2026 Fly Club Symposium Program

Monday, May 4. UConn Storrs, Engineering and Science Building (ESB) Room 121

Schedule

10:30 am - 10:50 am	Arrival and registration; Poster setup
10:50 am - 11:00 am	Opening remarks
11:00 am - 12:00 pm	**Keynote lecture: Dr. Harmit Malik** **Genetic conflicts during meiosis drive the rapid evolution of essential chromatin proteins**
12:00 pm - 1:00 pm	Lunch mixer
1:00 pm - 2:00 pm	Poster presentation
2:00 pm - 2:15 pm	**Sophie Bizink, Inaba lab** Investigating Sperm Quality Control Mechanisms in *Drosophila melanogaster*
2:15 pm - 2:30 pm	**Natalie Aloisio, Sun lab** ILP8 serves as a mature follicle sensor to prevent excessive accumulation of mature follicles in *Drosophila* ovaries and oocyte aging

2:30 pm - 2:45 pm	**Teng Long, Menuz lab** Identifying the Molecular Components that Generate the Transepithelial Potential in Insect Sensilla
2:45 pm - 3:00 pm	Break
3:00 pm - 3:15 pm	**Natalie Warsinger-Pepe, Hanlon lab** Exploring the underpinnings of B chromosome drag through the *Drosophila melanogaster* paternal lineage
3:15 pm - 3:30 pm	**Ruiyi Sun, Mellone lab** Testing the centromere drive hypothesis in *Drosophila melanogaster*
3:30 pm - 3:45 pm	**Akshada Shankar Ganesh, Erceg lab** The functional organization of chromosome territories in single nuclei during zygotic genome activation
3:45 pm - 4:00 pm	Poster awards and closing remarks

Abstracts: Short Talks

1. Sophie Bizink, Mayu Inaba Lab

Investigating Sperm Quality Control Mechanisms in Drosophila melanogaster
Sperm development in Drosophila melanogaster is a highly organized process essential for male fertility. Previous work from our lab has shown Bone Morphogenetic Protein (BMP) signaling from somatic cyst cells to germ cells as a key pathway during late spermatogenesis. BMP ligands such as Glass bottom boat (Gbb) activate receptors like Saxophone (Sax) in germ cells to promote elongation and differentiation of spermatids. When Sax is disrupted, sperm bundles become disorganized and mitochondria develop abnormal morphology, producing the sperm scattering phenotype. Although BMP signaling through Sax is required to maintain spermatid structure, it remains unclear how defects in mitochondrial or meiotic defects cause sperm scattering and lead to cyst disorganization. Live imaging of Sax RNAi testes suggests that sperm scattering may also result from separation between nuclei and axonemes at the basal body region. Centrosomes that are normally anchored to the nuclei start to drift away as elongation progresses. This separation causes the spermatid heads and tails to lose alignment, and the cyst begins to lose its structure. The defect becomes clear at the canoe and leaf stages, when many nuclei shift toward the apical side and the cyst starts to break apart. Mitochondria in these cysts also appear enlarged and lose their normal shape. These observations suggest that scattering begins when centrosome anchoring fails, disrupting the link between nuclei, mitochondria, and axonemes. To test this directly, bundled and scattered spermatids will be examined ultrastructurally using electron microscopy. This approach enables high-resolution comparison of internal architecture and will determine whether scattered spermatids exhibit defects in centrosome–nucleus attachment, axoneme organization, mitochondrial derivative structure, or other subcellular features not resolved by light microscopy. By comparing spermatids within the same developmental window, this analysis will identify which ultrastructural abnormalities are associated with the onset of scattering. Together, my project seeks to identify how sperm structure is maintained during reproductive checkpoints and how defects in this process contribute to infertility.

2. Teng Long, Karen Menuz Lab

Identifying the Molecular Components that Generate the Transepithelial Potential in Insect Sensilla
Insects rely on olfaction to navigate their environment, find food, locate mates, and avoid predators. Odorants are detected by sensory neurons housed in olfactory sensilla, the hair-like protrusions on the antenna. The olfactory sensilla share a similar structure with other types of sensilla that serve various sensory functions including taste, mechanosensation and hygrosensation. A key feature across all sensilla is a voltage difference between the sensillar lymph and hemolymph, called the transepithelial potential (TEP), with the sensillar lymph being more positive. This potential is thought to drive cations into sensory neurons when receptors are activated, initiating neuronal firing. The TEP may stem from high potassium concentrations in the sensillar lymph relative to the hemolymph, and is likely generated by support cells transporting potassium. However, the molecular basis of TEP generation remains entirely unknown. Here, we use the Drosophila olfactory system to characterize the TEP and investigate its role in odor detection. Using single sensillum recordings, we demonstrate that the TEP in large basiconic olfactory sensilla is consistent across sensillum functional subclasses, sexes, and ages. We went on to use an RNAi screen to identify candidate genes involved in TEP generation. Finally, we demonstrate that loss of the TEP in support cells reduces odor-evoked neuronal responses, confirming that the TEP plays a critical role in insect sensillar neurophysiology.

3. Natalie Aloisio, Jianjun Sun Lab

ILP8 serves as a mature follicle sensor to prevent excessive accumulation of mature follicles in Drosophila ovaries and oocyte aging

Excessive mature follicle accumulation in ovaries harms oocyte health and offspring viability while also wasting maternal energy. Therefore, the number of mature follicles in the ovary is tightly controlled. In Drosophila, each ovary is comprised of ~16 ovarioles, each typically containing 1-2 mature follicles, regardless of the female's mating status. However, the mechanism by which females count mature follicles in their ovaries remains a mystery. Recent work demonstrated that Drosophila insulin-like peptide 8 (ILP8) is expressed in mature follicle cells but not in younger follicles. Here, we found that ILP8 is not essential for mating-induced ovulation but plays an essential role in inducing ovulation and preventing excessive accumulation of mature follicles in virgin females. Excessive accumulation of mature follicles in ilp8-knockdown females leads to reduced oocyte quality. Additionally, knockdown of Lgr3 (Leucine-rich repeat-containing G protein-coupled receptor 3), encoding a previously identified ILP8 receptor, globally or in all neurons, resulted in similar ovulation and egg laying defects, accumulation of mature follicles, and poor oocyte quality in virgin females. In contrast, Lgr3 knockdown specifically in octopaminergic neurons that innervate the ovaries and reproductive tract did not impair ovulation/egg laying, indicating that ILP8-Lgr3 signaling acts through non-octopaminergic neurons to regulate virgin ovulation. Using an enhancer screening, I will demonstrate the specific Lgr3+ neurons involved in this process. Together, our findings indicate that ILP8 functions as a mature follicle sensor to prevent excessive accumulation of mature follicles and maintain oocyte quality likely through neuronal the Lgr3 receptor in virgin females. Our findings suggest that ILP8/Lgr3 is likely essential for maintaining the optimal reproductive fitness of virgin females and for species survival in the wild, where mating success is not guaranteed.

4. Natalie Warsinger-Pepe, Stacey Hanlon Lab

Exploring the underpinnings of B chromosome drag through the Drosophila melanogaster paternal lineage

B chromosomes are non-essential chromosomes that can exhibit drive (>50% transmission frequency) to be maintained in a population. The B chromosomes in a matrimony heterozygous mutant stock (mtrm126/TM3) of Drosophila melanogaster are subject to drive through females. Conversely, paternal B chromosome transmission in the mtrm126/TM3 stock was found to result in drag, where B chromosomes were transmitted at a lower than Mendelian frequency (<50%). In this study, we explored the underpinnings of B chromosome drag through the paternal lineage in D. melanogaster by testing the hypothesis that paternal drag is due to abnormalities in the germline of mtrm126/TM3 males. We found no difference in total progeny sired by mtrm126/TM3 males compared to control males in a standard fertility assay, suggesting that infertility is not a factor impacting B chromosome drag. Interestingly, sperm exhaustion assays revealed significantly higher total progeny sired by young mtrm126/TM3 males (2-4 days old) compared to controls. In concordance with this finding, we discovered that young mtrm126/TM3 males have more germline stem cells (GSCs) than control males, suggesting that the increase in progeny sired by mtrm126/TM3 males may be due to an increase in GSCs resulting in an increase in total sperm produced.

The sperm exhaustion assay also revealed two populations of mtrm126/TM3 males: as the males aged (11-15 days old), the fertility of some males dropped earlier than others revealing "low fertility" and "high fertility" aged males. Interestingly, low-fertility aged males sired progeny that carried a higher copy number of B chromosomes compared to the progeny of the high fertility aged males. Whether this negative correlation between paternal fertility and B chromosome copy number in the progeny is due to differences in paternal B chromosome copy number or variation in B chromosome transmission is unknown. Regardless of mechanism, it is intriguing to speculate that improper GSC regulation may be the source of B chromosome variation. As GSCs are believed to have a replicative lifespan, GSCs with higher numbers of B chromosomes may be negatively impacted as males age (resulting in lower fertility over time) compared to GSCs with lower numbers of B chromosomes (resulting in relatively higher fertility over time). Future studies are underway to test

whether B chromosome copy number impacts replicative aging of male GSCs and whether this phenomenon is a source of B chromosome drag.

5. Ruiyi Sun, Barbara Mellone Lab

Testing the centromere drive hypothesis in Drosophila melanogaster
Centromeres are essential for accurate chromosome segregation during cell division, yet both centromeric DNA and associated proteins evolve rapidly. The centromere drive hypothesis proposes that this paradox arises from a genetic conflict whereby expansion of centromeric DNA promotes the assembly of larger kinetochores, biasing chromosome segregation during female meiosis, while compensatory evolution of centromeric proteins acts to suppress this bias. Here, I tested whether variation in centromeric DNA composition and kinetochore protein levels can generate segregation bias in *Drosophila melanogaster*. Using hybrid FM7a/WT and FM7a/T(1;Y) backgrounds, I quantified parental contributions of the kinetochore protein CENP-C in the germarium and assessed chromosome segregation outcomes. Although some of these genotypes exhibit asymmetric kinetochores by immunofluorescence, I did not detect biased transmission associated with increased kinetochore signals, nor increased nondisjunction as a fitness cost. However, I did observe that T(1;Y) lines show a slightly lower segregation compared with wild type X. Together, these results suggest that kinetochore asymmetry alone is insufficient to drive biased segregation in this context. While there are several limitations associated with my system, my findings support nearly mendelian segregation in spite of kinetochore differences in *D. melanogaster*.

6. Akshada Shankar Ganesh, Jelena Erceg Lab

The functional organization of chromosome territories in single nuclei during zygotic genome activation
Chromosome territories (CTs) are intricately organized and regulated within the nucleus. Despite remarkable advances in our understanding of genome packaging and gene expression, the interplay among CTs, pairing of parental homologous chromosomes, and genome function during development remains elusive. Here, we employ an Oligopaints-based high-resolution imaging approach to examine variable CT organization in single nuclei during the developmental process of zygotic genome activation. We reveal large-scale chromosome packaging differences and high levels of homolog pairing at the whole-chromosome scale that decreases locally due to spatial variability in chromosome conformations. In the absence of one homolog copy, the dynamics of CT compaction and RNA polymerase II recruitment are associated with the transcriptional changes in haploid embryos. Finally, global inhibition of transcription results in decreased CT opening and no significant impact on CT pairing levels. These findings enhance our understanding of variable parental genome folding and regulation during development, which may inform strategies for chromosome-based diseases.

Abstracts: Posters

1. Shania Kalladanthyil, Stacey Hanlon Lab

Establishing the landscape of the D. melanogaster B chromosome centromere

Most models of karyotype evolution are inferred from comparative genomics of chromosomal rearrangements between species separated by millions of years of evolution, obscuring the context of how chromosomes change. Chromosome fusion is a structural rearrangement that plays a major role in shaping karyotypes but can lead to the formation of dicentric chromosomes that are prone to chromosome instability. Evidence of the successful stabilization and maintenance of dicentric chromosomes exists in nature, but research into the mechanism(s) behind dicentric chromosome stabilization is still ongoing. The supernumerary, non-essential B chromosomes that arose less than 25 years ago in Drosophila melanogaster are a newly established model system that has the potential to provide valuable insights into how new chromosomes form and stabilize. Our current model predicts that the B chromosomes arose from a fusion of two centric fragments that originated from chromosome 4, leading to a dicentric chromosome that is not prone to chromosome instability. To test this model, we performed fluorescent in situ hybridization (FISH) on metaphase chromosome spreads using probes that bind specifically to the chromosome 4 centromeric island Lampedusa. Our preliminary data indicates that Lampedusa is enriched on the B chromosomes compared to chromosome 4, which is consistent with our model. We are currently assembling a map of the B chromosome to give us further resolution about the structure of this genetically dicentric chromosome as well as further resolution fusion point and overall structure of this region. Together, this work will set up a foundation for using the B chromosomes as a model system for the empirical study of de novo chromosome formation and dicentric chromosome stabilization, ultimately leading to a deeper understanding of how new chromosomes arise and how chromosome stability is maintained after fusion.

2. Violet Xiao, Jianjun Sun Lab

Investigating mating-induced ovulation circuit in Drosophila melanogaster

Egg laying in female Drosophila melanogaster is controlled by a distributed neural circuit integrating mating status, central decision neurons, and peripheral motor execution. Previous work has identified a SPSN-SAG-pC1-oviDN/oviEN neuronal circuit that mediates mating-induced oviposition; however, it is unclear whether the same neuronal circuit regulates mating-induced ovulation. To address this question, we used the previously published tools to activate pC1 neurons, which were previously shown to reduce the egg-laying capacity of mated females. In contrast, we did not observe an obvious egg-laying defect as previously reported. We went on to activate SAG and SPSN neurons to test whether this can replicate the previous report. Unfortunately, activation of SAG and SPSN neurons does not affect mating-induced egg laying in our experimental setting. Furthermore, we went on to knock down sex peptide receptor (SPR), a receptor in SPSN neurons for relaying the mating signal to post-mating behavior response, in all neurons or globally. We still did not observe any reduction in egg laying after mating. We are seeking help to explain our inability to reproduce the reported phenotypes. Nonetheless, we were able to reproduce the phenotype that inactivation of oviENs using tetanus toxin (TNT) resulted in a significant reduction in egg laying. We went on to carry out the ovulation assay by measuring the ovulation time. We found that oviEN-inactive females have normal ovulation. The defective egg laying is due to the oviposition defect. Therefore, oviENs do not involved in mating-induced ovulation. Future work is required to figure out the entire circuit for mating-induced ovulation.

3. Sydney Ballou, Karen Menuz Lab

Role of adult antennal support cells in Drosophila olfaction

Insects utilize their antennal olfactory system to detect numerous environmental odorant stimuli to aid in behaviors such as identifying food sources, host seeking, and mating. While Olfactory Receptor Neurons (ORNs) and the odors which they respond to are extensively studied, there is much less known about another

major class of antennal cells, the support cells. The roles of support cells are defined during development, but in the adult antenna their function remains elusive. It's hypothesized that support cells might metabolize odors, since many putative Odorant Degrading Enzymes (ODEs) are found expressed in the antennal transcriptome. One abundant class of these putative ODEs are the Cytochrome P450 (Cyp) gene superfamily. Of the approximately 87 Cyps expressed in the Drosophila melanogaster genome, ~57 are detected in the antennal transcriptome at significant levels. However, the contribution of antennal Cyps to odorant detection and their localization within the antenna is poorly understood. Here, we selected thirteen antennal Cyps to investigate, including those most highly expressed and those with antennal-enriched expression compared to other tissues. Using newly generated transgenic LexA lines, immunohistochemical analysis revealed that most of the Cyps are broadly expressed across the antenna, whereas a few are more narrowly expressed. Comparison with the neuronal marker, elav, revealed that one Cyp is expressed in olfactory neurons, whereas the majority are found partially expressed in one or more support cell types of the antenna. Additionally, very few functional studies have been done to investigate Cyps role as ODEs. Here, we identify one Cyp, Cyp313a4, that is uniquely expressed in the support cells of a specific class of ORNs. Since the antennal ORNs odorant response profiles have been very well-studied, it makes it straightforward to test if Cyp313a4 has an effect on those particular odorant responses. Ongoing work is testing a Cyp313a4 null mutant for any differences in odorant responses and kinetics in comparison to wildtype responses. Together, this work will be the first map of Cyp expression in the Drosophila antenna and will serve as a roadmap for future studies on their functional roles in odorant degradation.

4. Daniel D'Souza, Barbara Mellone Lab

The RHINO R-loop Sensor Causes Mitotic Errors in Drosophila melanogaster
Centromeres are essential chromosomal loci that mediate faithful chromosome segregation during mitosis through the recruitment of the kinetochore and microtubules. The centromere is epigenetically defined by the histone H3 variant CENP-A, and disruption of centromere integrity may lead to aneuploidy and cell death. Studies in human cells have shown that important regulators of centromere structure and function are R-loops. These co-transcriptional nucleic acid structures form when nascent RNA hybridizes with template DNA and can be enzymatically resolved by the ribonuclease RNase H1. They have been shown to help facilitate recruitment of centromere proteins and activate the ATR-CHK1-Aurora B signaling pathway required for proper chromosome segregation. However, whether R-loops play a conserved role in Drosophila melanogaster centromere biology remains unclear. To investigate R-loop dynamics at Drosophila centromeres, we employed RHINO, a genetically encoded R-loop sensor comprising three catalytically dead RNase H1 domains that bind R-loops with high specificity. Validation by immunofluorescence and DNA FISH confirmed RHINO enrichment at the 18S rDNA locus, a well-characterized site of R-loop formation. Since RHINO lacks catalytic activity, it is predicted to block endogenous R-loop resolution, analogous to catalytically dead RNase H1 overexpression. Expression of RHINO in larval brain neuroblasts produced striking mitotic phenotypes including anaphase bridges, chromosome breakages, and aneuploidy. RHINO signal was absent from all mitotic spreads, suggesting R-loop clearance occurs prior to mitotic entry. These findings suggest that proper R-loop resolution is required for faithful chromosome segregation in Drosophila, and that unresolved R-loops cause genomic instability. Future experiments will employ live imaging and Topoisomerase II RNAi to further characterize the spatiotemporal regulation of R-loops during the cell cycle.

5. Aadya Deshmukh, Geoffrey Tanner Lab

Ketone Body Supplementation as a Possible Metabolic Intervention for Post-TBI Circadian Disruption in Drosophila Females
Traumatic brain injuries (TBIs) are characterized by an injury to the brain caused by an external force, such as a blow to the head or a violent impact on the body. Repeated TBI, especially concussions or mild traumatic brain injuries occur in many populations such as those involved in contact sports, military veterans, and victims of

domestic abuse. Such repeated TBIs often lead to the development of chronic traumatic encephalopathy, a neurodegenerative disorder associated with cognitive decline and sleep disturbances, particularly disturbances to circadian rhythm, the body's internal sleep and wake cycle. We investigated the relationship between metabolism and post-TBI circadian disruption using Drosophila melanogaster. We fed flies a diet supplemented with ketone bodies (KBs), circulating metabolites produced during periods of fasting, or on a ketogenic diet (KD). The KD is known for promoting therapeutic effects in disorders such as drug-resistant epilepsy and pyruvate dehydrogenase complex deficiency. We investigated whether ketone body supplementation (KBS) may alleviate or "rescue" the effects of TBI in Drosophila. Virgin female flies were raised on either a standard high carbohydrate (SHC) or KBS diet and subjected to TBI or sham intervention using a High-Impact Trauma device. Subsequently, 72 hours post-TBI, circadian rhythms were monitored for 72 hours using automated Drosophila Activity Monitoring Systems (DAMs), which track individual fly locomotion. Previous findings showed greater circadian disruption in SHC-fed virgin females compared to mated females following TBI, motivating a focus on virgin females in this study. We aim to determine whether KBS supplementation alleviates TBI-induced circadian disruption and to better understand the metabolic mechanisms underlying recovery. Additionally, this work may provide insight into how mating status influences physiological responses to TBI in Drosophila.

6. Kristine Zlotnick, Karen Menuz Lab

Markers of olfactory sensilla support cells in adult Drosophila
In the Drosophila antenna, olfactory sensilla are the fundamental functional unit of odor detection and processing. These sensilla house not only the well-studied Olfactory Receptor Neurons (ORNs), but also three distinct support cells: the thecogen, trichogen, and tormogen cells. Our understanding of the role of support cells in olfactory signaling has been limited by the lack of markers to distinguish these cells in adult flies. Genetic markers for these cell types have been identified for developmental studies, but their specificity and expression in the adult antenna remains poorly defined. Here, we systematically identified and validated tools to label the three support cells types as well as the epithelial cells found between sensilla. First, we confirmed previous suggestions that ASE5-GAL4 specifically and broadly labels tormogen cells, which express Su(H). In contrast, expression of nompA-GAL4, previously used as a thecogen cell marker, is strongly biased toward coeloconic sensilla and does not capture all thecogen cells. As an alternative, we identified prospero as a candidate thecogen marker using the Fly Cell Atlas. We found that an anti-prospero antibody widely labels thecogen cells, which are ASE5-negative. Because an adult trichogen cell marker has not been previously reported, we generated knock-in GAL4 lines for two candidate genes identified through a literature search. One line, atk-GAL4, broadly drives expression in cells that are distinct from those expressing ASE5 or prospero (which are also distinct from each other), supporting their identity as trichogen cells. Finally, we examined an Obp19d-GAL4 line based on prior reports that Obp19d is predominantly expressed in epithelial cells. Like the support cell markers, Obp19d-GAL4 widely labels non-neuronal cells. The Obp19d-positive cells are also distinct from support cells, confirming their epithelial identity. Together, our findings provide a validated framework for distinguishing antennal support cell classes in the adult fly, a crucial step for understanding their individual roles in olfactory sensilla.

7. Maddy O'Connor, Barbara Mellone Lab

Utilizing a new CRISPR technology to deplete centromere-derived transcripts in Drosophila melanogaster
The accurate segregation of chromosomes relies on the proper functioning of the centromere, the region of the chromosome where the kinetochore and microtubules attach during cell division. The centromere consists of a specialized chromatin domain containing histone H3 variant CENP-A, which recruits other essential centromeric proteins. If the centromere fails to assemble properly, this can cause detrimental chromosome segregation errors, leading to cell death and aneuploidy. The mechanism behind how the essential centromeric proteins (CENP-A, CENP-C, and CAL1) are recruited to the centromere has yet to be understood.

Studies in human cells suggest that centromere-derived transcripts interact with CENP-A and CENP-C, but whether this is conserved across species is unknown. Previous work from our lab revealed that in Drosophila melanogaster, the centromeres are made up of islands of retroelements flanked by simple satellite repeats. PRO-seq analyses showed that both islands and satellites are transcriptionally active while cytological localization revealed that, for the retroelement Jockey-3, the transcripts produced are retained in cis. Whether this localization has functional significance is unknown. Jockey-3 is the only transcribed element shared between all centromeres, while the varying satellites exhibiting transcriptional activity are chromosome-specific. Simple satellites comprise a substantial portion of both the centromeric and pericentric heterochromatin, yet the functional relevance of these transcripts is unknown. To elucidate how centromeric transcripts contribute to centromere integrity, maintenance, and function, I will use CRISPR-Csm, which specifically targets and degrades transcripts without affecting the underlying DNA, to degrade satellites and Jockey-3 transcripts and determine if loss of RNA has an impact on how the centromere forms and functions. I will assess the effects of transcript depletion on centromeric protein levels, missegregation events, and aneuploidy in different tissues to investigate effects on viability and fertility after transcript degradation. This work will provide key insights on the importance of centromeric transcripts on the overall integrity and function of centromeres through analyzing the consequence of their elimination.

8. Anna Lassota, Pedro Miura Lab

Compartmentalised alternative splicing of Dscam is conserved in Drosophila and honey bees
Alternative splicing generates molecular diversity from a limited number of genes. Drosophila Down Syndrome Cell Adhesion Molecule (Dscam) is the record holder producing 38,016 different proteins from one gene. Initial models proposed stochastic exon selection through RNA secondary structures. However, deleting the common base-pairing sequence does not alter splicing patterns, and no polar effect is observed. Since Dscam splicing adapts to generate isoforms with higher affinity for pathogens in mosquitoes and changes in honey bees after learning, exon selection seems regulated rather than stochastic. To test this, we used transgenic reporter lines with -1 nucleotide deletions to analyse variable exon inclusion in Drosophila. We found that exon inclusion was not entirely stochastic and showed a deterministic component in larval tissues. In larval photoreceptors repeated inclusion of the same exon variant across multiple ommatidia indicates cell-type-specific regulation. Moreover, splicing was not always productive in larval tissues, leading to suppressed expression. In honey bee mushroom bodies, RNA in situ hybridisation revealed compartmentalised patterns of variable exon inclusion in foraging bees, with inclusion levels and locations differing between individuals, suggesting experience-dependent modulation. These findings challenge a fully stochastic model and support a framework where Dscam alternative splicing integrates stochasticity and deterministic selection.

9. Roma Kale, Jianjun Sun Lab

Investigating the Role of Matrix Metalloproteinase 2 (MMP2) in Drosophila Border Cell Migration
Collective cell migration is a highly coordinated process essential for development and disease progression. The Drosophila melanogaster border cell (BC) cluster is an established in vivo model for studying these movements. While signaling pathways governing BC specification and migration are well understood, the role of extracellular matrix (ECM) remodeling via proteases like Matrix Metalloproteinase 2 (MMP2) remains to be fully elucidated. This study investigates whether MMP2, a membrane-tethered protease that cleaves ECM components, is required for the collective migration of the BC cluster at mid-oogenesis. Using two MMP2 reporter lines, we found that MMP2 is expressed in border cells after initiation of migration in Stage 9 and reaches the highest level of expression before the end of the migration in Stage 10B. We employed the Gal4/UAS system to knockdown MMP2 using two independent RNAi lines across different cluster cell types. Migration was quantified using a migration index (MI), comparing the cluster's progress to the mainbody follicle cells. We utilized three drivers, including c306-gal4 (expressed in border and polar cells), slbo-gal4 (expressed in border cells), and GR1-gal4 (expressed in all follicle cells). MMP2 knockdown using these drivers

yielded no significant migration defect at Stage 10A. Furthermore, validation of our RNAi lines using an MMP2::GFP fusion line confirmed successful knockdown of the protease despite the lack of a strong migratory phenotype. Our findings suggest that while MMP2 is dynamically expressed in the BC cluster, it may not play a primary role in regulating migration speed or completion. These results point toward potential functional redundancy between MMP family proteins or imply that MMP2's primary function may be reserved for later developmental stages, such as micropyle formation or follicle rupture. Ultimately, we seek to enhance our understanding of MMP2 and conserved MMP family proteins, contributing to a broader understanding of reproductive processes.

10. Alexandria Pacrin, Karen Menuz Lab

Role of jhamt in the chemosensory regulation of mating in Drosophila
Juvenile hormone (JH) is a sesquiterpenoid hormone known for its roles in controlling the development of insect larvae and in regulating reproduction in adult insects. More recently, global JH levels have been found to modulate olfactory receptor neuron (ORN) responses to pheromones in adult male Drosophila, resulting in higher success rates of older male flies when compared with younger flies in a mating assay (Lin et al., 2016). JH is known to be synthesized and secreted by the corpora allata (CA), an endocrine gland, but scRNASeq data from the FlyCellAtlas suggest that JH may be synthesized in the antenna due to its expression of jhamt, a key enzyme in JH synthesis. Here we demonstrate that JH is expressed in non-neuronal cells of the antenna, including those that enwrap pheromone-sensing ORNs to regulate mating behavior. Surprisingly, knockdown of jhamt expression specifically in these antennal cells leads to a decreased mating latency of male flies. We present a model that may explain this unexpected phenotype.

11. Emma Anderson, Barbara Mellone Lab

Identifying a kinetochore protein homolog in a non-traditional fly model
The fungus gnat, Sciara coprophila, is an emerging model system used for studying chromosome biology due to its unique patterns of chromosome elimination. Unlike most organisms that maintain strict genomic integrity, Sciara bypass canonical mechanisms ensuring DNA constancy, providing a rare opportunity to investigate non-Mendelian chromosome behavior. While oogenesis in females is typical, male meiosis is characterized by atypical divisions that produce a single functional sperm.
In males, the first meiotic division is defined by a monopolar spindle in which only maternal chromosomes are retained, while all paternal chromosomes are selectively eliminated in a bud. The mechanism behind the formation of the monopolar spindle and how chromosomes move along it remain unknown. In the second meiotic division, a bipolar spindle forms and sister chromatids segregate towards to opposite poles, except the X chromosome, which undergoes non-disjunction. This X-chromosome non-disjunction is governed by the controlling element (CE), a yet to be identified DNA region present on one of the X chromosomes. The resulting nullo X-product is eliminated in a bud, yielding a single sperm cell, consisting of maternal autosomes, 2 maternal X chromosomes, and 2 germline limited "L" chromosomes. The mechanism behind the CE and how it causes non-disjunction remains to be elucidated, although it has been hypothesized that the CE could act by inhibiting centromere function.
My work aims to define the centromere and kinetochore landscape during male meiosis in Sciara. To address this, I am identifying and characterizing putative centromere and kinetochore homologs in Sciara, which will be used for cytological analysis in testes chromosome spreads. Preliminary analyses have identified two putative kinetochore protein homologs of Ndc80 in Sciara. BLAST searches using Drosophila and mosquito Ndc80 sequences revealed two candidate homologs, which were further supported by sequence alignments and AlphaFold structural predications. A conserved region shared between the two Ndc80 candidates was used to generate an antibody for cytological analysis in testes chromosome spreads. Ongoing cytological analyses using this antibody indicates differential Ndc80 localization during male meiosis, however, whether this pattern is chromosome-specific or dependent on meiotic timing remains unresolved.

12. Suparna Dutta, Dr. Stacey Hanlon

Chromosomes in conflict: Uncovering how B chromosomes disrupt chromosome 4 segregation during female meiosis

The supernumerary B chromosomes recently discovered in Drosophila melanogaster are non-essential, heterochromatin-rich elements that are believed to have originated from chromosome 4. Although dispensable, B chromosomes disrupt normal female meiosis by inducing high levels of chromosome 4 missegregation. To investigate the relationship between B chromosome and chromosome 4, we genetically analyzed their co-segregation patterns during female meiosis. The transmission frequency of B chromosomes was assessed in progeny that inherited zero, one, or two copies of chromosome 4 from the female parent. We discovered an inverse relationship between chromosome 4 inheritance and B chromosome transmission: when both chromosome 4 homologs were inherited, the frequency of B chromosome transmission was low, and vice versa. This result indicates that the segregation of chromosome 4 and the B chromosomes are not independent from one another, suggesting that they may rely on similar mechanisms for their segregation during female meiosis. We then investigated if modulating the number of B chromosomes would affect chromosome 4 segregation during female meiosis by measuring chromosome 4 nondisjunction as a function of parental B chromosome copy number. Our results clearly demonstrate a positive correlation between B chromosome copy number and chromosome 4 missegregation, consistent with our previous result demonstrating these chromosomes do not segregate independently. We hypothesize that chromosome 4 and the B chromosomes are interacting during late oogenesis through the establishment of aberrant non-homologous heterochromatic threads. These threads normally arise between non-crossover chromosomes to establish a transient connection that provides the necessary tension for proper segregation in the first meiotic division. If multiple B chromosomes are present, they may potentially interfere with the thread formation between chromosome 4 homologs, leading to aberrant chromosome 4 segregation. To test this, we plan to examine the cytological interaction between B chromosomes and chromosome 4 by visualizing heterochromatic threads during late oogenesis. Overall, our research aims to enhance our understanding of how supernumerary, nonessential chromosomes influence the segregation of essential chromosomes during female meiosis.

13. Tyler McDermott, Barbara Mellone Lab

Centromeric targeting enables a retroelement to propagate and shape centromere evolution

Transposable elements—"jumping genes" that copy and paste themselves throughout the genome—face a fundamental challenge: how to spread without harming their host. Although typically deleterious due to insertional mutagenesis or genome instability, TEs can also act as powerful engines of genetic innovation, introducing new regulatory and structural variation. Centromeres, marked by chromatin containing the centromere-specific histone variant CENP-A, exhibit low recombination frequencies, tolerate sequence variation without loss of function, and are transcriptionally active. These characteristics make them attractive safe zones for transposable element insertion and propagation. Turnover between satellite repeats and TEs at centromeres is a major force driving of the rapid evolution of centromere DNA. The non-LTR retroelement *G2/Jockey-3 (Jockey-3)* shows strong centromere enrichment, with 60% of its copies localized to centromeric regions. It is conserved across all five centromeres of *D. melanogaster* and *D. simulans*, despite divergence in their centromeric satellite DNA. *Jockey-3* is also the most highly enriched repeat in CENP-A chromatin, and population analyses suggest that recent insertions preferentially occur within CENP-A-associated domains. However, the mechanisms by which this element recognizes, and targets centromeres remain unknown. To investigate this, we generated transgenic fly lines carrying an inducible full-length *Jockey-3* transgene (UASz-*eJockey-3*) with a unique barcode to track new insertions. Upon activation, *eJockey-3* induced DNA-damage foci and developmental defects, consistent with active transposition confirmed by digital droplet PCR analyses. Immunofluorescence staining revealed that *eJockey-3*–derived DNA damage occurs

preferentially at centromeres, with an over 30-fold enrichment over random expectation. By individually tagging the open reading frames of *eJockey-3*, we found that ORF1p, but not ORF2p, mediates nuclear import and centromeric localization. The bias of ORF1p to centromeres is supported by both immunofluorescence and CUT&Tag experiments. Remarkably, replacing the ORF1 nuclear localization signal (NLS) into ORF2 was sufficient to confer centromeric targeting, demonstrating that this short peptide is a driver of centromere bias. Centromeric localization of *eJockey-3* requires these ORFs to be translated in *cis* i.e., from the same transcript. Temporal analyses revealed that *eJockey-3* dissociates from chromosomes during mitosis and reforms centromeric foci in interphase, suggesting that its chromatin targeting is cell-cycle regulated. Together, our findings define how a mobile element biases the CENP-A chromatin for targeting to enhance its own spread, reshaping the sequence landscape and evolution of centromeres.

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