KU Center for Genomics

1st Annual Research Symposium

May 20, 2022

Contents

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KU Center for Genomics

Schedule at a Glance

8:00am	Breakfast, Registration, and Poster Setup	
	Session 1: Chromosome-level dynamics	
9:00am	Rob Unckless (KUCG Director, Associate Professor in MB at KU)	Welcoming Remarks
9:15am	Jae Choi (Postdoc at NYU, Assistant Professor in EEB at KU - 2023)	Natural variation in plant telomere length is associated with flowering time
9:30am	Martina Dalikova (Postdoc in EEB at KU)	Assembly and characterization of W chromosome in monarch butterfly (*Danaus plexippus*)
9:45am	Brandon Fagen (Technician at Stowers Institute)	Outer repeats of *Schizosaccharomyces pombe* centromeres experience high rates of gene conversion, expansion, and contraction
10:00am	Andrew Degar (Illumina)	Metagenomic Whole Genome Sequencing Using Illumina Products
10:15am	*Coffee/tea Break*	
	Session 2: Genomics in human health	
10:45am	Samalee Banerjee (Graduate student in MB at KU)	Quorum Sensing Regulation by the Nitrogen Phosphotransferase System in *Pseudomonas aeruginosa*
11:00am	Emily Nissen (Bioinformatician at KUMC)	Assessment of immune cell profiles among apparently healthy postmenopausal women in the Women's Health Initiative using DNA methylation-based methods
11:15am	Timothy Raben (Postdoc at Michigan State University)	Polygenic Risk Scores using Sparse Prediction: sibling validation, clinical application, and other methods
11:30am	Nanda Yellapu (Postdoc at KUMC)	Identification of treatment response signatures in the Protein-Protein interaction network maps of triple negative breast cancer
11:45am	Poster Session 1 (Odd Numbers)	
12:30pm	*Lunch Break*	
	Session 3: Gene Expression	
1:30pm	Luciana Castellano (Graduate student at Stowers Institute)	Codon-dependent regulation of gene expression during Dengue infection
1:45pm	Jeffrey Medley (postdoc at Kansas State University and KINBRE speaker)	Regulation of microRNA Strand Selection in vivo
2:00pm	Patrick Lansdon (Postdoc in MB at KU)	Wild-type *Caenorhabditis elegans* Isolates Exhibit Distinct Gene Expression Profiles in Response to Microbial Infection
2:15pm	Joanna Gress (Assistant Professor at Emporia State University)	What the Buzz about CBD: Does cannabidiol oil provide a protective effect against pesticide exposure in the honey bee gut?
2:30pm	*Coffee/tea Break*	
2:45pm	Poster Session 2 (Even Numbers)	
	Session 4: Microbiome and Genomics	
3:30pm	Tokee Tareq (Graduate student in EEB at KU)	Functional profiling of soil microbiome for elucidating maize heterosis
3:45pm	Sarah Mullinax (Graduate student in MB at KU)	The intersection of sex, immunity, and the microbiome on the maintenance of genetic variation in the Drosophila antimicrobial peptide diptericin A
4:00pm	Dingbo Lin (Associate Professor at Oklahoma State University)	Zeaxanthin-altered gut microbiome is associated with changes in whole body bone mineral density in mice
4:15pm	**Keynote Speaker: Helen E. Vuong (Assistant Professor at University of Minnesota)**	Understanding microbiome and nervous system interactions
5:15pm	*Happy Hour and Appetizers + Closing Remarks and Awards*	

General Information

Event Location and Information:

Marceli's Banquet Hall
1031 New Hampshire St.
Lawrence, Kansas 66044
(785) 331-2096
https://www.macelis.com/

Wi-fi login information
Network: Maceli's Guest or Maceli's Guest Ext
Password: Catering

Parking:

There is limited street parking. The nearest parking garage is a block north at 935 New Hampshire St., across from the Lawrence Arts Center. The top level is free, while the lower levels have 10-hour metered parking. See map on the following page for more information. To use a credit card to pay for parking, you can download the Park Mobile App.

Food and Drink at the Event:

Breakfast will be provided to symposium participants, courtesy of Illumina. It will be available at 8:00 am during registration.

Coffee and tea will be available throughout the day. Lunch will be available at 12:30 pm on site.

Happy hour with appetizers will begin at 5:15 pm. Drink tickets will be handed out to all participants 21 and over for beer or wine. A cash bar will be available for other drinks and cocktails.

Map of Downtown Lawrence

An interactive map of downtown Lawrence can be found here.

Venue & Parking

⭐ Maceli's Banquet Hall & Catering

🅿 935 New Hampshire St

🅿 824 New Hampshire St

🅿 One Riverfront Plaza Parking

Hotels

🏨 TownePlace Suites by Marriott Lawrence Downtown

🏨 Eldridge Hotel

🏨 SpringHill Suites by Marriott Lawrence Downtown

🏨 The Oread Hotel

Dining Suggestions

✖ Merchants Pub & Plate

✖ 715 Restaurant

✖ Mass St. Fish House & Raw Bar

✖ Zen Zero

✖ Free State Brewing Company

✖ Aladdin Cafe

✖ Molcajete's Lawrence Lawrence

✖ Beer Company

✖ RPG (Restaurant, Pub & Games)

✖ Limestone Pizza•Kitchen•Bar

✖ The Burger Stand at The Casbah

✖ Noodles and Company

✖ Taco Zone

Presentation Abstracts

Session 1: Chromosome-level dynamics

9:00 am	**Welcoming remarks** Rob Unckless, KUGC Director and Associate Professor of Molecular Biosciences at the University of Kansas
9:15 am	**Natural variation in plant telomere length is associated with flowering time** *Presenter:* Jae Choi, Postdoctoral researcher in the Department of Biology at New York University, Assistant Professor in Ecology and Evolutionary Biology at University of Kansas – 2023 *Coauthors:* Liliia R. Abdulkina[2], Jun Yin[3], Inna B. Chastukhina[2], John T. Lovell[3,4], Inna A. Agabekian[2], Pierce G. Young[5], Samsad Razzaque[3], Dorothy E. Shippen[5], Thomas E Juenger[3], Eugene V. Shakirovb[6,7,1], and Michael D. Purugganana [1]Center for Genomics and Systems Biology, Department of Biology, NYU; [2]Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Russia; [3]Department of Integrative Biology, UT Austin; [4]Genome Sequencing Center, HudsonAlpha Institute for Biotechnology; [5]Department of Biochemistry and Biophysics, Texas A&M University,;[6]Department of Biological Sciences, College of Science, Marshall University; [7]Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University Telomeres are highly repetitive DNA sequences found at the ends of chromosomes that protect the chromosomes from deterioration during cell division. Here, using whole-genome re-sequencing and terminal restriction fragment assays, we found substantial natural intraspecific variation in telomere length in Arabidopsis thaliana, rice (*Oryza sativa*), and maize (*Zea mays*). Genome-wide association study (GWAS) mapping in A. thaliana identified 13 regions with GWAS-significant associations underlying telomere length variation, including a region that harbors the telomerase reverse transcriptase (TERT) gene. Population genomic analysis provided evidence for a selective sweep at the TERT region associated with longer telomeres. We found that telomere length is negatively correlated with flowering time variation not only in A. thaliana, but also in maize and rice, indicating a link between life-history traits and chromosome integrity. Our results point to several possible reasons for this correlation, including the possibility that longer telomeres may be more adaptive in plants that have faster developmental rates (and therefore flower earlier). Our work suggests that chromosomal structure itself might be an adaptive trait associated with plant life-history strategies.

9:30 am	**Assembly and characterization of W chromosome in monarch butterfly (*Danaus plexippus*)** *Presenter:* Martina Dalikova, Postdoctoral researcher in Ecology and Evolutionary Biology at University of Kansas – 2023 *Coauthors:* Daniel Portik[2], Sarah B. Kingan[2], Hayley Mangelson[3], Liuqi Gu[1], James R. Walters[1] [1] Department of Ecology and Evolutionary Biology, The University of Kansas; [2]Pacific Biosciences, Menlo Park, CA; [3] Phase Genomics, Seattle, WA Degenerated sex chromosomes have a precedent of being notoriously difficult to assemble. Recent advances in long-read sequencing are catalyzing increases in the number and quality of W and Y assemblies, providing novel insights concerning the content and evolution of these elusive chromosomes. Here we report a novel genome assembly for the monarch butterfly (*Danaus plexippus*), focusing on the W chromosome. This species harbors a neo-Z chromosome, arising from the fusion of the ancestral Z with an autosome. Previous cytogenetic analyses indicated a similarly large and bipartite W chromosome, suggesting the possibility of a comparable neo-W, but much ambiguity remains concerning the sequence and history of monarch W chromosome. We generated PacBio HiFi reads with Hi-C data from females to support de novo assemblies of maternal and paternal haplotypes using Trio binning. Putative W contigs from the maternal genome were determined based on male to female coverage and sex specific kmers. The paternal assembly and W contigs were scaffolded using Hi-C data. This produced chromosomal level scaffolds for the Z and all autosomes, including three autosomes assembled from telomere to telomere. The W chromosome scaffold length was around 10 Mbp, thus leaving about one third of this chromosome in unscaffolded contigs. While primarily composed of transposable elements (TEs), the W contains at least two protein coding genes that arose through retroposition and ectopic recombination from other chromosomes. Neither of these W-linked copies appear pseudogenized and one appears to be evolving adaptively. The prevalent repetitive content of the W chromosome are elements from the LINE and LTR retrotransposon groups. Surprisingly, the TEs on W chromosome have lower divergence compared to the rest of the genome, suggesting their ongoing accumulation. Finally, despite this novel W assembly, strong evidence for or against a neo-W chromosome origin remains elusive.
9:45 am	**Outer repeats of *Schizosaccharomyces pombe* centromeres experience high rates of gene conversion, expansion, and contraction** *Presenter:* Brandon Fagen, Technician at the Stowers Institute *Coauthors*: Sarah Gilmour[1], Blake Billmyre[1], Andrew Price[1], Lexy Cockrell[1, 2], SaraH Zanders[1, 2]

[1]The Stowers Institute for Medical Research, [2]Department of Molecular and Integrative Physiology, University of Kansas Medical Center.

Eukaryotic genomes are full of repetitive regions, such as centromeric repeats, but these have only recently become accessible with the advent of long-read sequencing technology. Centromeres are sequences on which kinetochores assemble and spindles attach during cell division. Human centromeres, as well as those of many other eukaryotes, include large heterochromatinized repeats. These repeats regularly homogenize, expand, and contract, which makes them highly variable. However, the mechanisms underlying centromere evolution are still unclear. We addressed this question from a genomics perspective using the fission yeast *Schizosaccharomyces pombe*. We conducted long-read Nanopore sequencing on seven *S. pombe* natural isolates and assembled their centromeres. We then performed phylogenetic analysis of the individual repeats, as well as other centromeric loci. Each of the three fission yeast centromeres has four to forty-six outer repeats, named dh and dg repeats. These are largely homogenized, and the length of centromere III (CenIII) varies between 35kb and 180kb due to outer repeat expansion. Our analysis uncovered rapid expansion and contraction on CenIII. In four closely related isolates, the CenIII outer repeat copy number varies by twenty. Homogenization has also occurred between dh and dg repeats in a conserved ~350bp sequence. We hypothesize that there is a high level of gene conversion between outer repeats on non-homologous chromosomes. Unexpected, we even found a likely gene conversion event between the mating-type locus and the centromere in one isolate, which are separated by ~500kb. This investigation provided insights into the evolution of fission yeast centromeres with previously unobserved gene conversion events and CenIII repeat expansion. Advancing our understanding of centromere evolution is essential for developing a deeper knowledge of centromere function in health and disease.

10:00 am	**Metagenomic whole genome sequencing using Illumina products** *Presenter:* Andrew Degar, Illumina Illumina offers a streamlined end-to-end solution for species level metagenomic profiling utilizing Illumnia's library preparation, sequencing platform, and analysis tools. Illumina DNA Prep offers a fast, flexible approach to library preparation for small and large genomes, a perfect fit with the NextSeq 2000 providing additional scalability with various flow cell configurations to accommodate deep or shallow metagenomic sequencing. BaseSpace Sequencing Hub offers analysis via the DRAGEN Metagenomics app to perform taxonomic classification of reads. The app provides interactive visualizations and raw classification output for per sample and aggregate analyses. In this presentation we will discuss ways in which Illumina can support you in your unique applications of metagenomic sequencing.

Session 2: Genomics in human health

10:45 am	**Quorum sensing regulation by the Nitrogen Phosphotransferase System in *Pseudomonas aeruginosa*** *Presenter:* Samalee Banerjee, Graduate student in Molecular Biosciences at the University of Kansas *Coauthor:* Josephine R. Chandler[1] [1] Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA The opportunistic bacterial pathogen, *Pseudomonas aeruginosa* is a major cause of hospital-acquired infections. *P. aeruginosa* uses a population-density dependent gene regulation system called quorum sensing to regulate expression of hundreds of genes, including many that contribute to virulence. *P. aeruginosa* virulence is also regulated by a nitrogen-phosphotransferase system (PTSNtr). This system is a conserved phosphotransfer system found in many gram-negative bacteria, which is poorly studied but believed to be important for integrating information on carbon and nitrogen availability to alter metabolism. We and others have demonstrated *P. aeruginosa* strains with null mutations of the first PTSNtr gene ptsP have enhanced quorum sensing activity as well as increased production of pyocyanin, a quorum sensing controlled toxin. The purpose of this study is to investigate the mechanism of PtsP-dependent quorum sensing activation. To carry out this objective, single and double deletions of the three PTSNtr genes were prepared and their effects on quorum sensing activity were assessed. We also constructed mutations affecting predicted phosphorylation sites of the PTSNtr proteins. Our genetic studies show that deleting PtsO or PtsN in a PtsP-null mutant restores quorum sensing activity back to wild type levels. Results also show that unphosphorylated versions of PtsO and PtsN positively regulate quorum sensing activity. These results together suggest that phosphate flow through the PTSNtr system is important for regulating quorum sensing. Deleting ptsP blocks phosphate flow, leading to unphosphorylated PtsO and PtsN and subsequent activation of quorum sensing. Results of our studies may reveal new insights into *P. aeruginosa* biology and could ultimately inform the development of anti-*P. aeruginosa* therapeutics.
11:00 am	**Assessment of immune cell profiles among apparently healthy postmenopausal women in the Women's Health Initiative using DNA methylation-based methods** *Presenter:* Emily Nissen, Bioinformatician, Department of Biostatistics & Data Science at the University of Kansas Medical Center *Coauthors:* Devin C. Koestler[1], Alexander Reiner[2], Simin Liu[3], Lucas A. Salas[4], Brock C. Christensen[4,5,6], Karl T. Kelsey[7], John K. Wiencke[8,9] [1]Department of Biostatistics & Data Science, University of Kansas Medical Center; [2]Division of Public Health Science, Fred Hutchinson Cancer Center, Seattle, WA; [3]Departments of Epidemiology, Medicine, and Surgery, Brown University, Providence, RI; [4]Department of Epidemiology, Geisel School of Medicine, Dartmouth College,

Lebanon, NH; [5]Department of Molecular and Systems Biology, Geisel School of Medicine, Dartmouth College, Lebanon, NH; [6]Department of Community and Family Medicine, Geisel School of Medicine, Dartmouth College, Lebanon, NH; [7]Departments of Epidemiology and Pathology and Laboratory Medicine, Brown University, Providence, RI; [8]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA; [9]Institute for Human Genetics, University of California San Francisco, San Francisco, CA

Peripheral blood immune cell counts are a snapshot of individual immune status that can be used to characterize disease risk and clinical response to therapeutic interventions. Central to interpreting these data is developing clear reference limits of cell counts in healthy populations. Using flow cytometry methods (FCM), genetic, demographic, and environmental factors have been identified that modulate the immune profile in healthy populations. However, FCM has some limitations; namely, it requires fresh blood and provides measurements of a limited number of cell types at one time. Over the past decade, DNA-methylation (DNAm) based deconvolution methods that leverage cell-specific DNAm markers of immune cell types have been developed to provide accurate estimates of the proportions of leukocytes in peripheral blood. Immune cell phenotyping using DNAm markers is termed immunomethylomics and offers a solution for determining the body's immune cell landscape. However, the reference ranges of immune cell proportions and counts using only DNA from whole blood in healthy populations remain sparse. We applied a DNAm-based deconvolution method to a large sample of apparently healthy postmenopausal women enrolled in the Women's Health Initiative Long Life Study (WHI-LLS) cohort (N=1,295) to determine the reference ranges for proportions and absolute counts of 12 immune cell-types (Neutrophils, Basophils, Eosinophils, Monocytes, CD4-memory, CD4-naïve, CD8-memory, CD8-naïve, T-regulatory, B-memory, B-naïve, Natural Killer). From these 12 cell types, we also derived the metrics for various other immune cell types (e.g., Total T-cells, Total B-cells, Total CD4-cells) and cell ratios (e.g., Neutrophil-to-Lymphocyte ratio, Lymphocyte-to-Monocyte ratio, Total-CD4-to-Total-CD8 ratio). Further, all reference ranges of immune cell counts were characterized according to age, race/ethnicity, and other known demographic and clinical covariates among women in the WHI-LLS. This study illustrates how immunomethylomics can provide a comprehensive characterization of an individual's immune profile using peripheral blood samples.

| 11:15 am | **Polygenic Risk Scores using Sparse Prediction: sibling validation, clinical application, and other methods**
 Presenter: Timothy Raben, Postdoctoral researcher in the Department of Physics and Astronomy at Michigan State University
 Coauthors: Louis Lello[1,2], Erik Widen[1,2], Stephen D.H. Hsu[1,2]
 [1]Department of Physics and Astronomy, Michigan State University; [2]Genomic Prediction, Inc. |

The prediction of complex human traits using just SNP information has become a feasible goal. In the last 10 years, polygenic scores (PGS) have been trained and validated for a wide range of human phenotypes from height to blood lipoproteins. In this talk we will first review why PGS is important and what are its potential and current benefits. A particular vein of complex trait prediction involves *sparse* prediction, i.e. predictor training methods that only use a subset of all possible nucleotides. These predictors are especially useful as they can be faster to compute, easier to use, and more straightforward to interpret.We will review some general aspects of sparse prediction and show how sparse predictors have been validated across several biobanks and using *sibling analyses*, i.e. characterizing how well sparse predictors perform between siblings where many environmental factors can be controlled. Next we will show that sparse PGS has been used to generate clinical risk scores that are competitive with traditional risk scores for atherosclerotic disease. Finally we will highlight current ethical issues---a lack of genetically diverse PGS, exacerbating healthcare inequalities, etc.---that are intrinsically tied to much of this research. Some additional details about technical methods will be given throughout the talk. This talk primarily presents research from a series of papers:
https://doi.org/10.1534/genetics.118.301267, https://doi.org/10.1038/s41598-020-69927-7, https://doi.org/10.3390/genes12070991,
and a forthcoming, unpublished preprint.

11:30 am	**Identification of treatment response signatures in the Protein-Protein interaction network maps of triple negative breast cancer** *Presenter:* Nanda Kumar Yellapu, Postdoctoral researcher in the Department of Biostatistics & Data Science at the University of Kansas Medical Center *Coauthors:* Shane Stecklein[2], Priyanka Sharma[3], Mihaela Sardiu[1], Jeffery A. Thompson[1], Devin C. Koestler[1] [1]Department of Biostatistics & Data Science, University of Kansas Medical Center; [2]Department of Radiation Oncology, University of Kansas Medical Center;[3]Division of Medical Oncology, Department of Internal Medicine, University of Kansas Medical Center The comprehensive exploration of molecular signatures in the triple negative breast cancer (TNBC) is remains to be fully understood. The available therapeutics for TNBC are not completely successful where few patients respond to specific treatment and other patients do not respond to the same treatment. Identification of treatment response signatures for a specific treatment greatly helps to provide the right treatment in right time. To accomplish this, we have considered 43 pre-treated TNBC patients who were identified as responders (n=25) and non-responders (n=18) to treatment with anthracycline containing chemotherapy. The differentially expressed genes (DEGs) among responders and non-responders were used to derive Protein-Protein network (PPI) network maps. The molecular complex detection (MCODE) and CytoHubba analysis of the PPI network revealed 10 hub nodes, which include: AHSG, SERPINC1, ALB, FGA, FGB, FGG, APOH, TTR, AMBP and APOB. Gene ontology

enrichment analysis of hub nodes demonstrated their association with cancer specific biological processes and molecular functions such as apoptotic and ERK signaling pathways. In addition, cancer related proteins in the node points tend to interact with their partners through distinct interfaces that are crucial for cellular pathogenicity. Protein-protein docking studies among 10 hub proteins, revealed similar binding mechanisms in terms of binding energies and bonding patterns. The docking complexes showed several similar residual bonding patterns. The identified intermolecular connections represent crucial interactions that define cancer pathogenesis among PPI networks. Any ideal target site of a cancer protein can be evaluated for such specific residues and intermolecular connections. These hub nodes represent the treatment-response signatures among TNBC for anthracycline containing chemotherapy.

Session 3: Gene Expression

1:30 pm	**Codon-dependent regulation of gene expression during Dengue infection** *Presenter:* Luciana A. Castellano, Graduate student at the Stowers Institute *Coauthors:* Ryan McNamara[1], Diego E. Alvarez2 and Ariel A. Bazzini[1] [1]Stowers Institute for Medical Research; [2]Instituto de Investigaciones Biotecnológicas, CONICET, Universidad Nacional de San Martin, Argentina Dengue is the most prevalent mosquito-borne viral disease in the world, causing an estimated 100 million infections and 40,000 deaths each year. Dengue infection results in dramatic changes in host gene expression creating an environment that favors viral replication in both human and mosquito hosts. Recent work indicates that translation can affect mRNA stability in a codon-dependent manner in different species through a phenomenon known as codon optimality. Codons that enhance mRNA stability have been defined as 'optimal' and codons that decrease mRNA stability as 'non-optimal'. However, whether this codon-mediated regulation is exploited during viral infections and how host optimality shapes codon composition of the dengue genome remains unknown. To study whether dengue virus uses optimal or non-optimal codons relative to its hosts, we first defined the codon optimality code in mosquito cells through blocking transcription and studying the correlation between stability and codon composition of endogenous genes. Surprisingly, analyses of codon choice in thousands of worldwide isolated dengue genomes revealed that dengue uses non-optimal codons more frequently than most human and mosquito genes, suggesting the existence of an evolutionary pressure to select non-optimal codons. This preference for non-optimal codons is conserved in over 300 human viruses, including Zika and SARS-CoV-2. Thus, we hypothesized that dengue infection affects host gene expression in a codon-dependent manner. RNA-seq analysis indicated that human genes upregulated during infection are enriched in codons preferred by dengue.

	Interestingly, analysis of the fitness effect of mutations in the dengue genome during host-restricted serial passages in human or mosquito cells revealed that mutations toward dengue's preferred codons tend to be beneficial, while mutations away from dengue's preferred codons frequently appear to be deleterious. Altogether, our findings uncover a novel mechanism underlying virus-host adaptation and underscore codon optimality as a driving force of virus evolution.
1:45 pm	**Regulation of microRNA Strand Selection in vivo** *Presenter:* Jeffrey Medley, Postdoctoral researcher at Kansas State University and KINBRE speaker *Coauthors:* Ganesh Panzade[1] and Anna Zinovyeva[1] [1]Division of Biology, Kansas State University, Manhattan, KS Gene expression must be tightly regulated to maintain cellular homeostasis and ensure proper animal development. Improper gene expression is the underlying mechanism of numerous human diseases, highlighting the importance of regulated gene expression. microRNAs (miRNAs) play a central role as regulators of gene expression by repressing the expression of target genes. A critical step in miRNA-mediated gene regulation is the processing of miRNA precursors into miRNA duplexes. Each miRNA duplex comprises two strands destined for different fates: one strand is loaded into an Argonaute effector protein to form the miRNA-induced silencing complex (miRISC), while the other strand is degraded. As either miRNA strand can be functional, the decision of which strand is loaded into Argonaute effectively determines the target repertoire of miRISC. Improper strand choice can have severe consequences, as alternative miRNA strand selection has been observed in several human diseases. Despite the importance of strand selection, our understanding of how a single miRNA strand is selected by Argonaute remains incomplete. Previous studies have suggested that the sequence of the miRNA duplex is important for strand selection in vitro. However, it remains unclear whether those sequence cues play a role in determining strand selection in vivo. To test the role of miRNA sequence features in vivo, we used CRISPR/Cas9 genome editing to mutate several Caenorhabditis elegans miRNAs. Our findings suggest that the sequence of miRNA duplexes plays a key role in determining strand selection in vivo and reveal unexpected effects on the relative stabilities of each miRNA strand. Furthermore, we found that miRNA mutations often resulted in altered distribution of miRNA variants (isomiRs), which might indicate a relationship between miRNA processing and strand selection. Collectively, our findings have expanded our understanding of how miRNA strand selection is regulated in the complex context of a developing organism.

2:00 pm	**Wild-type *Caenorhabditis elegans* Isolates Exhibit Distinct Gene Expression Profiles in Response to Microbial Infection** *Presenter:* Patrick Lansdon, Postdoctoral researcher in Molecular Biosciences at the University of Kansas *Coauthors:* Maci Carlson[1], and Brian D. Ackley[1] [1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS The nematode *Caenorhabditis elegans* serves as a model system to study innate immunity against microbial pathogens. *C. elegans* have been collected from around the world, where they have presumably adapted to regional microbial ecologies. Here we use survival assays and RNA-sequencing to better understand how two isolates from disparate climates respond to pathogenic bacteria. We found that, relative to N2 (originally isolated in Bristol, UK), CB4856 (isolated in Hawaii), was more susceptible to the Gram-positive pathogen, *Staphylococcus epidermidis*, but equally susceptible to *Staphylococcus aureus* as well as two Gram-negative pathogens, *Providencia rettgeri* and *Pseudomonas aeruginosa*. Transcriptome analysis of infected worms found gene expression profiles to be considerably different in an isolate-specific and pathogen-specific manner. We utilized geneset enrichment analysis to categorize differential gene expression in response to *S. epidermidis*. In N2, genes that encoded detoxification enzymes and extracellular matrix proteins were significantly enriched, while in CB4856, genes that encoded detoxification enzymes, C-type lectins, and lipid metabolism proteins were enriched, suggesting these isolates have different responses to the pathogen, despite being the same species. Finally, to examine changes in transcriptional networks post-infection, we cross-referenced our list of differentially expressed genes with a list of *C. elegans* transcription factors. In total, 22 transcription factors were differentially expressed between N2 and CB4856 worms following *S. epidermidis* infection, 13 of which were members of the nuclear hormone receptor family. Overall, discerning gene expression signatures in an isolate-by-pathogen manner can help us better understand the different possibilities for the evolution of immune responses within organisms.
2:15 pm	**What the Buzz about CBD: Does cannabidiol oil provide a protective effect against pesticide exposure in the honey bee gut?** *Presenter:* Joanna Gress, Assistant Professor of Genetics at Emporia State University *Coauthors:* Tania Wiest[1] and Nicholas Rutherford1 [1]Department of Biological Sciences, Emporia State University Pesticides used in agriculture can be harmful to non-target insects including pollinating bees. When exposed foragers return to the hive, they inadvertently expose their fellow workers to toxic pesticide residues. Neonicotinoid and organophosphate pesticides alter the levels and expression of antioxidants in forager bees causing oxidative stress and damage to tissues, especially in the gut. Cannibidoil oil (CBD) is a non-psychoactive phytocannabinoid from Cannabis sativa that has been shown in vitro to have antioxidant properties. To examine if CBD has an antioxidant effect on the bee gut in

terms of gene expression, we measured the impact of pesticides on foragers exposed to 10nmol of either Imidacloprid or Coumaphos alone or in combination with 100nmol of CBD in a 1M sucrose solution using qPCR and RNA-seq. Our initial study found that that the addition of CBD to foragers dosed with either coumaphos or imidacloprid caused an upregulation of several genes involved in the antioxidant/detoxification pathway in the gut. For honeybees given the combination of CBD+ coumaphos, a significant upregulation of Sod1 and 2 occurred. These genes help to rearrange superoxides to oxygen and hydrogen peroxide. Additionally, SelT was upregulated, which acts as a peroxidase and can assist in protein re-folding, redox signaling, and hormone metabolism. The addition of CBD+ imidacloprid caused a significant upregulation of several antioxidant genes including Sod1, Cat, Trx1 and MsrA. To further examine the protective effect of CBD in the bee digestive system, we conducted RNA-Seq analysis of the bee abdomen after pesticide and CBD exposure. CBD does appear to activate important genes in the antioxidant pathway in the gut in the presence of these two pesticides. This indicates that CBD may help in the detoxification of harmful ROS in the gut and should be explored for new potential management strategies.

Session 4: Microbiome and Genomics

3:30 pm	**Functional profiling of soil microbiome for elucidating maize heterosis** *Presenter:* Tokee Tareq, Graduate student in Ecology and Evolutionary Biology at the University of Kansas Heterosis or hybrid vigor, which is the enhanced performance of the progeny derived from the cross of two diverse (preferably inbreed) parents, has a profound effect in the evolutionary aspect of outcrossing wild plants as well as agricultural productivity of multiple crop species including maize. In terms of production of maize seeds, heterosis is the major source of the enhanced growth, biomass and productivity of the currently commercially available cultivar lines. Despite being widely used as a driving force for the current success of the maize production, the underlying mechanism of hybrid vigor remains under investigation. Recent evidence suggests that interactions between plants and microbes play a role in heterosis - however, the functional mechanisms remain unknown. Differential taxonomic distribution is just the indicative parameter of a distinctive functional profile of the associated microbiomes, but due to functional redundancy, microbial composition difference doesn't directly translate to difference in microbial functional profiles of the heterosis. To fully illustrate the functional attributes of the microbial community that may have an impact on the heterosis, we need to investigate the gene content of the microbiome. Here, we represent the comparative whole genome analysis of the soil microbiomes associated with a total 42 maize samples. Our analysis revealed that inbred and hybrid mazes have distinctive functional category distributions and their compositions. Among the

	different gene functions, when classified according to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database, we found more than 2000 genes that have some level of contribution to the distinctive functional composition between the microbiomes of inbred and hybrid maize plants. The most promising gene functions are related to secondary metabolites synthesis, complex carbohydrate synthesis, xenobiotic compound synthesis, transport proteins and intracellular signaling. Such results indicate the possibility of different microbial community interaction among the microbes and distinctive signal transduction with corresponding host plants. Although we need more detailed experiments before specifying the microbial genes which have contribution towards the heterosis, our study has demonstrated the presence of functional composition difference between the two breeds.
3:45 pm	**The intersection of sex, immunity, and the microbiome on the maintenance of genetic variation in the Drosophila antimicrobial peptide diptericin A** *Presenter:* Sarah Mullinax, Graduate student in Molecular Biosciences at the University of Kansas *Coauthor:* Rob Unckless, Department of Molecular Biosciences. University of Kansas, Lawrence, Kansas. The innate immune system provides hosts with a crucial first line of defense against pathogens. Immune genes are often among the fastest evolving genes in the genome, but in Drosophila, antimicrobial peptides (AMPs) are notable exceptions. Instead, they are under the influence of balancing selection such that multiple alleles are maintained in populations over evolutionary timescales. In this study we focus on the Drosophila antimicrobial peptide Diptericin A which has an amino acid polymorphism segregating in Drosophila populations. Diptericin A is especially important in the defense against a systemic infection by the Gram- bacteria *Providencia rettgeri* and the survival probability is highly dependent on the genotype of diptericin A. As well, Diptericin A is important in gut immunity to control opportunistic infections from common Drosophila gut microbes, especially those of Lactobacillus plantarum. In addition to genotypic effects on gut immunity we also see strong sex effects that are most prominent in flies that do not have functional diptericin A. To further characterize differences in microbiomes between different genotypes we used 16S metagenomics to look at the microbiome composition as a whole. We used both lab reared and wild caught flies for our sequencing and will be looking at overall composition as well as the differential abundance of individual bacterial families. Overall, we find flies that are homozygous serine in diptericin A are better equipped to survive a systemic infection from *P. rettgeri*, but homozygous arginine flies have a longer lifespan after being fed certain common gut commensals. Based on our results we find evidence for the maintenance of genetic variation of diptericin A through the complex interactions of sex, systemic immunity, and the maintenance of the gut microbiome.

4:00 pm	**Zeaxanthin-altered gut microbiome is associated with changes in whole body bone mineral density in mice** *Presenter:* Dingbo Lin, Associate Professor of Nutritional Sciences at Oklahoma State University *Coauthors:* Peiran Lu[1], Yashu Tang[1], Siau Yen Wong[1], Jianmin Chai[2], Paniz Jasbi[3], Lei Wu[1], Edralin A Lucas[1], Hui He[1], Jiangchao Zhao[2], Haiwei Gu[3], Tyrrell Conway[4], Adrian Wyss[5], Brenda J Smith[6] [1]Department of Nutritional Sciences, Oklahoma State University; [2]Department of Animal Science, University of Arkansas; [3]Department of Nutrition Science, Arizona State University; [4]Department of Microbiology and Molecular Genetics, Oklahoma State University; [5]DSM Nutritional Products Ltd; [6]Department of Obstetrics and Gynecology, Indiana University School of Medicine Objective. Zeaxanthin is commonly known as a macular carotenoid pigment, with antioxidant properties in humans. An increased intake of zeaxanthin lowers the risk of osteoporosis. It remains unknown whether and how zeaxanthin mediates bone formation and health. In this study, we sought to investigate the association of zeaxanthin intake with the gut microbiome homeostasis and bone mineral density in mice. Methods. Six-week-old male and female C57BL/6J wild type (WT), beta-carotene oxygenase 2 (BCO2) knockout (KO) mice were fed with AIN93M chow diets supplemented with or without zeaxanthin (0.02% w/w) for 10 weeks. At the termination of the study, mice were fasted for 3 hrs prior to anesthesia and PIXImus scan. Cecal contents, colon, serum, feces, and other tissues were collected for laboratory assessments.16S rRNA sequencing and LC-MS/MS were performed for gut microbiota profiling and serum and fecal metabolomics analyses, respectively. Results. Significant zeaxanthin accumulation occurred in BCO2 KO, but not WT mice. Zeaxanthin accumulation was associated with the alteration of cecal gut microbiota composition, for example, increased richness in Lachnospiraceae, Proteobacteria, and Parabacteroides. The results of fecal and serum metabolomics revealed that zeaxanthin significantly altered tyrosine metabolism, branched-chain fatty acid oxidation, fatty acid biosynthesis, and phospholipid biosynthesis, and indo metabolites production, but suppressed levels of kynurenine and trimethylamine N-oxide. Further, zeaxanthin significantly enhanced whole body bone mineral density (BMD) in BCO2 knockout mice. Conclusion. The results suggested that zeaxanthin accumulation promotes gut microbiome homeostasis and enhances BMD in mice. The causality of zeaxanthin-altered gut microbiome in BMD warrants further investigation.

4:15 pm	**Understanding microbiome and nervous system interactions** *Keynote Speaker*: Helen E. Vuong, Assistant Professor at University of Minnesota

Bio: Helen Vuong earned her B.S. in Neuroscience from the University of California, Los Angeles (UCLA). She continued her graduate education at UCLA, earning her Ph.D. in Molecular, Cellular and Integrative Physiology under the mentorship of Dr. Nicholas C. Brecha. During her graduate studies, Helen investigated the anatomical and electrophysiological regulation of retinal microcircuits by neuropeptides, including somatostatin and vasoactive intestinal polypeptide. She did her postdoctoral training with Dr. Elaine Y. Hsiao's. In her postdoctoral research, Helen aimed to elucidate the role of the maternal microbiome in fetal brain development, including modulation of neuronal connectivity, activity and function, and behavior. She was a National Institutes of Health (NIH)-Institutional Research and Academic Career Development Award fellow, where she conducted research while pursuing her passion for education and outreach. In addition, Helen was awarded the NIH-National Institute of Child Health and Human Development Pathway to Independence Award which will facilitate her transition into an independent research position. Helen just started her lab at the University of Minnesota, Department of Pediatrics, to study the role of the microbiome in nervous system development and behaviors.

Abstract: Alterations of the maternal gut microbiome, in response to environmental challenges such as infection, altered diet and stress during pregnancy, has been increasingly associated with abnormalities in offspring brain function and behavior. Here we investigate how depletion, and selective reconstitution of the maternal gut microbiome influences fetal neurodevelopment in mice. Embryos from antibiotic-treated and germ-free dams exhibit widespread transcriptomic alterations in the fetal brain relative to conventionally-colonized controls. Depletion of the maternal microbiome yields offspring that exhibit altered sensorimotor behaviors. Metabolomic profiling reveals that the maternal microbiota regulates levels of numerous small molecules in the maternal serum and embryonic brains. Moreover, maternal supplementation with select metabolites abrogates deficiencies in fetal neurodevelopmental processes and prevents abnormalities in sensory behavior in offspring from microbiome-depleted dams.

Poster Abstracts:

Posters will be presented at the following poster sessions:

Poster Session 1	11:45am – 12:30pm	Odd Number Posters
Poster Session 2	2:45pm – 3:30pm	Even Number Posters

1. Dogs about the Strait: Using dog genomes to assess human interactions
   Lauren E. Y. Norman, Brittany Bingham, Justin Tackney, Kristine G. Beaty, and Dennis O'Rourke
   Department of Anthropology, University of Kansas

   Dog-human relationships have been an important part of many cultures since the Pleistocene. These relationships often mirror human-human relationships, with dogs and humans travelling together on long migratory journeys, interacting with new people and dogs, and aiding each other in subsistence pursuits. Dogs accompanied people into the Americas on numerous occasions. The most recent joint venture began around AD 1000, with new populations of dogs and humans, specifically the Thule Inuit, spreading across the Canadian and Greenlandic Arctic. However, the Thule Inuit were not the first people in the region carrying the larger Inuit cultural tradition. We recently showed that the Birnirk (AD 800-1200), a potential cultural predecessor of the Thule, carried the same mitochondrial haplotype gene pool as the Thule Inuit. We set out to investigate if Birnirk dogs were similarly related to Thule dogs, by attempting to capture and sequence whole mitochondrial genomes from ancient dog remains at a Birnirk site, Cape Espenberg, on the Bering Strait in Alaska. We compare these ancient dog mitochondrial genomes to Siberian and Alaskan wolves to see if there is any geographic or temporal affinity. Our results help to identify if Birnirk people were migrants to the area or were long-term residents and push back in time our record of the last pre-colonial migration of dogs into the Americas. Our work on dog genomes from the Birnirk will continue to clarify relationships between dogs and humans, and among human groups, without the need for destructive analysis on ancestral human remains.

2. ZWYX: Software for detecting sex-linked regions and chimeras in genome assemblies
   James Walters
   Ecology and Evolutionary Biology, University of Kansas

   I present a new R-language software package "ZWYX" which employs sex-specific sequencing coverage data to delineate regions of a genome assembly corresponding to sex chromosomes. The X or Z chromosomes should show a two-fold differences between sexes, while the sex-specific Y or W chromosomes should show an opposing and far more biased

pattern of coverage; autosomes should show no differences. Using sex-specific sequencing coverage is an established and increasingly common method for identifying scaffolds derived from X, Y, W, and Z chromosomes. However, such analyses are typically bespoke, highlighting the need for software to streamline and standardize this approach. ZWYX aims to meet this need, and is implemented in the context of the Bioconductor Project, to take advantage of existing R-language infrastructure for genome analysis. ZWYX provides a structure and functions for identifying and visualizing scaffolds with sex-biased coverage on average as a whole, but also via windows along each scaffold. Importantly, ZWYX incorporates "changepoint" algorithms for detecting shifts in sex-specific coverage that occur within a scaffold, which often indicate a mis-assembly that erroneously joins autosomal and sex-linked regions. Thus, in conjunction with relevant sex-specific sequencing data, ZWYX offers a valuable method for assessing the quality of genome assemblies. For demonstration, I apply ZWYX to a version of the Drosophila melanogaster genome computationally rearranged to include Z-Autosome and Y-Autosome chimeric scaffolds.

3. Single-cell RNA Sequencing of Oligodendrocytes to Map how Nuclear Hormone Receptor Ligands Regulate Differentiation
Ziyu Zhu
Department of Chemistry, University of Kansas

Oligodendrocyte-derived myelin is the foundation of a properly functioning central nervous system (CNS), while demyelination – the loss of the myelin sheath – occurs in many neurodegenerative diseases, such as multiple sclerosis, Parkinson's diseases, and Alzheimer's disease. In the CNS, oligodendrocyte progenitor cells (OPCs) differentiate into mature oligodendrocytes, which form myelin. Historically, thyroid hormones and other nuclear hormone receptor ligands (NR ligands) have a well described role in regulating oligodendrocyte differentiation and myelination during development, however, it remains unclear which NR ligands are required to drive and promote these effects. In our project, we aim to apply both phenotyping and genotyping to OPCs treated with NR ligands to evaluate the effects on the differentiation of OPCs. We will evaluate Triiodothyronine (T3), Progesterone, Vitamin D3, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), 9-cis-Retinoic acid, β-estradiol, and Testosterone). The OPCs were isolated from P7-8 Sprague-Dawley rats and were differentiated in the presence of the NR ligands for 3-6 days. We performed single-cell RNA sequencing on day 4 of differentiation and analyzed the transcriptomic profiles using Partek Flow software. Immunophenotyping was performed with neuron-glial antigen 2 (NG2) to identify OPCs, and myelin basic protein (MBP) to label mature oligodendrocytes. qPCR assays were performed using cells differentiated for 3 or 5 days to investigate the expression level of three genes: cgt, mag (both are early oligodendrocyte markers), and mbp (encodes the myelin sheath). The single-cell RNA sequencing data in combination with other analyses offers a high-resolution view that could reveal the regulatory relationships between NR ligands and OPC differentiation.

4. Analyzing the Cellulolytic Capabilities of a Bacterial Subcommunity Isolated from Soil

Scott Romeiser, Katrina Basore, and Stephen Fields
Department of Biology, Emporia State University

Cellulose is a major energy source for many microbial soil communities, but the community interactions between cellulolytic and non-cellulolytic species during cellulose degradation is poorly understood. We grew ten subcommunities from a cultivated soil community from east central Kansas on carboxymethylcellulose (CMC) plates. Metagenomic sequencing revealed a high level of variability in species diversity and composition between the ten randomly established bacterial assemblages on CMC even though they were from the same temperate soil sample. We then isolated eight of the dominant species onto CMC plates, sequenced them with Illumina NextSeq and Oxford Nanopore MinION platforms, assembled the genomes with the Galaxy-based Unicycler algorithm and annotated them using RAST and KEGG servers. In hopes of correlating the growth capabilities on CMC with the genomic features we have identified the genes most closely associated with cellulose utilization, namely the exoglucanase, endoglucanase, and beta-glucosidase classes of cellulase enzymes. The number of identified cellulase genes is highly variable, ranging from zero in the gram-negative Pseudomonas sp. strain ES-006 and Achromobacter sp ES-001 to 34 cellulases in both *Kitasatospora albolonga* ES-004 and *Gordonia sp* ES-007, each of which are gram-positive Actinobacteria. We have also quantified CMC digestion for each species using the Congo Red Analysis of Cellulose Concentration (CRACC) assay and found a general positive correlation with the number of cellulase genes. Perhaps of greater importance is the potential synergy in cellulose degradation observed between cellulosic and non-cellulosic species. RNA-seq will be employed to determine which genes in the non-cellulosic species are most highly regulated in the presence of cellulose and/or cellulose-degrading species. This study indicates that integrated waste management practices and cellulose-based biofuels industries would benefit by optimizing cellulolytic species composition

5. Evidence for advanced generation hybridization between two prairie milkweeds.

Victor Andreev[1], Joshua Puzey[2], Lizzy Davies[2], Carrie Olson-Manning[3], Sydney Kreutzmann[3], Mark Fishbein[1]
[1]Department of Plant Biology, Ecology and Evolution, Oklahoma State University; [2]Biology Department, College of William and Mary; [3]Biology Department, Augustana University

Two species of milkweeds, *Asclepias speciosa* and *A. syriaca*, meet on the Great Plains, and the contact zone spreads from Kansas to Minnesota. Several researchers have observed multiple individuals of intermediate morphology in this area, and they proposed introgressive hybridization as the explanation for the observed pattern of morphological variation. However, there are alternative explanations, which include a preservation of ancestral polymorphism (i.e., Incomplete Lineage Sorting) or the action of selective pressure imposed by environmental clines. The last explanation seems especially plausible, since the species meet on a strong precipitation gradient that may have resulted in a geographical cline in plants' morphology. The goals of this research were to characterize *A. speciosa, A. syriaca* and the intermediates morphologically and genetically and validate the hybrid origin of the

individuals of intermediate morphology. We analyzed a range-wide sample of 552 individuals obtained from herbarium and field-collected samples. Our morphological analyses were based on measurements of 15 floral and vegetative traits, and genetic analyses were based on 7480 genome-wide SNPs. The analyses showed that the morphological variation between *A. speciosa* and *A. syriaca* is not correlated with geographic distance, which allow us to rule out the environmental clines as the explanation for the morphology of the intermediates. At the same time, these individuals are genetically and morphologically intermediate between *A. speciosa* and *A. syriaca*. There is also an evidence of interspecific gene flow in sympatric populations of these species. Individuals in the zone of sympatry demonstrate various degrees of admixture, and the pattern of shared genetic variation is consistent with that of advanced generation hybridization. Our results demonstrate that the individuals of intermediate morphology are indeed hybrids, and their existence cannot be explained by environmental clines or incomplete lineage sorting. This research helps us to understand the spatial distribution of morphological and genetic variation in prairie plants and the processes that shape this variation.

6. The influence of the oral microbiome on head and neck squamous cell carcinoma

Austin Gregory Morrison[1], Melissa Shadoin[1], Levi Arnold[1], Soumyadev Sarkar[2], Kellen Wright[1], Matthew Kriet[1], Shahid Umar[3], Sonny T M Lee[2], Sufi Mary Thomas[1, 4, 5]
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The oral microbiome is an emerging field with high potential for uncovering new avenues for cancer therapy. Over 700 microbial species have been identified within the human aerodigestive tract, however the impact of many of these is still unknown. Head and neck squamous cell carcinoma (HNSCC) is the most frequently occurring malignancy of oral cavity cancers with an incidence of over 90%. Treatment options for HNSCC include surgery, chemotherapy, and radiotherapy; all of which still provide a low 5-year survival rate which indicates a need for additional treatment options. The overall goal of this research is to provide information on how *Fusobacterium nucleatum*, and other significant microorganisms, influence HNSCC, as well as increasing the scientific knowledge of their role within the oral microbiome. We hypothesized that the oral microbiome is distinct in patients from paired cancer-free controls. We have interrogated the profile of the oral microbiota by use of 16S rRNA amplicon sequencing of saliva samples from HNSCC patients and their significant others. The patients showed a higher relative abundance in *Prevotella*, *Neisseria*, *Fusobacterium*, and *Campylobacter* when compared to their significant others. *Fusobacterium nucleatum* is one of the most common cultivable microorganisms found within the oral cavity that has been shown to exhibit pathogenic properties. Here, we observed significantly higher relative abundance of *Fusobacterium nucleatum* when comparing patients to their significant others. In this research, we detailed the key properties involved with the anaerobic cultivation of *Fusobacterium nucleatum*, including optimized growth conditions and medium, as well as increased *in-vivo* proliferative properties in conjunction with metagenomic and metabolomic

data. Further studies will provide information on the biological mechanisms *Fusobacterium nucleatum*, in conjunction with other microorganisms, utilizes to alter the progression and therapeutic response of HNSCC.

7. Role of maternal RNA decay during zebrafish embryo development

J. Rene Wong[1], Gopal Kushawah[1], Ariel A. Bazzini[1,2]
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In animals, the earliest stages of development are driven by maternally deposited mRNAs. The decay of most of these molecules and their substitution by zygotic-transcribed mRNA during a process known as the Maternal-to-Zygotic transition (MZT) has been extensively studied in many bulk RNA-seq experiments. However, it has been shown that some mRNAs during the MZT are distributed in specific cells of the early embryo. Here, we are focused on the maternally deposited and specific-localized mRNAs, hypothesizing that these RNAs play an important role in the early developmental process through their specific localization. To address this question, we took the advantage of CRISPR-Cas13d tool, which was recently developed to degrade RNA. Using the CRISPR-Cas13d system, we have previously shown that CRISPR-RfxCas13d is an effective and precise system to deplete specific mRNA transcripts in zebrafish and other animal embryos. We demonstrate that zygotically-expressed and maternally provided transcripts are efficiently targeted. We have shortlisted a set of maternally deposited cell-specific RNA in the zebrafish embryo. Our preliminary data suggest that perturbation of the expression levels of cell-specific maternal RNA using the CRISPR-Cas13d system leads to abnormal early embryo development. Together with these results, we aim to dive deep to decipher the mechanism of cell-specific maternal RNA decay and their role in the regulation of early embryo development in zebrafish.

8. Next Generation Sequencing at KU Genome Sequencing Core

Jennifer Hackett[1,2,3,4], Mary R. Reed-Weston[1,2,3,4], Erik A. Lundquist[1,2,4], Susan M. Lunte[1,5,6]
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The Genome Sequencing Core (GSC) is one of three research service core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq, Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure already at KU by providing a range of Illumina sequencing platforms including the NextSeq 2000 and NextSeq 550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted

amplicon sequencing projects) to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, including project consultation, sample quality check, sequencing library construction, Illumina sequencing, and FASTQ generation and demultiplexing. For latest pricing, current sequencing queue, or other information, visit the Genome Sequencing Core's website: https://gsc.ku.edu/.

9. iCodon: Harnessing mRNA stability to customize gene expression
Michay Diez[1], Santiago Gerardo Medina-Muñoz[1,2], Luciana Andrea Castellano[1], Gabriel da Silva Pescador[1], Qiushuang Wu[1], Ariel Alejandro Bazzini[1,3]
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The codon composition of messenger RNA (mRNA) conveys regulatory information that strongly affects transcript stability, allowing cells to fine-tune gene expression. Further, specific codons are enriched in stable mRNAs, whereas others occur in unstable mRNAs. Yet, current codon optimization methods revolve around codon usage metrics that omit these observations, and therefore weakly correlate with mRNA stability. In addition, these methods only work in one direction, which means that they only seek to increase expression, limiting their applications for RNA biology research. To address these challenges, we hypothesized that by using our knowledge of mRNA stability, we could create a tool that customizes gene expression. Hence, we trained a machine learning model using stability profiles generated after blocking transcription and measuring mRNA decay over time. These profiles from several vertebrate species allowed our model to predict mRNA stability based on codon composition. This led us to develop iCodon, a tool that predicts mRNA stability and produces a list of synonymous sequences covering a range of expression levels by introducing synonymous codon substitutions. First, using a massive reporter library, we showed in a transcription-independent manner that the stability of more than 2500 mRNAs significantly correlates with the stability predicted by iCodon. Next, we validated that our predictions correlated with gene expression by testing 18 constructs designed by iCodon encoding different fluorescent proteins and endogenous genes in human cells and zebrafish embryos. Additionally, iCodon synonymous variants had higher expression levels than sequences designed by codon usage based methods. In conclusion, iCodon provides a powerful tool to interrogate mRNA stability and design strategies to modulate gene expression in vertebrates, for a wide range of applications for research, and for the potential optimization of RNA-based therapeutics and vaccines.

10. Ancient genomes from the Plains: Preliminary evidence for long-distance population continuity
Lydia Sykora[1], Justin Tackney[1], Brooke Morris[2], Caroline Kisielinski[1], Kristine G. Beaty[1], Lauren E. Y. Norman[1], Austin Reynolds[2], Jennifer A. Raff[1]
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The genetic diversity of past peoples within the Central and Southern Plains, a region that is crucial for understanding the population histories of the Americas, is poorly characterized. The Plains served as a place of extensive migration and trade from the dispersal of the First Peoples through present day. In a community-led collaboration with a Plains tribe we performed human genome-wide targeted SNP capture from two individuals from different archaeological sites: one in the Central Plains (Kansas, direct radiocarbon dated between AD 1280 and 1390) and one in the Southern Plains (Texas, likely lived around AD 1150-1450). We successfully genotyped 346,199 SNPs from the Kansas individual and 164,390 SNPs from the Texas individual. Preliminary analyses using principal component analysis show that these two individuals cluster with the "Southern Native American" (SNA) lineage. Maximum likelihood estimates of ancestry components supports this observation. Furthermore, genetic kinship modeling indicates that these two individuals are second-degree relatives (e.g. grandparent-grandchild, half-sibling, or aunt/uncle-niece/nephew). We show the potential for modeling long-distance population continuity between the Central and Southern Plains during the Plains Village Tradition (AD 1100 to 1700). Beyond its contribution to micro-scale population modeling and the specific interests of the collaborating tribe, this project fills a critical gap as there have been no ancient genomes published from individuals living in the Plains prior to European contact. These individuals, and others, will inform larger research aims investigating mortuary practices, dietary shifts, and population migrations throughout the Plains.

11. What is *Salvinia molesta*? Determining the Genetic Composition and Number of Origins of the Invasive Giant Salvinia

Stacy Holt Jr[1], James Beck[1], Erin Sigel[2], Brittany Sutherland[3], Pedro Bond Schwartsburd[4], Cecília Vieira Miranda[4]
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The occurrence of polyploidy, having multiple complete genomes, is now recognized as a major influence on the evolution, genetic composition, and diversification of many plant lineages. Polyploidy is widely viewed as following two pathways. An autopolyploid is one that is the result of genome doubling involving a single species. In contrast, an allopolyploid is a polyploid that is the result of hybridization between two or more species. This can involve multiple independent hybridization events, a process which creates allopolyploid species that have several independently constructed genomes. The aquatic fern *Salvinia molesta* D.S. Mitch. belongs to a clade of closely related species known as the "*Salvinia auriculata* complex," and is an allopentaploid hybrid with unknown parentage. *Salvinia molesta* is an invasive that can have devastating ecological effects on the freshwater ecosystems it colonizes. The lack of clarity surrounding the genomic composition of *S. molesta* complicates current eradication methods, as it is not clear how many genotypes are present and what these genotypes are. This research focuses on identifying the maternal genome of *S. molesta* and determining if this species consists of a single or multiple independently derived lineages. To answer these questions chloroplast genomes (plastomes) will be sequenced from field and

herbarium samples of *S. molesta* and several closely related species. These samples will be used to identify the maternal genome of S. molesta, as well as to construct a phylogeny that delineates species level relationships within the genus. Plastome diversity within *S. molesta* will also be examined, and the presence of multiple divergent genotypes will strongly suggest multiple origins of this hybrid.

12. RNA Sequencing Reveals Donor Variability in Sex- and Age-Matched Human Meniscal Fibrochondrocyte Response to Estrogen

Kelsey E. Knewtson[1], Adam R. Podgorny[2], Cuncong Zhong[3], Donna M. Pacicca[4], Jennifer L. Robinson[1]
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INTRODUCTION: More than 650 million people worldwide have knee osteoarthritis (OA). Meniscal tears pose significant risk in the development of OA. Recent epidemiological data suggests differences in meniscal injury rates, repair, and correlation to subsequent OA between males and females. These data and others indicate estrogen could be fundamental for knee joint health. However, the cell response to estrogen, and differences in response of cells from different donors, is unknown. Thus, the purpose of this study is to investigate the role of pulsed and continuous dosing of estrogen on gene expression of meniscal cells harvested from 2 female patients of similar age and injury. We hypothesized that cells from both patients would respond similarly to treatment and that cell response would vary based on estrogen dose and dosing kinetics. METHODS: Cells were harvested from tissue resected from the right medial meniscus of 2 female patients aged 16 and 17 at Children's Mercy Hospital (IRB Exemption #STUDY00000746). RNA was isolated using a Qiagen RNeasy Mini Kit. Libraries were generated for full transcriptome sequencing using an NEBNext Ultra II Directional RNA Library Prep Kit and sequenced utilizing Illumina NextSeq 550 at the KU Genome Sequencing Center. RESULTS: Contrary to our hypothesis, there were few DEGs that overlapped between the two donors within the same treatment group. Further, there were marked differences in the number of DEGs between the donors. DAVID analysis revealed that diverse groups of genes related aspects of basic cellular functions were differentially expressed between the donors even without estrogen treatment. DISCUSSION: Elucidating the role of estrogen signaling on knee meniscal fibrocartilage could provide starting points for the development of new patient-centered therapies to reduce the onset of knee osteoarthritis. This study serves as an important reminder of the inherent differences in cells sourced from different donors and the importance in considering donor source when designing studies.

13. Tracking SARS-CoV-2 variants through municipal wastewater

Justin M. Hutchison, Yasawantha Hiripitiyage, Belinda S. M. Sturm

Civil, Environmental, and Architectural Engineering, University of Kansas.

The COVID-19 pandemic has highlighted the potential role that wastewater-based epidemiology can play in assessing aggregate community health. The University of Kansas (KU) has been monitoring the prevalence of SARS-CoV-2 variants in wastewater for the state of Kansas, covering 95 counties. However, efforts to translate Sars-CoV-2 information obtained from wastewater samples into meaningful community health indicators are nascent. KU has undertaken two approaches to uses this data to protect public health. The first measured quantities of Sars-CoV-2 nucleocapsid (N) genes (N1 and N2). Four biomarkers (human mitochondrial gene NADH dehydrogenase subunit 5 (mit5), creatinine, ammonia, and biological oxygen demand (BOD)) were quantified and used to normalize Sars-CoV-2 gene copy numbers to account for variations in sewershed conditions. The normalized values were correlated to daily new case data and one-, two-, and three-week cumulative case data. For early stages of the pandemic, the wastewater samples may have indicated active COVID-19 cases before clinical indications. In addition to prevalence, wastewater measurements can be used to assess the emergence of novel variants in communities. KU has been sequencing the spike (S) gene to detect variants including Delta and Omicron.

14. Fishing with CRISPR/Cas13d: Elucidating micropeptide functions in zebrafish development

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'Omics-based techniques have revealed messenger RNA (mRNA) sequences once defined as non-coding that produce short proteins (< 100 amino acids) called micropeptides. Emerging studies have shown the importance of micropeptides in diverse biological processes. For example, the micropeptide APELA maintains pluripotency in human embryonic stem cells and is critical for heart patterning in zebrafish and mice. Additional micropeptide functions during vertebrate development, however, remain largely unexplored. Zebrafish is an outstanding model for interrogating developmental vertebrate gene function(s) due to its genetic tractability and synchronous, external embryogenesis. In fact, zebrafish express over 400 micropeptides during early development whose characterization is hindered by three key barriers: 1) many early mRNAs are maternally provided and can mask the effects of a targeted gene disruption, 2) conventional RNAi is ineffective in zebrafish, and 3) established, morpholino-based mRNA targeting has been questioned over toxicity and off-target effects. To overcome these barriers, our lab has established the CRISPR/Cas13d system in zebrafish that targets and degrades mRNA, including maternally provided mRNA. In this study, we selected five maternally provided micropeptide mRNAs with high abundance across the first eight hours of zebrafish development to assess for function with CRISPR/Cas13d. RT-qPCR revealed that CRISPR/Cas13d significantly reduced mRNA levels in 4 out of 5 targeted micropeptide mRNAs at 6 hours post-fertilization (hpf). Notably, CRISPR/Cas13d targeting of candidate "micropeptide 4" mRNA produced over 98% knockdown and developmental delay

at 6 hpf. RNA-seq in knockdown embryos at 6 hpf revealed specific targeting of "micropeptide 4" mRNA and suggests a loss-of-function defect in zygotic transcriptional activation. Ongoing work will determine the localization of "micropeptide 4" mRNA and protein and determine its molecular function. Micropeptides that are critical for zebrafish development are expected to provide novel insights into vertebrate, and ultimately human, biological processes.

15. Family-based whole exome sequencing identifies *BUD13* variants involved in specific language impairment

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Specific language impairment (SLI) is a neurodevelopmental disorder that displays high heritability estimates and aggregation in families. While the language development of individuals with SLI has been well-documented, the genetic architecture underlying this disorder remains unclear. We performed whole-exome sequencing (WES) in multiple members of one family with SLI (family 489; N = 11) and prioritized co-segregating rare variants (MAF ≤ 0.005 in the 1000 Genomes Project) in three genes: *BUD13*, *APLP2* and *NDRG2*. To determine the significance of rare variants, we Sanger sequenced all the coding regions of these three genes in unrelated individuals with SLI (N= 175). We observed a total of 13 additional variants in 18 unrelated individuals with SLI. Gene level burden analysis revealed *BUD13* is involved in SLI. Additionally, variant level multiple in silico analyses predicted the pathogenicity of five *BUD13* variants. Bud13 is a component of the retention and splicing (RES) complex, an important mechanism of RNA splicing, which was found to be important for neural phenotypes observed in an animal model. Recent report of a copy number variant spanning *BUD13* was observed in an individual with a neurodevelopmental phenotype. Previous reports and our family-based investigation suggest *BUD13* could be a new target for the neural basis of language.

16. A workflow for the identification of novel transposable element insertions in *Drosophila virilis* pooled long reads

Eva Morrison and Justin P. Blumenstiel
Department of Ecology and Evolutionary Biology, University of Kansas

Transposable elements, first discovered by Barbara McClintock in the 1940s, are known to jump around the genome. Currently, there is a lack of robust tools to discover where these elements insert. This can be attributed to the limitations of short sequencing reads and the diverse nature and characteristics of each transposable element. Recent advancements in long-read sequencing technology have now made it possible to identify new insertions without ambiguity. I have created a workflow that enables the detection of de novo transposable element insertions using nanopore sequencing. The workflow begins with running RepeatMasker on available genome assemblies and long nanopore reads collected from pooled individuals. Nanopore reads masked for transposable elements are then

mapped to the masked assemblies to provide a normalized genomic coordinate system for later comparison. bedtools is then used to compare coordinates of known transposable element insertions in the genome assemblies to the location of insertions identified within the nanopore reads. Using this approach, we have validated the mobilization of transposable element families that are known to become activated in a syndrome of hybrid dysgenesis in *Drosophila virilis*. This workflow could be influential in future transposon research by providing a singular tool for the annotation of de novo transposable element insertions in pooled long-read sequencing experiments.

17. Digging up DNA: Sedimentary ancient DNA as an interdisciplinary tool to reconstruct past biodiversity
Caroline Kisielinski and Dennis O'Rourke
Department of Anthropology, University of Kansas

Sediments and paleosols are known repositories of both micro- and macrofossils commonly utilized to reconstruct past environments. Recent advances in DNA extraction methodologies and high-throughput sequencing technologies have allowed for the isolation and sequencing of genetic material directly from ancient sediments (sedaDNA). SedaDNA complements traditional methods of paleoenvironmental reconstruction by providing greater taxonomic resolution as well as by generating data from sources with no visible fossil evidence. To date, sedaDNA from a variety of depositional settings has been successfully analyzed to survey local and regional biodiversity, to detect the presence of specific plants and animals (including humans), and to track vegetation changes as a response to climatic shifts. The rapid growth of the field has garnered broad interest to use sedaDNA to address their own disciplinary questions. However, working with genetic material from ancient sources is notoriously difficult as the molecules are degraded, often extracted in low concentrations, and very susceptible to contamination from multiple sources. Additionally, the taphonomy of sedaDNA molecules is vastly understudied--especially in terrestrial sedimentary deposits from lower latitudes. In response, we present a guide outlining what considerations must be taken when working with ancient sedimentary DNA, best practices for sample collection and storage from a variety of sedimentary deposits, and recommendations of different sampling strategies to collect the most relevant data to address specific research questions.

18. Membrane-anchored UNC-6/Netrin reveals roles of both close- and long-range interactions in regulating VD growth cone dorsal outgrowth
Kelsey Ferguson, Snehal Mahadik, and Erik Lundquist
Department of Molecular Biosciences, University of Kansas

UNC-6/Netrin directs dorsal-ventral axon pathfinding and is expressed in ventral cord neurons. Classically, UNC-6 was thought to form a ventral-to-dorsal gradient that was interpreted by growth cones. Our previous studies on VD growth cones indicated a more complex mechanism, involving discrete aspects of growth cone polarity coupled with regulation of protrusion. This polarity/protrusion model parts from the classical gradient model in important ways. First, growth cone polarity is separable from growth cone

protrusion. Second, UNC-40/DCC and UNC-5 receptors both have roles in growth cones that grow away from UNC-6. To further test our model, we constructed a membrane-anchored UNC-6 called *unc-6(lq154).* Diffusible UNC-6 is predicted to be absent in this mutant. During development, the AVM neuron extends an axon ventrally toward UNC-6. As there is no contact between AVM and UNC-6-expressing cells, diffusible UNC-6 is predicted to guide the axon. unc-6(lq154) animals display axon guidance defects to the same extent as *unc-6(ev400)* null mutants, consistent with the idea that diffusible UNC-6 is absent. In contrast, VD/DD axon guidance defects of *unc-6(lq154)* were less severe than those of *unc-6(ev400). unc-6(ev400)* VD growth cones were unpolarized, whereas polarity in VD growth cones near the ventral surface in *unc-6(lq154)* was normal. Growth cones further from the ventral surface, in the dorsal half of the animal, were unpolarized in *unc-6(lq154)*. This result suggests that initial polarity is normal in *unc-6(lq154),* but polarity is lost as growth cones migrate dorsally. Possibly, a close-range or contact-mediated interaction of UNC-6 and UNC-5 polarizes the growth cone, but longer-range diffusible UNC-6 is required to maintain polarity. Preliminary studies using unc-5 hypomorphs, which affect only the long isoforms of UNC-5, show a similar phenotype. This indicates the short unc-5B isoform mediates close-range polarity, possibly through a contact-mediated event, whereas the long isoforms might mediate maintenance of polarity requiring diffusible UNC-6.

19. Protein Quality Control in Early Vertebrate Development
Gabriel da Silva Pescador and Ariel A. Bazzini
Stowers Institute for Medical Research

Maternal-to-zygotic transition (MZT) is a crucial step during embryogenesis where the zygotic genome assumes developmental control from maternally provided gene products. During this step, embryos must activate their genome after clearing maternally deposited products, failure to do so prevents development and is consequently lethal. Maternal RNA degradation has been extensively studied during MZT, yet maternal protein dynamics have remained largely unexplored. Recently, one of the protein degradation pathways, the ubiquitin-proteasome pathway, was implicated in MZT in *Drosophila*, but a conserved mechanism in vertebrates has yet to be described. Thus, we hypothesize that vertebrate genome activation requires degradation of maternal proteins. To test this, we employ zebrafish embryos to perform biochemical assays, proteomics, RNA sequencing, and genetic manipulations with the CRISPR-Cas13d system. The latter is an effective and fast system to deplete specific mRNA transcripts in vertebrates. First, we established a fluorescence-based assay to visualize proteasome degradation activity in zebrafish which is active since fertilization. Then, we employed the CRISPR-Cas13d system to knock down several proteasome subunits that are highly deposited as mRNA in zebrafish oocytes. These embryos are arrested at the onset of MZT and die right after. We also confirmed that knocked down embryos have lower proteasome activity that correlates with reduced protein expression seen by Western Blot. Further, knocked down embryos display downregulation of zygotic genes, whereas otherwise cleared maternal RNA targets are upregulated, supporting a failure to fully activate their genomes. Upon GO term analysis, we observe enrichment of differentially expressed genes related to apoptosis and protein stress. Next, we will analyze protein abundance by mass

30

spectrometry to identify candidate maternal proteins to dissect for their roles during zebrafish genome activation. In conclusion, these results suggest that maternally deposited proteins require degradation and highlight the importance of looking beyond RNA clearance regulation during early development.

20. SNPfiltR: an R package for interactive and reproducible SNP filtering
Devon A. DeRaad
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I have developed the R package SNPfiltR which can form the backbone of a customizable, reproducible single nucleotide polymorphism (SNP) filtering pipeline implemented exclusively via the widely adopted R programming language. SNPfiltR extends existing SNP filtering functionalities by automating the visualization of key parameters such as sequencing depth, quality, and missing data proportion, allowing users to visually optimize and implement filtering thresholds within a single, cohesive work session. All SNPfiltR functions require vcfR objects as input, which can be easily generated by reading a SNP dataset stored in standard variant call format (vcf) into an R working environment using the function read.vcfR() from the R package vcfR. Performance and accuracy benchmarking reveal that for moderately sized SNP datasets (up to 50M genotypes, plus associated quality information), SNPfiltR performs filtering with comparable accuracy and efficiency to current state of the art command-line-based programs. These results indicate that for most reduced-representation genomic datasets, SNPfiltR is an ideal choice for investigating, visualizing, and filtering SNPs as part of a user friendly bioinformatic pipeline. The SNPfiltR package can be downloaded from CRAN with the command install.packages("SNPfiltR"), and the current development version is available from GitHub at: (github.com/DevonDeRaad/SNPfiltR). Thorough documentation for SNPfiltR, including multiple comprehensive vignettes detailing realistic use-cases, is available at the website: (devonderaad.github.io/SNPfiltR/).

21. Role of Epistasis in Differential Resistance Outcomes
Kervens Accilien, Tiffany Chan, and Dr. Robert Unckless
Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas.

Microbes evolve resistance to antibiotics at an astonishing rate. Only 20 years after the discovery of penicillin in 1928, penicillin-resistant *Staphylococcus aureus* had become a global pandemic. By 2050, more than 20 million people are expected to die annually from antimicrobial resistant infections. Development of new antimicrobials does little to alleviate this problem; once a microbe is exposed to an antimicrobial, resistance follows shortly thereafter. Combatting antimicrobial resistance require that we adjust how we study the phenomenon itself. Rather than looking at the final outcome i.e., resistance to a set of antimicrobials, we need a better understanding of the evolutionary steps leading to resistant genotypes. Ultimately, this will enable us to proactively mitigate the occurrence of resistance. Accordingly, we want to investigate the importance of epistasis in adaptation against

antimicrobials using empirically derived fitness landscapes. We hypothesize that antibiotics present more epistasis than antimicrobial peptides, as measured by global fitness landscape ruggedness. In addition, we expect epistasis to correlate with naïve resistance (resistance from standing genetic variation before new mutations). In contrast, antimicrobial peptides will present less epistasis and lower incidence of resistance from standing genetic variation. This approach will both enrich our understanding of genetic adaptation and provide a new way to approach the crisis of antibiotic resistance.

22. Corn-y Cultures: Maize Root Endophyte Culture Collection
Felicity Tso[1], Nichole Ginnan[1,2], Maggie Wagner[1,2]
[1]Ecology and Evolutionary Biology, University of Kansas; [2]Kansas Biological Survey and Center for Ecological Research

Maize (*Zea mays*) is a model plant system and an important staple crop grown around the world. Understanding the complex relationship between maize and its associated microbial community is vital to sustainably increase yield. Axenic microbial cultures are crucial tools for studying microbiome function, conducting synthetic community experiments, and investigating evolutionary-based questions, which cannot be interrogated with sequencing alone. There has not been an extensive collection of maize root endophytes reported. To increase the number and diversity of axenic microbial cultures, we collected soil from four pristine tallgrass prairie remnants along the natural Kansas precipitation gradient. Then, maize seedlings were grown in sterile clay inoculated with soil slurries. After one month of growth, endophytes were cultured from surface-sterilized roots. To date, over 900 bacterial strains have been isolated and taxonomically identified using full-length 16S Sanger Sequencing. Of the 27 unique genera in our collection, *Luteibacter spp.* (553 isolates) were most frequently isolated. Other frequently isolated bacterial genera include *Burkholderia spp.* (128 strains), *Pantoea spp.* (83 strains) , *Rhizobium spp.* (82 strains), and *Paraburkholderia spp*. (61 strains). These wild isolates will be screened for a myriad of traits including, antibiotic resistance and drought tolerance, and be used in synthetic community studies. Furthermore, this maize root endophyte culture collection will be made available to the maize-microbe research community.

23. The More You Watch, the Less You Know: How Genetic Literacy Relates to Consuming Media About Genetic Ancestry Tests
Peter Bobkowski
School of Journalism and Mass Communications, University of Kansas

This poster draws on data from two national U.S. surveys to discuss the role of popular media about genetic ancestry tests in individuals' genetic literacy. First, the Health Information National Trends Survey V (n = 3,865), establishes media content about ancestry tests as the chief genetics topic that individuals see in the media. The public is significantly more familiar with genetic ancestry tests than with genetic screening tests for cancer or other health risks.

The data also shows that significantly larger shares of the public identify television and the internet as sources of information about genetic tests than identify other sources (e.g., social media, family member). These results justify further research into genetic ancestry test media messages, and into the potential consequences of this content on genetic literacy. Second, data from an online survey of 413 U.S. adults identifies the factors that predict whether individuals pay attention to media messages about genetic ancestry tests, and how this relates to their genetic literacy. Individuals who are engaged in genealogy research, who express greater certainty in their ancestry, who have second-hand test experience, who are younger, and who are more religious, tend to pay more attention to media messages about genetic ancestry tests than their counterparts. In turn, those who pay greater attention to media about genetic ancestry tests know less about genetics than those who pay less attention to such media messages. Moreover, among those who pay attention to media about genetic ancestry tests, those with first-hand ancestry test experience know less about genetics than those without first-hand test experience. In all, these data suggest that while media messages about genetic ancestry tests are a key source for learning about genetics, consuming such media in higher quantities may contribute negatively to individuals' understanding of genetics.

24. Computer-Aided, Resistance-Gene-Assisted Genome Mining for Proteasome and HMG-CoA Reductase Inhibitors

Cory B. Jenkinson[1], Adam R. Podgorny[2], C. Elizabeth Oakley[1], Cuncong Zhong[2], Berl R. Oakley
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Fungi produce biologically active small molecules, called secondary metabolites (SMs), many of which are medically valuable. The genes that encode SM biosynthetic pathways are usually clustered together in the genome, forming biosynthetic gene clusters (BGCs). Genome sequencing reveals that the number of SM BGCs vastly exceeds the number of known SMs, and, thus, that huge numbers of potentially valuable SMs are yet to be discovered. Resistance-gene-assisted genome mining is a strategy to exploit the greater fungal secondary metabolome efficiently, by identifying SM BGCs that are likely to make useful products. It takes advantage of the fact that some SM BGCs contain a gene encoding a resistant version of the protein targeted by the compound produced by the BGC. This allows the producing organism to survive while its competitors are inhibited. The bioinformatic signature of such SM BGCs is that they contain an allele of an essential gene with no SM biosynthetic function, and there is a second allele elsewhere in the genome. Manually searching thousands of sequenced genomes for this signature is daunting, so we have developed a computer-assisted approach that allows users to query large databases for SM BGCs that putatively make compounds that have particular targets of therapeutic interest. We have applied this approach to look for SM BGCs that target the proteasome β6 subunit, the target of the proteasome inhibitor fellutamide B, or HMG-CoA reductase (HMGCR), a key enzyme in sterol biosynthesis and the target of cholesterol reducing therapeutics such as lovastatin. Our approach proved effective, finding known fellutamide and lovastatin SM BGCs as well as fellutamide- and lovastatin-related BGCs with variations in the SM genes that suggest they

may produce structural variants of fellutamides and lovastatin. Gratifyingly, we also found SM BGCs that are not closely related to lovastatin BGCs but putatively produce novel HMGCR inhibitors.

25. Maximizing the phylogenomic utility of formalin-fixed museum specimens
Kevin Chovanec
Ecology and Evolutionary Biology, University of Kansas

Formalin-fixed natural history specimens preserve snapshots in time that are increasingly accessible to genomic investigation. A fundamental challenge of working with degraded DNA from such material is that rare or extinct taxa of interest typically lack suitable (i.e. phylogenetically close) reference genomes for validation and comparison. Following protocols optimized in the field of paleogenomics, we sequenced multiple single-stranded DNA libraries from a 50-year-old specimen of *Anolis distichus*, a common West Indian lizard within a genetically well-characterized genus. These libraries allow us to better quantify post-mortem DNA damage in formalin-fixed museum specimens and to explore the phylogenomic utility of resulting datasets. Using novel alignment and assembly strategies, we recover thousands of informative loci with sufficient depth and coverage for downstream phylogenetic analysis. Across multiple datasets, we reliably place the specimen within a clade of living relatives. Our results are replicable and robust to increasing degrees of reference divergence, suggesting these methods are appropriate for extinct taxa whose closest living relatives are unknown. Wet bench and bioinformatic strategies presented here contribute to a growing literature on how to appropriately analyze ancient DNA from formalin-fixed museum specimens.

26. Verifying insertion of a plasmid by non-homologous end joining using PCR and sequencing
Nicholas Lacy, Justin P. Blumenstiel, and Kelley Van Vaerenberghe
Ecology and Evolutionary Biology, University of Kansas

CRISPR has revolutionized genetic modification. One method of inserting genes using CRISPR involves creating plasmids with homology arms, which requires significant expertise and time. An alternative method is to use a plasmid without homology arms and allow it to insert by non-homologous end joining. In our experiment, a pUASz plasmid was inserted into *Drosophila melanogaster* lines using CRISPR-Cas9 and NHEJ. Here we evaluate this method. The presence of the insertion was confirmed and the direction determined in forty-four lines using PCR and gel electrophoresis of the junction between the existing genomic DNA and inserted plasmid. Four lines had double insertions of the plasmid with two copies back-to-back in opposite directions. The exact location of the insertions in 16 lines was determined using Sanger sequencing. Unique patterns of genetic damage around the insertion site act as signatures that can be used to distinguish between different insertions using Sanger sequencing.

27. Identifying DNA transposable element excisions in a Drosophila virilis syndrome of hybrid dysgenesis using long-read sequencing

Stefan Cerbin[1], Danny Miller[2], and Justin Blumenstiel[1]
[1]Ecology & Evolutionary Biology, University of Kansas; [2]Department of Genome Sciences, University of Washington

DNA transposons are sequences that are capable of moving in the genome resulting in profligate DNA damage and genome instability. Estimating transposon mobilization rates and the damage they cause is important for understanding genome evolution and genome stability. *Drosophila virilis* has several strains with varying copy number of DNA transposons and differences in germline piRNAs profiles. Transposons become activated when females lacking the requisite piRNAs that silence transposons inherited paternally, resulting in hybrid dysgenesis. With these strains we can study global transposon mobilization to determine the rates of excision and the overall change in copy number. In this study, we use pooled long-read DNA sequencing to identify excision events for DNA transposons. This excision analysis will be performed to estimate an excision rate using a likelihood model for estimating global excision rates for DNA transposons. This model will incorporate specific DNA transposon insertions, family, and piRNA profiles as parameters. We predict that differences in piRNA profiles, DNA transposon family identity, location, and internal deletion status will jointly determine the excision rate.

28. Efficacy of CRISPR-assisted insertion tagging

Jordan X. Lyerla, Ana P. Dorador, Mia X. Willingham, and Justin P. Blumenstiel
Department of Ecology & Evolutionary Biology, University of Kansas

CRISPR has revolutionized genetic analysis by allowing targeted break formation at any DNA sequence. Using CRISPR, one can target transgene insertion to specified sites within the genome. One method of targeted insertion using CRISPR employs homology-dependent insertion. This is achieved by generating homology arms that flank the transgene and also the target cut site. However, this requires specialized constructs to be made for each insertion location. CRISPR-assisted insertion tagging serves as an alternative method to homology-dependent CRISPR/Cas9 transgene insertion. Instead, this approach uses non-homologous end joining (NHEJ) to repair the DSB made by the Cas9-sgRNA machinery. Using *Drosophila melanogaster*, CRISPR-assisted insertion tagging was used to target three genes for disruption. These genes were *p53*, *chk2*, and *CG6325*. Here we evaluate the efficacy of this method.

29. CRISPR-Cas13d induces efficient mRNA knock-down in animal embryos

Gopal Kushawah
Stowers Institute for Medical Research

Early embryonic development is driven exclusively by maternal gene products deposited into the oocyte. Although critical in establishing early developmental programs, maternal gene functions have remained elusive due to a paucity of techniques for their systematic disruption and assessment. CRISPR-Cas13 systems have recently been employed to degrade RNA in

yeast, plants and mammalian cell lines. However, no systematic study of the potential of Cas13 has been carried out in an animal system. Here, we show that CRISPR-RfxCas13d is an effective and precise system to deplete specific mRNA transcripts in zebrafish embryos. We demonstrate that zygotically-expressed and maternally-provided transcripts are efficiently targeted, resulting in a 75% average decrease in transcript levels and the recapitulation of well-known embryonic phenotypes. Moreover, we show that this system can be used in medaka, killifish and mouse embryos. Altogether our results demonstrate that CRISPR-RfxCas13d is an efficient knock-down platform to interrogate gene function in animal embryos.

30. Understanding the features of highly diverged Wtf proteins to elucidate their mechanism of action
Sam Campbell
Stowers Institute for Biomedical Research

Meiotic drivers are selfish genes that unfairly influence gametogenesis to increase their transmission into the offspring. The wtf meiotic driver was first discovered in the recently diverged fission yeast species *Schizosaccharomyces pombe* and *S. kambucha*. Since then, wtf drivers have been identified across diverse fission yeast species including *S. octosporus*, *S. osmophilus*, and *S. cryophilus*. These gamete-killing *wtf* genes drive by encoding both a poison protein and an antidote protein. The Wtfpoison targets all fission yeast spores during gametogenesis, while only the spores carrying the wtf+ allele are rescued by the Wtfantidote. First, the Wtfpoison forms punctae throughout the ascus. The Wtfantidote then colocalizes with the Wtfpoison in wtf+ spores. The meiotic driver is maintained in the population as only wtf+ gametes survive. In this work, we aim to compare features of other highly diverged Wtf proteins to identify key properties that are relevant to their function. The results of this work will elucidate if the highly diverged wtf genes function similarly, and further the understanding of the mechanism of wtf drive in fission yeast.

31. The *tom-1/tomosyn* locus encodes different isoforms with opposing roles in growth cone protrusion
Snehal Mahadik
Molecular Biosciences, University of Kansas

Previous work from the Lundquist lab showed that the UNC-6/Netrin receptors UNC-40 and UNC-5 regulate growth cone protrusion. UNC-40 stimulates protrusion whereas UNC-5 inhibits protrusion, and asymmetric distribution of protrusive activity across the growth cone results in directed growth cone migration away from UNC-6/Netrin (the Polarity/Protrusion model). *unc-5* mutant VD growth cones display unpolarized and excessive protrusion. To explore the role of vesicle fusion in growth cone protrusion, we analyzed *tom-1/tomosyn* mutants. Tomosyn normally occludes formation of the SNARE complex by interacting with and inhibiting *syntaxin-1*. VD growth cones of tom-1 null mutants were similar to wild-type. However, tom-1 null mutants suppressed the effects of constitutively-activated MYR::UNC-5, which alone causes small growth cones with little protrusion. This suggests that TOM-1 is

normally required for the inhibitory effects of MYR::UNC-5 on growth cone protrusion. Mutations specifically affecting tom-1 long isoforms showed small and non-protrusive growth cones, and did not suppress MYR::UNC-5. This suggests that TOM-1 short and long isoforms might have opposing roles, with TOM-1 short normally inhibiting protrusion, and TOM-1 long stimulating protrusion. A short isoform specific mutation was generated by CRISPR/Cas9 genome editing, and suppressed MYR::UNC-5, consistent with this idea. Similarly, *tom-1* null and short isoform specific mutation did not suppress protrusive phenotype of *unc-5* mutants, but mutation specifically affecting tom-1 long isoform did. This is consistent with differential role of two isoforms of TOM-1. Finally, transgenic expression of full-length tom-1 short(+) in tom-1 null background resulted in small and non-protrusive growth cones, consistent with a role of TOM-1 short in inhibiting protrusion downstream of UNC-5. In sum, these studies show that the genomic organization of the *tom-1* locus produces isoforms with opposing roles in growth cone protrusion. It is possible that the long isoform inhibits the activity of the short isoform in a potential autoregulatory manner.

32. Annotation of isomiR dynamics across the *C. elegans* developmental stages

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microRNAs (miRNAs) are short, ~22 nucleotides, small RNAs that repress genes through translational repression and mRNA destabilization. During canonical miRNA biogenesis, several miRNA species, or isoforms, known as isomiRs, are produced from a single precursor miRNA. Templated isomiRs are generated through Drosha or Dicer cleavage at alternate positions on either the primary or the precursor miRNAs, generating truncated or extended 5' or 3' miRNA ends. Because mature miRNA sequence modifications can alter their gene target repertoire, we wished to investigate the extent of isomiR prevalence and dynamics across *C. elegans* developmental stages. We performed small RNAseq from staged animals to assess isomiR variability across developmental stages. Using isomiR-SEA (Urgese et al 2016) for isomiR identification and quantification, we provide an isomiR profiling map against the miRBase annotated miRNAs at all stages of *C. elegans* development. We found that many miRNAs display isomiR level variability at different developmental stages, suggesting that the functional specificity of isomiRs to the developmental stage may exist. Not surprisingly, 3' end miRNA alterations were more frequent than the potentially seed-altering 5' end extensions or truncations. Some miRNA loci produced templated isomiRs that were just as, or more abundant than their annotated canonical mature miRNAs. These isomiRs included those with 5' end truncations and extensions, predicted to target new, potentially distinct sets of genes. Overall, we will present annotation of isomiR dynamics across *C. elegans* developmental stages, which we hope can provide us with insights into miRNA biogenesis and the intriguing potential of isomiR function.

33. Incorporating bioinformatics and genomics in undergraduate curriculum
Anuradha Ghosh
Department of Biology, Pittsburg State University

This poster is a reflection on the efforts made to incorporate advanced topics in undergraduate curriculum over a span of 5 years. The goal was to engage undergraduate students in scientific discovery using modern tools of genomics and bioinformatics. The specific experience involved isolating and culturing of microbes from various sources and their characterization by whole genome sequencing. This process engaged the students in core principles of microbiology, genetics, and cell biology while providing a substantial introduction to core skills in bioinformatics. This talk also elaborates on how graduate research was benefited from this new curriculum component.

34. Oral administration of water extract from *Euglena gracilis* alters the intestinal microbiota and metabolites and prevents lung carcinoma growth in mice
Deepa Upreti
Anatomy and Physiology, Kansas State University

Euglena gracilis, a single-celled alga used as a nutritional dietary supplement, possesses a broad range of medicinal properties including anticancer activity against a few types of cancers. The antitumor effects of a partially purified water extract from *Euglena gracilis* (EWE) and EWE treated by boiling (bEWE) were evaluated using orthotopic lung cancer syngeneic mouse models with Lewis lung carcinoma (LLC) cells. Daily oral administration of either EWE or bEWE started three weeks prior to the inoculation of LLC cells significantly attenuated tumor growth. The intestinal microbiota compositions in both extract-treated groups were more diverse than that in the PBS group. Fecal microbiota transplantation using bEWE-treated mouse feces attenuated tumor growth to an extent equivalent to bEWE treatment. Further, *Euglena* water extract's anti-cancer properties and effects on the gut metabolic landscape were investigated in a tobacco smoke carcinogen: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung carcinoma mouse model. Oral administration of bEWE 2 weeks prior (pre-bEWE) or 10 weeks post (post-bEWE) NNK injection greatly attenuated NNK-induced tumorigenesis in the mouse lungs. A clear difference in fecal metabolites between the PBS and bEWE treated groups was observed. High-throughput metabolic profiling identified succinate, malate, triethanolamine, acetylserine, glyceric acid, aspartic acid, glutamic acid, and threonine, which can be metabolized to short-chain fatty acids (SCFAs), were increased with the bEWE treatment. Furthermore, a significant increase in SCFAs, such as acetic acid, butyric acid, and propionic acid, was observed in the feces collected from either pre- or post-bEWE treated mice. Moreover, treatment with SCFAs significantly suppressed the proliferation of both human and murine lung cancer cell lines in cell culture via induction of apoptosis. Our studies strongly suggest that daily oral administration of partially purified water extracts from *Euglena gracilis* attenuates lung carcinoma growth via the alteration of the intestinal microbiota and altering the gut metabolites to increase SCFA production.

35. A telomere-associated system of paramutation in *Drosophila virilis* mediated by maternally provisioned piRNAs

Ana Dorador
Department of Ecology and Evolutionary Biology, University of Kansas

Paramutation is the phenomenon by which a silent alle can turn off a normal allele in trans in an epigenetic manner. The silenced state of the wildtype allele can persist through generations even in the absence of the original paramutagenic allele. The mechanisms underlying paramutation are poorly understood. Further, little is known about how paramutation shapes gene expression under natural conditions. In this study, we investigate a system of genic paramutation in *Drosophila virilis*. Previous studies have shown that maternally transmitted piRNAs that target the center divider (*cdi*) gene in *D. virilis* have the capacity to silence expression of *cdi* in the next generation. In addition, it has been shown that piRNAs that target cdi can be maintained in subsequent generations in the absence of the original silencing allele. However, it is not known whether this pattern of piRNA biogenesis and maternal transmission coincides with epigenetic repression of cdi expression across multiple generations. To determine if cdi piRNA biogenesis mediates paramutation, we measured the expression of cdi in the ovaries of females heterozygous for the silencing allele, as well as their daughters that lack the silencing allele. In two independent experiments, cdi expression was quantified in 20 F1 heterozygous mothers and 20 first-generation backcross daughters, lacking the original silent allele, using RT-qPCR. Confirming previous studies, we found that heterozygous females that maternally inherited the piRNA producing allele had low expression of cdi in ovaries. We further found that the first-generation backcross daughters - lacking the paramutagenic allele – had significantly lower cdi expression in the ovaries compared to a genotypically identical strain with no piRNAs mapping to cdi. This study thus describes a new system of paramuation in gene expression of *Drosophila virilis,* which can serve as a baseline for future studies that seek to understand how paramutation is regulated.

Index of presenters in alphabetical order

Presenter	Speaker Session/ Poster number	Page
Romeiser, Scott	Poster 4	21
Sykora, Lydia	Poster 10	24
Tareq, Tokee	Session 4	15
Treichel, AJ	Poster 14	27
Tso, Felicity	Poster 22	32
Upreti, Deepa	Poster 34	38
Vuong, Helen E	Session 4	18
Walters, James	Poster 2	19
Wong, J. Rene	Poster 7	23
Yellapu, Nada Kumar	Session 2	11
Zhu, Ziyu	Poster 3	20

About the KU Genomics Center

Established in August 2021, KU's Center for Genomics (KUCG) capitalizes on the ongoing COBRE Center for Molecular Analysis of Disease Pathways, an NIH-funded program, which founded and supports the KU Genome Sequencing Core and focuses on disease-related research. The KUCG seeks to develop an integrated community of scientists at KU that includes both biomedical investigators and those using genomics to study fundamental biological questions of development, behavior, evolution, and ecology. Members of the KUCG will have access to COBRE CMADP resources through the Genome Sequencing Core and other campus core facilities.

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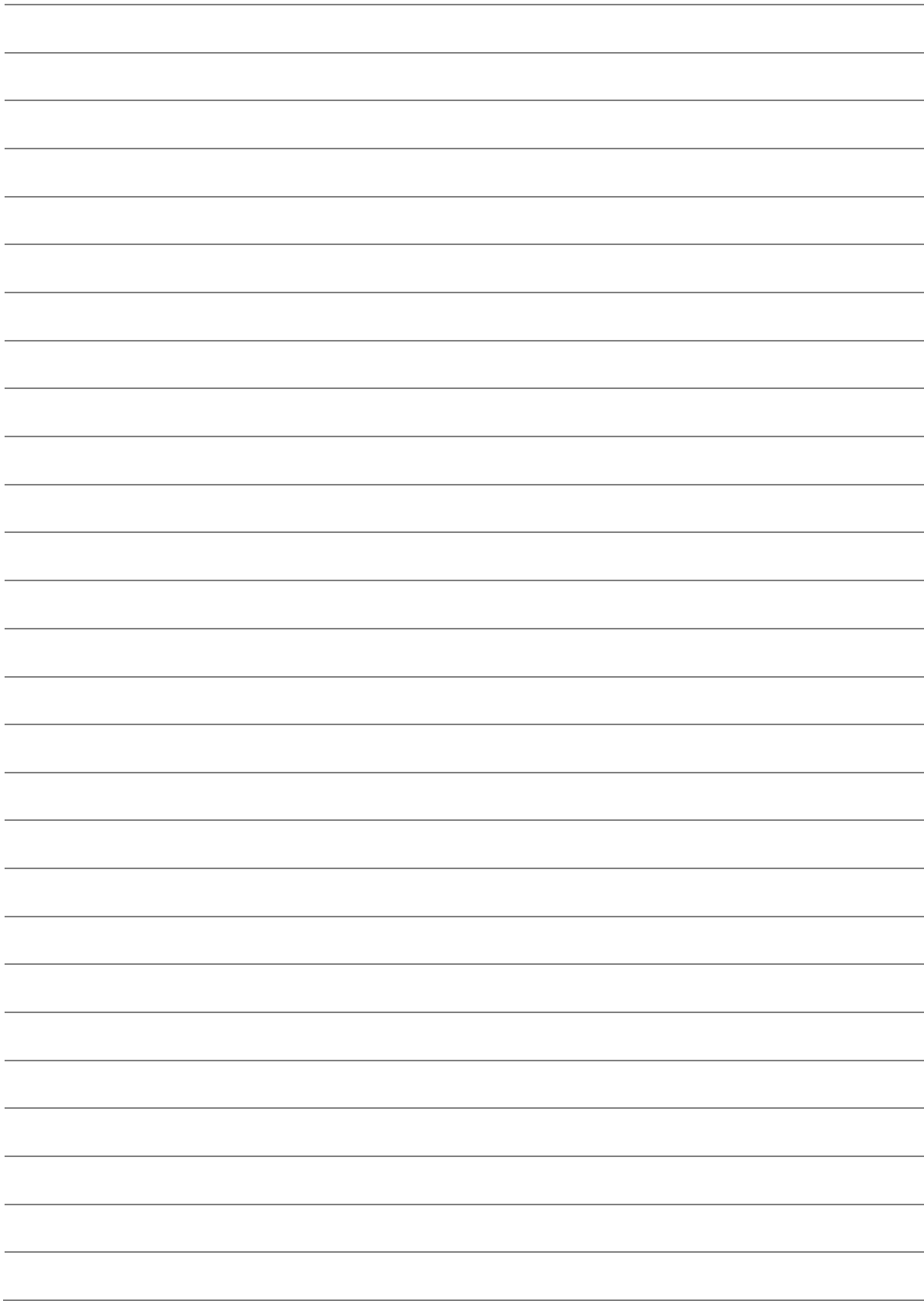Department of
Anthropology, KU

Lauren Norman
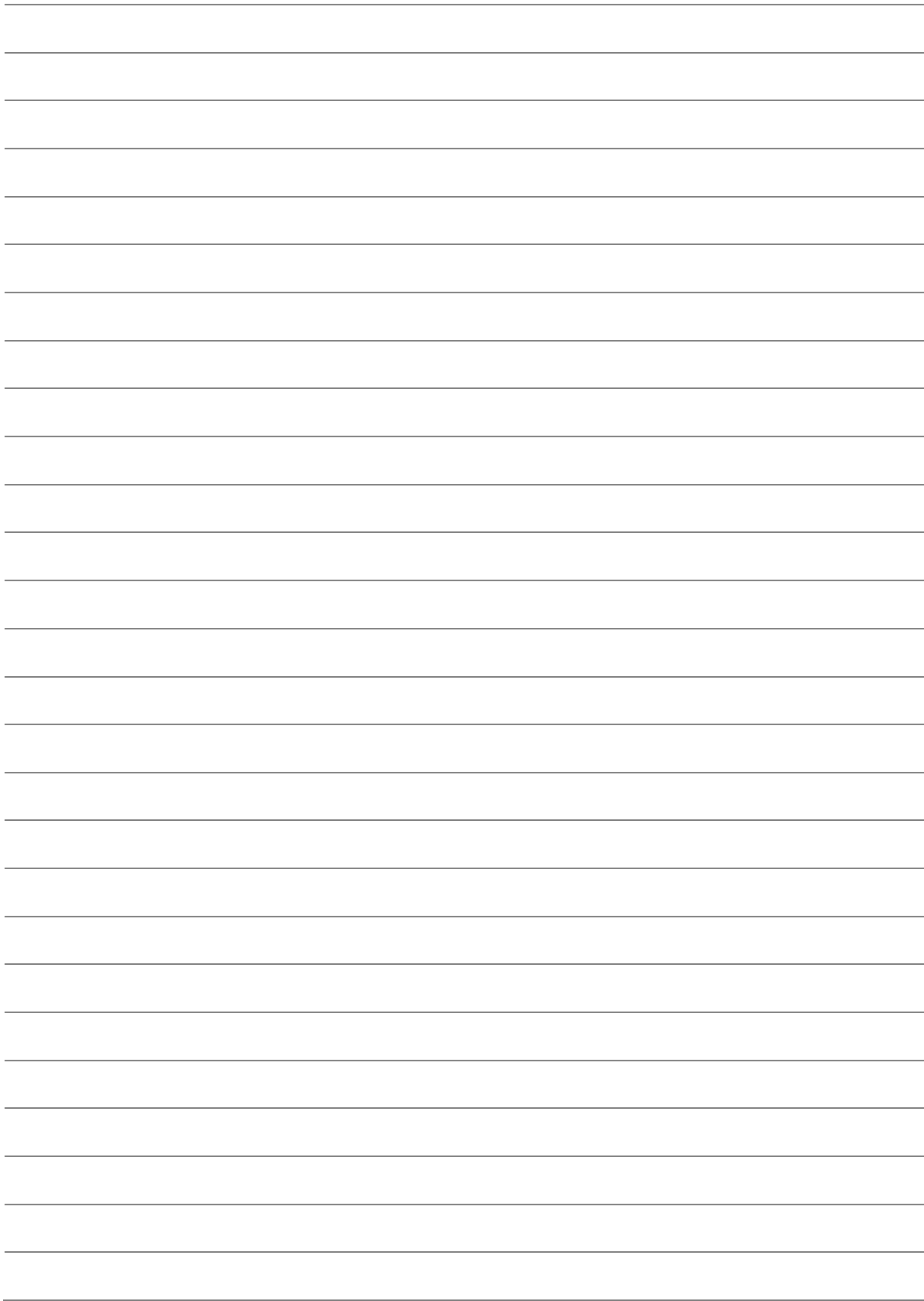Visiting Assistant Professor
Department of
Anthropology, KU

Nicole Ginnan
Postdoctoral Researcher
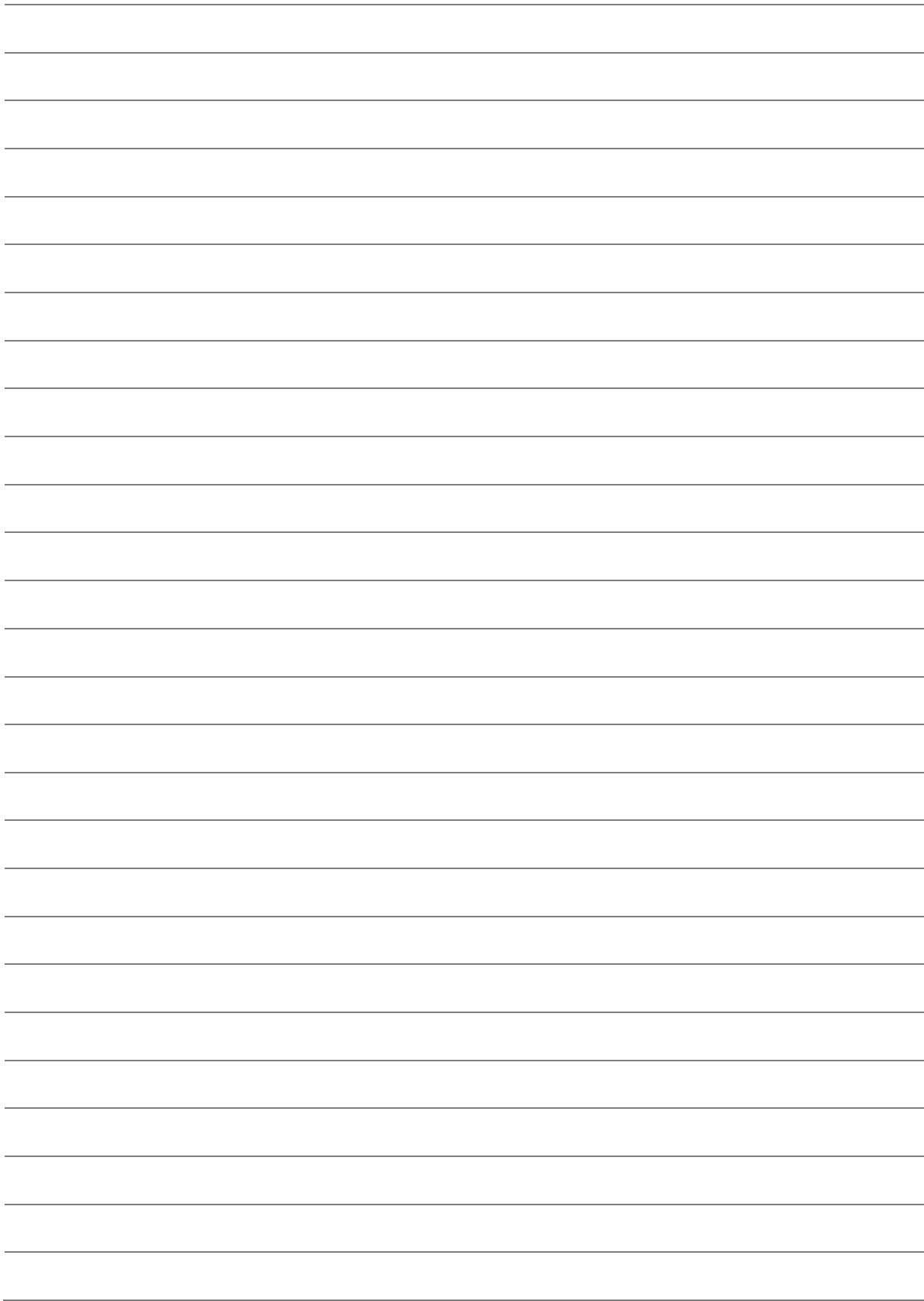Ecology and Evolutionary
Biology, KU

Patrick Lansdon
Postdoctoral Researcher
Molecular Biosciences, KU

Wen-Juan Ma
Research Associate
Associate
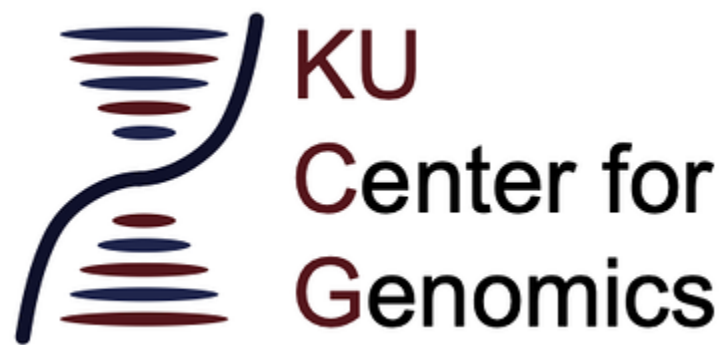Molecular Biosciences, KU

Notes Pages:

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