 to 302 genes, only 30 contained two or more non-trivial components. Most of the 32 analyzed ODE models are very small, which may help explain why we do not find many modules. Altogether, this research provides a somewhat inconclusive answer to the question whether biological networks are indeed modular. This may be because most published models are small and focus on one specific biological process. We conclude with a discussion of alternative approaches to answer this question.

Keywords – systems biology, gene regulatory networks, ordinary differential equations and dynamical systems

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23052 – Poster

Exploring the Role of Connexins in the Establishment of Left-Right Asymmetry during Embryonic Development

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Directional nodal flow initiated by motile cilia in the left-right (L-R) organizer of developing embryos is required for proper establishment of L-R asymmetry. Previous work in zebrafish has shown that morpholino knockout of the gap junction (GJ) protein, connexin43.4 (gjc4b), causes disruption of the L-R organizer, Kupffer's Vesicle (KV), and correlated randomization of L-R asymmetry (Hatler et al., 2009). Interestingly, Zygotic indel mutants of connexin43.4 (gjc4b) do not disrupt L-R development of the heart, but maternal zygotic mutants do. Here, we present phylogenetic analysis of proteins related to gjc4b and various approaches for generating mutant lines in these genes. Guides generated for injection of in connexin52.8 (gjc1) and connexin47.1 (gjc2) use the Geneweld method for integrating a cassette with a primary and secondary marker into an exon of this gene. Whole gene knockout guides were also generated for connexin44.2 (gjc4a.1) and it's tandem duplicate gjc4a.2 involving the deletion of both genes and the ~65kb region between them. Injection of a gRNA targeting gjc1, homozygous embryos for gjc4b and mutated gjc1 caused defects in L-R asymmetry. This indicates that loss of function in gjc4b is compensated or has overlapping function with other related genes.

Keywords – left-right axis formation, knockout, gene compensation, zebrafish

23053 – Poster

Definition of regulatory elements and transcription factors controlling immune cell gene expression at single cell resolution using single cell ATAC-seq

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The cis-regulatory networks of differentially expressed genes (DEG) in porcine peripheral blood mononuclear cells reported in Herrera-Uribe et al., 2021 are not well understood. By profiling the chromatin accessibility of porcine PBMC collected from two pigs using snATAC-seq, we assigned 11 cell types to this snATAC-seq dataset. We found 11872 unique cell-type differentially accessible peaks (DAP) and predicted the transcription factors (TFs) by performing TF binding motif (TFBM) enrichment analysis using such identified DAP. We found both general and cell-type specific TFsin the porcine PBMC. Unsurprisingly, the binding motif of TFs playing a crucial role in multiple cell types or lineages, like PU.1 and ETV2 are ubiquitously enriched in all cell types. At the same time, some novel regulators were predicted in this TFBM analysis. The predicted regulative function of Spi-B and TCF12 in pDCs DAPs supported a barely studied field. To identify potential regulatory regions of DEG, cis-co-accessibility networks (CCAN) were generated for each DEG whose TSS overlaps with a DAP in the matching cell type. Across 11 cell types, we identified 244 such CCANs which were used as input to conduct TFBM analysis aiming at linking the TFs to target genes As a result, the binding motif of 41 unique TFs were found enriched in CCANs associated with 43 unique DEG. 22 of the 41 TFs and their target genes were reported in ENCODE Transcription Factor Targets dataset. Notably, for these 22 TFs, 55% predicted TF-target gene pairs described are highly consistent with the ENCODE result. Our results demonstrate the power of snATAC-seq to annotate cell types, predict cell type TFs and decipher the cis-regulatory network by connecting the regulator and targeted genes across the whole genome. Supported by USDA-NIFA-AFRI-2018-67015-27501.

Keywords – single cell genomics, bioinformatics, regulatory network, pig, snATAC-seq, scRNA-seq

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23054 – Poster

Response of maize transcriptome to infection by maize lethal necrosis

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Maize lethal necrosis (MLN) is an economically important disease in SSA, with few lines displaying tolerance. This created the need to establish host resistance. Previous studies have revealed a QTL associated with MLN resistance in unadapted maize line KS23-6. Established phenotypic data on response to MLN on adapted maize lines reveal a variation in response to MLN. The spectra of MLN phenotypes displayed by these lines show that they possess some functional host resistance mechanisms, albeit low compared to KS23-6. Therefore, whether or how these lines confront MLN infection remains to be explored. Here, we report a transcriptome analysis study of five CIMMYT maize inbred lines under MLN. An RNA-Seq analysis revealed a cluster of differentially expressed genes involved in plant disease resistance. In addition to identifying the presence of plant innate immune systems, such as the upregulation of some major R genes, the study revealed the presence of well-established viral resistance mechanisms, such as RNAi silencing and ubiquitin-proteasome systems (UPS). Various RING/U-box families of genes of the UPS system were differentially expressed in the five lines. We also showed that translation initiation factors such as eIF4Es and eiF4Gs were deferentially expressed across these lines. In conclusion, this study reveals the presence of viral resistance mechanisms within inbred lines in SSA. Exploring why these genotypes cannot establish strong resistance to MLN will provide new opportunities for resistance.

Keywords – maize lethal necrosis, transcriptomics, RNA-seq

23055 – Poster

Butyrate modulation of inflammatory responses to lipopolysaccharide in porcine monocytes

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Butyrate is a short-chain fatty acid produced through microbial fermentation of dietary fibers in the lower intestinal tract. Butyrate production is associated with enhanced mucosal immunity, increased barrier integrity, and modulation of immune cells. Butyrate driven immunomodulation occurs through a variety of mechanisms, including histone deacetylase (HDAC) inhibitor activity and inhibition of NF-kB signaling. Butyrate impacts lipopolysaccharide (LPS)-induced cytokine production and gene expression by porcine monocytes in a dose-dependent manner. To investigate the mechanisms driving butyrate modulation of porcine monocyte responses to LPS, peripheral monocytes were cultured with LPS +/- butyrate. After incubation, cells were processed for collection of cytoplasmic and nuclear fractions. HDAC activity in nuclear fractions and NF-kB signaling state (IkBa, p-IkBa, p65, p-p65) in cytoplasmic fractions was assessed. Butyrate did not inhibit HDAC activity after 1 hour (h) but at 24 h, butyrate exposure resulted in inhibition of HDAC activity regardless of LPS exposure, in a dose-dependent manner. Neither the low nor high concentration of butyrate altered IkBa, p-IkBa, p65, or p-p65 status, though p-IkBa and p-p65 status was dependent on LPS exposure. Overall, the impact of butyrate on HDAC activity was time and dose-dependent, and butyrate had little to no effect on NF-kB signaling induced by LPS exposure. Continued investigation is warranted to understand the potential mechanisms driving butyrate modulation of monocyte responses to LPS to enhance abilities to modulate immune status.

Keywords – butyrate, HDAC inhibitor, NF-kB, monocytes, pigs

23056 – Poster

A multidisciplinary approach to assess the roles of Glossy2 and Glossy2-like in maize cuticular wax biosynthesis

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The cuticle is a hydrophobic barrier that covers all surfaces of the aerial organs of land plants. It provides the first line of defense from biotic and abiotic stresses that are detrimental to plant health. The cuticle is composed of a network of solvent extractable cuticular waxes that are both intercalated within and laid atop an insoluble cutin polyester matrix. Depending on the organ and stage of development, the cuticular waxes are comprised of combinations of different lipid classes, namely very long chain fatty acids (VLCFAs) and their derivatives, including hydrocarbons, alcohols, aldehydes, ketones, and wax esters. Classical genetic strategies have identified approximately 30 glossy genes required for normal cuticle deposition in maize, and molecular characterization of these genes is providing insights on cuticle formation. This study focuses on the maize Glossy2 (Gl2) gene, which encodes a protein that is archetypal for the BAHD class of acyltransferases. Although the biochemical function of GL2 remains unclear, homozygous gl2 mutant seedlings exhibit cuticular waxes of shorter chain lengths, presumably due to an alteration of the maize fatty acid elongase complex (FAE). Recently, a GL2-paralog, called GLOSSY2-LIKE has been identified, and these 2 proteins share 63% amino acid similarity. Heterologous expression of GL2 and GL2-like in Arabidopsis indicate that they play a role in VLCFA elongation. To assess the in planta physiological function of Gl2-like, six unique maize mutant alleles were generated via CRISPR-Cas9 genome editing. These gl2-like mutants, in combination with gl2 mutants, will enable the characterization of the functional relationship between these two paralogous genes. In parallel, the functionality of GL2 and GL2-LIKE proteins are being investigated using a yeast heterologous expression system, in which each paralog is being expressed in an engineered strain in which the entire maize FAE system has been reconstructed. This combination of strategies is unraveling the roles that Gl2 and Gl2-like serve in maize cuticle biosynthesis.

Keywords – cuticular wax, fatty acid elongation, maize

23058 – Poster

Maize orphan genes and their potential association with cuticle synthesis

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Orphan genes are genes encoding species-specific proteins that do not share orthology to any other annotated genes in the biosphere. Recent studies on the functionality of orphan genes demonstrate their impact on the development and metabolism of an organism. For example, the dau1 orphan found in nematodes controls larval development according to the copy number variations among different strains. (Mayer et al., 2015). In plants, the QQS (Qua-Quine Starch) orphan unique to Arabidopsis modulates carbon and nitrogen allocation (Li et al., 2015). In maize, approximately 39,000 putative orphan transcripts have been identified from publicly available maize transcriptome data, however their potential functions are unknown. The goal of this work is to identify putative orphan genes that are associated with plant cuticle development, particularly in the reproductive silks. The hydrophobic cuticle covers the plant epidermis and protects the plants against stressful environments. It is composed of a cutin polyester matrix and cuticular waxes that are infused within or laid on top of the cutin matrix. In this study, we identified putative silk-specific orphan genes via a phylostratigraphy approach, examined differential expression of the putative orphan genes along the silk length and between B73 and Mo17 inbred lines, and queried potential associations between these orphans and the cuticular waxes. We discovered significant expression differences of silk-related orphan genes between husk-encased and emerged portions of the silks, and have identified interesting correlations between orphan gene expression and cuticle composition. These discoveries will pave the way toward the broader understanding of the functions of orphan genes and the genetic networks that underlie important metabolic pathways.

Keywords – orphan genes, cuticular wax, phylostratigraphy, maize, transcriptomics

Notes

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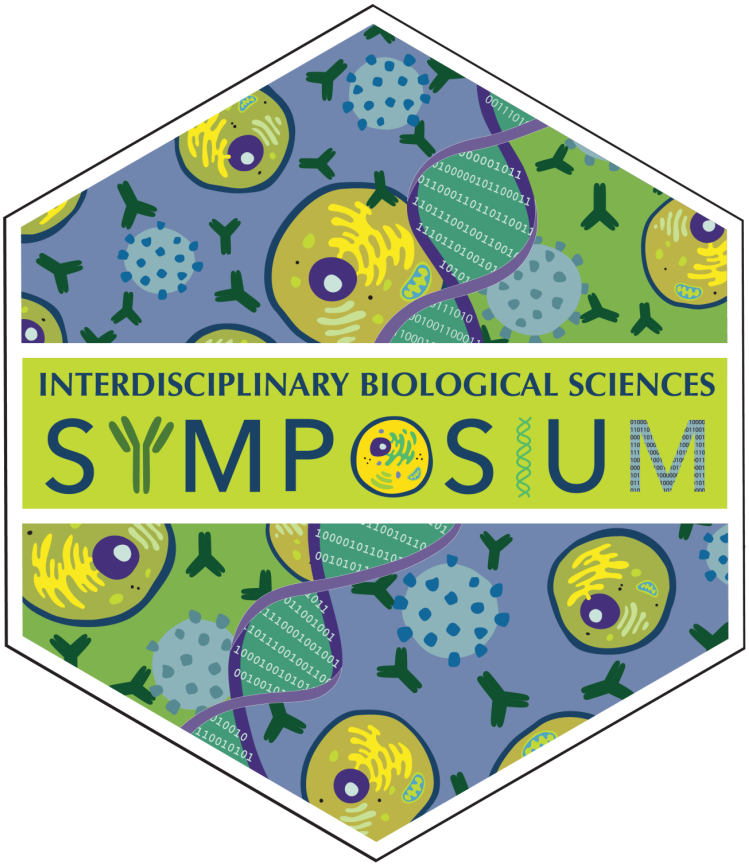

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