2008
Midwest Drosophila Conference

October 3 & 4
Allerton Park and Retreat Center
Monticello, Illinois
Meeting Schedule

Friday, October 3

12:30 - 1:40 Registration & Check-In (Allerton House)

Hang posters according to assigned number (pp. vi – viii).

1:45 Welcome (Allerton Library)

Cell & Developmental Biology (John Manak, moderator)
Allerton Library

1:50 – 2:20 Seth S. Blar, University of Wisconsin
Fat, Dachsous and Approximated – growth, patterning and polarity in the wing

2:20 – 2:40 Aishwarya Swaminathan, Wayne State University
The role of Drosophila SIN3 in wing development

2:40 – 3:00 Leonard Dobens, University of Missouri – Kansas City
Opposing interactions between Drosophila

3:00 – 3:20 Belinda Pinto, University of Iowa
Genetic analyses reveal tissue-specific contributions of the Drosophila LEM domain proteins in the nuclear lamina

3:20 – 3:40 Jing Chen, Miami University
Developmentally Regulated dADAR mRNA Isoforms in Drosophila: A Novel Role for a Truncated Isoform in rnp-4f 5'-UTR Intron Splicing Regulation

3:40 – 4:00 Scott Barolo, University of Michigan Medical School
Complex regulatory circuitry, structural rules, and a novel “Remote Control” element govern a Notch- and EGFR-regulated eye enhancer

4:00 – 4:30 Break (Solarium)
Neurobiology (Dan Eberl, moderator)
Allerton Library

4:30 – 5:00  Ravi Allada, Northwestern University
Sleep and Circadian Rhythms in Drosophila

5:00 – 5:20  Harald Vaessin, The Ohio State University
A Novel Role for Friend-of-echinoid (Fred) in Sensory Organ Precursor Formation

5:20 – 5:40  Scott Kreher, University of Illinois, Urbana-Champaign
Functional analysis of novel honey bee neuropeptide genes using Drosophila melanogaster

5:40 – 6:00  Lauren Wegman, University of Iowa
Peripheral nervous system control of developmental timing and larval behavioral transitions

6:00 – 6:30  Free Time
6:30  Dinner (Dining Room)

Techniques & Data Blast
Allerton Library

8:00 – 8:20  Jennifer Hackney, Indiana University
The Drosophila Genomics Resource Center

8:20
Data Blast
Justin Blumenstiel, University of Kansas
Robert Holmgren, Northwestern University
Bin He, University of Chicago
Susan Spencer, Saint Louis University
Bryant McAllister, University of Iowa

Poster Session

8:45  Cash Bar
9:00 – 9:45  Authors of odd-numbered posters available
9:45 – 10:30  Authors of even-numbered posters available
### October 4
7:30 – 8:30  **Breakfast (Dining Room)**

#### Evolutionary Biology (Bryant McAllister, moderator)
**Allerton Library**

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<td>Hope Hollocher, University of Notre Dame</td>
<td>Species Collisions and Aftershocks: Using Hybrids to Dissect Patterns of Gene Expression Divergence</td>
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<td>9:00 – 9:20</td>
<td>Kristi Montooth, Indiana University</td>
<td>Functional and evolutionary consequences of Drosophila mitochondrial-nuclear interactions</td>
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<td>9:20 – 9:40</td>
<td>Ian Dworkin, Michigan State University</td>
<td>Genetic background effects: what can they tell us about genetics and evolution</td>
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<td>9:40 – 10:00</td>
<td>Luke Hoekstra, Indiana University</td>
<td>Sexually dimorphic heat shock survival in knockdown selected <em>Drosophila melanogaster</em></td>
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<td>10:00 – 10:20</td>
<td>Sara Sheeley, University of Iowa</td>
<td>Contrasting selection on homologous metabolic genes in <em>Drosophila</em></td>
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<td>10:20 – 10:50</td>
<td><strong>Break</strong></td>
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#### Genes & Genomics (Lori Wallrath, moderator)
**Allerton Library**

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<td>John Manak, University of Iowa</td>
<td>Microarray-based integrative genomics: from genome annotation to mapping mutations to genes</td>
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<td>11:20 – 11:40</td>
<td>Justin Blumenstiel, University of Kansas</td>
<td>Identifying EMS mutants by whole genome sequencing</td>
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<td>11:40 – 12:00</td>
<td>Vaughn Cleghon, Cincinnati Children’s Hospital</td>
<td>An amino-terminal region of class 2 DYRKs is essential for autophosphorylation of the molecule and for protein kinase enzymatic activity</td>
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<td>12:00 – 12:20</td>
<td>George Dialynas, University of Iowa</td>
<td><em>Drosophila Lamin C</em> linked to hormone signaling</td>
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12:20  **Business Meeting, followed by Lunch in Dining Room**
1. Identification of factors involved in heterochromatin spreading and gene silencing  
   Kaitlin M. Flannery, Luka N. Zirbel, Diane E. Cryderman and Lori L. Wallrath, University of Iowa

2. Comparative analysis of the D. mojavensis dot chromosome  
   Matthew Dothager, Taylor Cordonnier, Chris Shaffer, Wilson Leung and Sarah C.R. Elgin, Washington University in Saint Louis

3. Imprinting of the Drosophila melanogaster Y chromosome is a potent modifier of the roX1-roX2- male lethality  
   Debashish Menon and Victoria Meller, Wayne State University

4. The role of a Su(Hw) binding region in transcriptional regulation at the IA locus in Drosophila  
   Alexey A. Soshnev, Xingguo Li, Misty D. Wehling and Pamela K. Geyer, University of Iowa

5. "Enhancer" Movement as a Possible Evolutionary Mechanism  
   Gizem Kalay, University of Michigan

6. Asymmetry in the Evolution of Transcription Factor Binding Sites Affinity Between Two Closely Related Drosophila Species  
   Bin HE and Martin Kreitman, University of Chicago

7. The Effects of Repetitious Element 1360 on Heterochromatin Formation in Drosophila melanogaster  

8. Elucidating the evolutionary mechanisms of pigmentation differences between Drosophila species  
   Lisa Arnold, Laura Shefner, Yainna Hernaiz-Hernandez, Adam Neidert and Trisha Wittkopp, University of Michigan

9. Artificial selection for divergent egg size in wild Drosophila melanogaster  
   Cecelia M. Miles, Martin Kreitman and MZ Ludwig, University of Chicago

10. Sexual Antagonism, Adaptation to Climate, and the Evolution of Neo-Sex Chromosomes in Drosophila americana  
    Rebecca A. Hart-Schmidt and Bryant F. McAllister, The University of Iowa
11. Analysis of Glutamate receptor transcript in the *Drosophila* neuromuscular junction
Subhashree Ganesan and D.E. Featherstone, University of Illinois

12. Atg1 positively regulates glutamate cluster formation at the *Drosophila* NMJ
Faith Liebl, Southern Illinois University Edwardsville

13. A reverse genetics screen for genes involved in regulating *Drosophila* sleep
Cory Pfeiffenberger, Northwestern University

14. BS mutant-like behavior in three wild *Drosophila* species
Zhe Wang, University of Iowa

15. A Forward Genetic Screen for Mutations that Affect Postsynaptic Glutamate Receptor Clustering at the *Drosophila* Neuromuscular Junction
Jackie Kostelac, Stephanie Matteson, Emma James, Hassan Yassin and Faith Liebl, Southern Illinois University Edwardsville

16. Regulation of glutamate receptor subunit availability by microRNAs
Julie Karr\(^1\), Vasia Vagin\(^2\), Kaiyun Chen\(^1\), Vladimir Gvozdev\(^2\) and David E. Featherstone\(^1\), \(^1\)University of Illinois at Chicago, \(^2\)Institute of Molecular Genetics, Moscow, Russia

17. Trophic and transmitter roles for serotonin at the same neural circuit
Wendi Neckameyer and Steve Dubec, St. Louis University School of Medicine

18. Effects of social isolation on neuromuscular excitability and aggressive behaviors in *Drosophila*: Altered responses in mutants affecting cellular oxidation state
Atsushi Ueda and Chun-Fang Wu, University of Iowa

19. Phenotypes of mutations in *nervana* 3, a beta subunity of Na/K ATPase, in *Drosophila*
Madhuparna Roy and Daniel Eberl, University of Iowa

20. Modulation of behavior by dopamine and juvenile hormone
Kathryn Argue and Wendi Neckameyer, St. Louis University School of Medicine

21. The vertebrate Wnt Inhibitory Factor-1 regulates Hedgehog activity during fly wing development
Andrei Avanesov, University of Wisconsin
22. **Isolation and Characterization of SIN3 Complexes in Drosophila**  
Marla Spain and Lori A. Pile, Wayne State University

23. **Spatial regulation of achaete via global activation and repression by Hairy and Delta**  
Ji Inn Lee, Meghana Joshi and Teresa V. Orenic, University of Illinois at Chicago

24. **Food processing in Drosophila (or “Seal a Meal” redux)**  
Shane Regnier, Gloria Mazock and Ron Dubreuil, University of Illinois at Chicago

25. **The regulation of signaling cascades by two cell adhesion molecules: Echinoid and Friend of Echinoid, during the Drosophila retinal development**  
Julius Woongki Kim and Susan A. Spencer, Saint Louis University

26. **Regulation of the Sex combs reduced gene within the transverse row bristle primordia of legs in the first thoracic segment**  
Emily R. Jensen, Stuti Shroff and Teresa Orenic, University of Illinois, Chicago, IL

27. **Control of Hdc expression: initial results from reporter-gene fusion studies**  
Elise Miller, Martin G. Burg† and Eric Anderson†, Grand Valley State University

28. **Using fruit fly to dissect honeybee worker division of labor**  
Chen Fu and Charles W. Whitfield, University of Illinois at Urbana-Champaign

29. **Biomonitoring of Environmental toxicants using UAS-GAL4Transgenic Drosophila melanogaster**  
Ajai Kumar and Rabindra Kumar, Feroze Gandhi College, INDIA
Fat, Dachsous and Approximated – growth, patterning and polarity in the wing

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Signaling via the large protocadherin Fat (Ft), regulated in part by its binding partner Dachsous (Ds) and the Golgi-resident kinase Four-jointed (Fj), is required for a variety of developmental functions in Drosophila. Ft, and to a lesser extent Ds, suppress overgrowth of the imaginal discs from which appendages develop at least in part via regulation of the Hippo/Warts pathway. Ft, Ds and Fj are also required for normal planar cell polarity (PCP) in the wing, abdomen and eye, and for the normal patterning of appendages, including the spacing of crossveins in the wing and the segmentation of the leg tarsus. We have shown that much of these effects are mediated by the intracellular domain of Ft. Ft signaling was recently shown to be negatively regulated by the atypical myosin Dachs. We have now identified an additional negative regulator of Ft signaling in growth control, PCP and appendage patterning, the Approximated (App) protein. We show that Approximated encodes a member of the DHHC family, responsible for the palmitoylation of selected cytoplasmic proteins, and provide evidence that App acts by controlling the normal subcellular localization and activity of Dachs.

The Role of Drosophila SIN3 in Wing Development

Aishwarya Swaminathan and Lori A. Pile
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SIN3 is a part of the SIN3 histone deacetylase complex. It is an essential gene that encodes a transcription co-repressor. To determine the role of SIN3 in Drosophila development and to identify developmental pathways affected by loss of SIN3, we performed SIN3 RNAi in the wing discs of Drosophila melanogaster. Induction of SIN3 RNAi produced flies with curly wings. SIN3-deficient wing discs show reduction in phospho-serine 10 of histone H3, indicative of failure to enter mitosis. This could possibly lead to asymmetric cell division between the dorsal and ventral layers of the wing discs resulting in the curly-wing phenotype. This phenotype can be modified by mutations in genes that have been previously established to interact with the SIN3 complex. The SIN3-deficient curly wing phenotype is suppressed by overexpression of STG, a protein required for G2/M progression. Our results are consistent with our previous findings showing that SIN3 is required for cell cycle progression of tissue culture cells. Taken together, our data supports a model whereby SIN3 regulates histone acetylation levels that are critical for cell cycle progression and organism viability.
**Opposing interactions between Drosophila Cut and the C/EBP encoded by Slow Border Cells mediate Notch regulation of apical constriction and epithelial invagination**

Benjamin Levine, Jennifer F. Hackney, Angela Truesdale, Leonard Dobens III, Andrew Bergen and Leonard Dobens

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Stage 10 of Drosophila oogenesis can be subdivided into stages 10A and 10B based on a change in the morphology of the centripetal follicle cells (FC) from a columnar to an apically constricted shape. After 10B, the centripetal FC coordinately involute between the oocyte and nurse cell complex to pattern the anterior operculum structure of the eggshell. We have shown previously that proper centripetal FC migration requires transient expression of slow border cells (slbo) at 10A, due in part to Notch activation followed by slbo autorepression (Levine et al., 2007). Here we show that decreased slbo expression in the centripetal FC after 10A coincides with increased expression of the transcription factor Cut, a Cut/Cux/CDP family member. The 10A/10B temporal switch from slbo to high levels of cut expression requires cross repression between Slbo and Cut and Cut auto activation in the centripetal FC. We demonstrate that high levels of Cut are required for polarized accumulation of actin, DE-cadherin and Armadillo necessary for apical constriction of the centripetal FC. Slbo and Cut interactions are similar in the border cells and restrict Fas2 accumulation to the pole cells, necessary for proper border cell migration. Thus Cut and Slbo regulate two distinct FC migrations – migration of a cell cluster and a cell sheet – and their interactions resemble the opposing effects of their homologs CAAT Displacement Protein (CDP; now CUX1) and CAAT Enhancer Binding Protein (C/EBP), respectively, on cell differentiation in mammalian tissue culture.
Genetic analyses reveal tissue-specific contributions of the Drosophila LEM domain proteins in the nuclear lamina

Belinda S. Pinto, Shameika R. Wilmington, Emma E. L. Hornick, Lori L. Wallrath and Pamela K. Geyer.
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The integrity and organization of the nucleus depends upon the nuclear lamina, a protein network underlying the inner nuclear membrane. Lamina components include the lamins, as well as the LEM domain proteins, named for LAP2, emerin and MAN1. The LEM domain associates with a small, double stranded DNA binding protein called Barrier-to-autointegration factor (BAF) to establish a bridge between the nuclear envelope and interphase chromosomes. LEM domain proteins play a central role in nuclear lamina function; mutations in genes encoding these proteins cause a spectrum of human diseases that include cardiomyopathies, muscular dystrophies and bone density disorders. Mechanisms of lamina pathogenesis are unclear, as LEM domain proteins are globally expressed, yet phenotypic defects are tissue-restricted. We are using Drosophila as a model to provide insights into how LEM domain proteins contribute to lamina function during development. The Drosophila genome encodes four LEM domain proteins – Bocksbeutel (Bocks), Otefin (Ote), dMAN1 and dLEM3. Bocksbeutel and Otefin are the putative emerin homologues, dMAN1 is the MAN1 and LEM2 homologue, and dLEM3 is the LEM3 homologue. To date, we have generated null mutations in bocksbeutel (bocks) and dMAN1, and identified null mutations in otefin (ote). Phenotypic analyses demonstrate that loss of Bocksbeutel produces no discernable developmental defects, whereas loss of dMAN1 or Otefin reduces viability, and produces a spectrum of non-overlapping, tissue-specific phenotypes. Genetic rescue experiments indicate that many of the dMAN1 mutant phenotypes are due to defects in neuronal tissues, while the ote mutant phenotype is due to defects in germ cells. Interestingly, double mutant analyses show that loss of any two of the three LEM domain proteins, dMAN1, Otefin and Bocksbeutel, causes lethality. Taken together, these data suggest that Drosophila LEM domain proteins make both distinct and over-lapping contributions to lamina function during tissue-development.
Developmentally Regulated dADAR mRNA Isoforms in Drosophila: A Novel Role for a Truncated Isoform in rnp-4f 5’-UTR Intron Splicing Regulation

Jing Chen, Danielle L. Hays, Katherine M. McDowell and Jack C. Vaughn
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Proteomic diversity in eukaryotes is greatly enhanced by alternative splicing of nuclear introns, which is regulated by differing molecular mechanisms. Drosophila rnp-4f encodes a splicing assembly factor which delivers U4/U6-snRNP dimer to the developing spliceosome. We have recently reported that alternative splicing of an intron in the 5’-UTR of rnp-4f is developmentally regulated, so that two major mRNA isoforms arise which are designated “long” (unspliced) and “short” (alternatively spliced by removal of a 177-nt tract including the intron and part of the adjacent downstream exon). The coding potential for the two isoforms is identical, suggesting the hypothesis that an essential cis-acting component behind regulation lies in one of the two untranslated regions (UTR). We found that the entire 177-nt tract potentially folds into a long, stable stem-loop (-40 kcal/mole) by intron-exon pairing. Previous work has shown that the long rnp-4f mRNA isoform localizes primarily to the developing embryonic CNS, as does dADAR mRNA. It is known that the single-copy dADAR gene encodes an editase in adults which utilizes long duplex RNAs as substrate. To test the hypothesis that embryonic dADAR is a component of the postulated trans-acting splicing silencer protein, we carried out developmental quantitative RT-PCR for the long isoform utilizing mRNAs from both wild-type and dADAR mutant fly lines. The results show that levels of long rnp-4f isoform diminish prior to the MBT, rise to a peak at 8-12 h, then diminish by larval stages in wild-type. In contrast, the elevation of the 8-12 h peak is diminished by 30% in the mutant. This is interpreted to show that embryonic dADAR is a component of the trans-acting splicing silencer, but that another component remains to be identified. It is known that several different dADAR mRNA isoforms exist, but these have not been characterized for embryo stages, and we asked: a) which isoforms are present in embryos; b) are these expressed into protein; c) if so, do any of these show a peak at 8-12 h; and d) if so, which isoform is responsible for the observed down-regulation of rnp-4f intron splicing. Developmental Westerns show that embryo stage dADAR mRNAs are expressed into protein, which was not previously known. Quantitative RT-PCR showed that each of the two major embryonic dADAR isoform classes shows a peak at 8-12 h. We are using DIG-labeled RNA probes capable of recognizing each of the two major isoform classes specifically to learn which one(s) localize with rnp-4f long isoform in embryos. Preliminary results suggest that a truncated mRNA isoform, predicted to encode a non-catalytic protein, preferentially localizes to the developing CNS. A model is presented to summarize our results. Work now in progress will enable us to determine if dADAR protein binds directly to the long rnp-4f stem-loop, by using in vitro RNA-protein coupling techniques. Our results are novel, since dADAR has not previously been shown to regulate alternative splicing.
Complex Regulatory Circuitry, Structural Rules, and a Novel “Remote Control” Element Govern a Notch- and EGFR-Regulated Eye Enhancer

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Enhancers integrate spatiotemporal information to generate precise patterns of gene expression during development. How complex is the regulatory logic of a typical developmental enhancer, and how important is its internal organization? Here, we examine in detail the structure and function of sparkling, a Notch- and EGFR/MAPK-regulated enhancer of the Drosophila Pax2 gene, which is activated specifically in cone cells of the developing eye.

We find that a “synthetic” sparkling enhancer, made by combining the twelve known transcription factor binding sites within sparkling, is unable to activate gene expression in vivo, even when those sites are placed in their native arrangement and spacing. Our fine-scale transgenic structure-function analysis of the sparkling enhancer has revealed several interesting features of its transcriptional activity in vivo:

Sequence: We find that, in addition to the twelve known TF binding sites, the sparkling enhancer is densely crowded with novel regulatory sequences. Despite surprisingly poor conservation of most critical regulatory sequences, the function and cell-type specificity of the enhancer are well conserved.

Mechanism: We will present evidence for a novel “remote control” element within sparkling that is required only for gene activation at a distance, but is dispensable for other activation functions and for patterning. This is the first report of a dedicated long-range regulatory sequence within an enhancer.

Structure: Unexpectedly, rearranging the regulatory sites within sparkling converts it from cone cell to photoreceptor specificity. We find that the internal organization of the enhancer is constrained by an intricate network of short-range cooperative and repressive interactions.
Sleep and Circadian Rhythms in Drosophila

Ravi Allada, Bridget Lear, Luoying Zhang, Jena Pitman, Brian Chung, Cory Pfeiffenberger, Tim Requarth, Gang Liu, Kevin Keegan, Jermaine McGill, Ji-Ping Wang*, Suraj Pradhan

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The fruit fly Drosophila melanogaster displays many of the core properties of behavioral sleep including regulation by a sleep homeostat and a circadian clock. Early indications are that genes important for sleep regulation in the fruit fly are conserved with those in higher organisms, including humans, indicating an evolutionarily ancient origin of sleep. We have used genetic technologies to map neural circuits important for sleep in the fly and identified a central role for the mushroom bodies in promoting sleep. Notably, the mushroom bodies are also involved in learning and memory, a process that has been intimately associated with the regulation and function of sleep. We have also analyzed the function of the circadian clock and found that a core set of clock neurons that express the neuropeptide PIGMENT DISPERSING FACTOR (PDF) are important for promoting wakefulness, especially in the morning. Using cell-specific rescue of PDF receptor mutants, we have identified a major neural target of PDF. To identify genetic pathways important for sleep, we have re-analyzed genomic analyses of circadian gene expression and identified a core set of genes which robustly oscillate over the course of the day. We have begun to analyze functionally these and other candidate sleep genes using a combination of standard genetics and RNA interference to identify new players in sleep control. Given the conservation of fly and human genes, these may provide insight into the regulation of human sleep.
A Novel Role for Friend-of-echinoid (Fred) in Sensory Organ Precursor Formation

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Fri
end-of-echinoid (Fred) is a paralog of Echinoid (Ed). Ed is a negative regulator of the EGFR signaling during eye development. ed gene function has also been shown to be required to suppress neurogenesis and to interact with the Notch signaling pathway (NSP). Furthermore, Ed acts as a modulator of cell adhesion in developing epithelia.

Using inducible RNAi, we have shown that fred gene function is required to suppress sensory organ precursor (SOP) cell fate in the wing disc and that fred phenotypes can be suppressed by increasing the activity of the NSP. However, ectopic SOPs arise not only in proneural clusters (PNC) but also in regions where normally no PNCs form. We have used microarray analysis to identify genes that are mis-regulated upon fred knock-down in the wing disc. Apart from genes known to be involved in the process of SOP specification, a number of known and predicted genes with no reported function in this process have been identified in this analysis. Among the known genes, our analysis has identified a novel role of Pnr in neurogenesis. We will present evidence that suggests that apart from its known role of promoting neurogenesis in certain regions of the wing disc, Pnr cooperates with Fred to suppress neurogenesis the non-neurogenic regions of the wing disc.
Functional analysis of novel honey bee neuropeptide genes using *Drosophila melanogaster*

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Previous analyses of the honey bee (*Apis mellifera*) genome resulted in the discovery of novel honey bee neuropeptide genes, via a combination of mass spectrometry and bioinformatic analysis. We are transgenically expressing some of them in *Drosophila melanogaster* in order to understand their function. The function of these genes is entirely unknown; they may be evolving rapidly and some may be unique to the bee lineage. The honey bee is a eusocial insect, and *Drosophila* lives a largely solitary lifestyle. Nevertheless, we believe it is possible to use genetics and molecular biology of *Drosophila* to understand bee biology in a rational and systematic way. Our research plan is to transgenically express these neuropeptide genes in *Drosophila* using standard methods and then to assess behavior using standard assays.

We initially have expressed a neuropeptide gene, named *ITGQGNRIF*, using the GAL4/UAS system in *Drosophila*. We have found that overexpression of this gene causes a perturbation of circadian rhythms and may affect circadian-mediated locomotor activity. We also have found that this gene is expressed in mushroom bodies, optic lobes, and antennal lobes of the honey bee brain by *in situ* hybridization. *ITGQGNRIF* may thus be a signaling component of neurons which mediate circadian rhythms. The *ITGQGNRIF* gene has a conserved ortholog in *Drosophila* and we are next examining the function of this gene. In addition, we are extending our analysis to more honey bee neuropeptide genes in order to understand their function.
Peripheral nervous system control of developmental timing and larval behavioral transitions

Lauren Wegman, Joshua Ainsley and Wayne Johnson
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Innate behavioral responses to changing internal or external environmental conditions must often be modified according to the developmental stage of the animal and coordinated with hormonally controlled gene expression cascades. *Drosophila* larval development requires a distinct developmental transition in food associated behavior, shifting from foraging(in food) to wandering(out of food) behavior in time to locate a safe pupation site. This transition must occur after the animal has sufficient nutritional stores for metamorphosis so is associated with larval growth, size and nutritional resources. Behavioral analysis showed that food exit and climbing behavior associated with wandering stage larvae is immediately preceded by an intermediate surfacing transition (IST) when foraging animals emerge to transiently roam the surface of the food. The class IV multiple dendritic (mdIV) neurons of the larval peripheral nervous system extend extensive dendrites tiling the entire inner surface of the larval body wall, and specifically express PPK1, a DEG/ENaC channel subunit. Hyperactivation of mdIV neurons causes a premature shift from foraging to surfacing behavior and ultimately leads to decreased adult size. Electrically silencing the neurons abolishes the surfacing behavior and generates delays in wandering behavior. These results suggest that developmental timing of the major food-associated behavior reversal ending foraging stage is dependent upon input from the mdIV neurons.
The Drosophila Genomics Resource Center

Justen Andrews, Kris Klueg, Lucy Cherbas, and Jennifer Hackney.
Indiana University, Bloomington IN

The Drosophila Genomics Resource Center (DGRC) is an NIH-funded non-profit organization which distributes molecular and cell line materials to the fly community. It is housed at Indiana University, but entirely distinct from the Bloomington Stock Center and FlyBase.

The DGRC now has an inventory of over 1,000,000 Drosophila DNA clones and common vectors through the generosity of individual community members, the BDGP, and the CuraGen Corporation. The collection includes transformation vectors, cDNA clones from eight Drosophila species, fosmids from the Drosophila Species Sequencing Project, and the CuraGen yeast two-hybrid collection. We distribute a majority of the clones and vectors on Whatman FTA discs. Some of the more common large collections are available for purchase in bulk as glycerol stocks.

The DGRC’s cell line collection consists of over 100 permanent cell lines donated by members of the community; we continue to acquire new lines as they become available. We have lines derived from embryos, imaginal discs, the central nervous system, and tumorous blood cells. While most of the lines come from D. melanogaster, 9 other species are represented in the collection. Much of our effort is devoted to characterization of the lines (primarily by microarray analysis) and to user support (guiding users in the choice of line and in overcoming difficulties in growing them).

At the urging of our study section, the DGRC no longer provides transcriptome microarrays to the community, although we continue to distribute genomic tiling arrays designed and fabricated by Kevin White’s group.

The DGRC is currently collaborating with FlyBase to link our collections with FlyBase. The August update of FlyBase includes links from the gene report pages to cDNAs in the DGRC collection. We are now helping FlyBase to design and populate new FlyBase cell line reports, which will also be linked to the DGRC web-site.

Materials can be ordered from the DGRC at dgrc.cgb.indiana.edu. Questions should be addressed to dgrc@cgb.indiana.edu.
Species Collisions and Aftershocks: Using Hybrids to Dissect Patterns of Gene Expression Divergence

Hope Hollocher
University of Notre Dame
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The expression of hybrid sterility in crosses between Drosophila simulans and Drosophila melanogaster is often considered an all or nothing trait. This bias comes from the predominate research focus on hybrid sterility in males, where this is the case. However, considerable variation exists for the expression of fertility phenotypes in hybrid females that is currently underutilized for understanding the evolution of this trait. Here we assayed whole genome microarray expression levels for D. simulans/D. melanogaster female hybrids exhibiting different degrees of fertility in order to understand how patterns of gene expression are impacted during speciation. Earlier microarray studies examining completely sterile hybrids have consistently shown the expression profiles of these hybrids to be vastly different from either parental species supporting True and Haag’s hypothesis (2001) that developmental pathways diverge quite significantly during speciation without accompanying change in phenotype (termed ‘developmental system drift’). By examining hybrid females that are not completely sterile we have discovered that hybrids do not always show these extreme expression profile differences, but can actually converge on one parental expression pattern over the other indicating that master switches controlling gene expression exist that can override incompatibilities in gene regulation that typically accumulate during speciation.
Functional and evolutionary consequences of Drosophila mitochondrial-nuclear interactions

Kristi Montooth, Colin Meiklejohn and David Rand
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The mtDNA encodes genes with well-defined physiological function that must co-evolve with the nuclear genome. One prediction is that increasing molecular divergence would cause increasing disruption of physiological function when divergent mtDNAs are paired with a foreign nuclear genome. We created strains of Drosophila that contain mtDNAs of increasing divergence from within the D. melanogaster species subgroup paired with multiple common D. melanogaster nuclear backgrounds. Despite the 100 amino acid substitutions that have accumulated among these mtDNAs, we find that disruption of metabolic fitness does not scale with mitochondrial molecular divergence. Mito-nuclear interactions affecting fitness are strongest between X-chromosome and mitochondrial variants segregating within D. melanogaster populations. In contrast, more diverged mtDNAs have only slight effects on mito-nuclear function. This suggests that populations harbor variation for mito-nuclear interactions that does not persist as between species divergence. There is, however, one extraordinary exception. A D. simulans mtDNA causes delayed development, reduced fecundity and decreased COX activity but only in one D. melanogaster nuclear background. The sequence of this mtDNA is virtually identical to another D. simulans mtDNA that shows no phenotypic effects, implying that a small number of mutations can lead to severely disruptive intergenomic epistasis for metabolic fitness. Preliminary mapping results point to the specific mutations underlying this interaction in both the mitochondrial and nuclear genomes.
Genetic background effects: What can they tell us about genetics and evolution

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Genetic background effects contribute to the phenotypic consequences of mutations, and are pervasive across all domains of life that have been examined, yet little is known about how they modify genetic systems. Indeed, such genetic variation may be “cryptic” in natural populations, and may contribute to standing genetic variation. However, it is unclear if this variation will affect phenotypes under selection, and how it may contribute to the evolutionary process. In this talk, I will demonstrate that such effects are ubiquitous, and the underlying alleles can be mapped and studied within both an evolutionary and functional genetic context. I will introduce a new model system for the study of genetic background effects in Drosophila, utilizing mutations in the scalloped (sd) gene. I will demonstrate that the expressivity of the sd$^{E3}$ mutation is background dependent, and is the result of at least one major modifier segregating between two standard lab “wild-type” strains. I will provide evidence that at least one of the modifiers is linked to the vestigial region, and may contribute to quantitative variation for wing shape. We also examine the functional genetic consequences of this effect, and demonstrate that the background effects modify the spatial distribution of known Sd target genes in a genotype dependent manner. Transcriptional profiling was utilized to test between several competing models to explain how genetic background modulates phenotypic expressivity. I also demonstrate that the epistatic interaction between sd$^{E3}$ and numerous mutations are background dependent, suggesting that the effects of background extend beyond the mean phenotype of a mutation. These results are discussed within the context of developing a complex but more realistic view of the consequences of genetic background effects with respect to mutational analysis, studies of epistasis and cryptic genetic variation segregating in natural populations.
Sexually dimorphic heat shock survival in knockdown selected *Drosophila melanogaster*

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Extreme environmental temperatures challenge the dynamics of cellular processes, forcing change or system failure. For aerobic organisms, life itself is constrained by the temperature-dependent limitation of oxygen delivery. Despite the clear importance of thermal limits, the physiological mechanisms underlying variation in thermal performance and associations between measures of thermotolerance remain poorly understood. Using flow-through respirometry, we analyzed the metabolic and locomotory thermotolerance of *Drosophila melanogaster* artifically selected for high temperature knockdown tolerance. We show that correlations among measures of thermotolerance vary among selection treatments and that physiological tradeoffs associated with knockdown selection are few. However, evidence of a sexually dimorphic heat stress response highlights the importance of understanding physiological mechanisms in both sexes and may reveal alternative survival strategies.

Contrasting selection on homologous metabolic genes in *Drosophila*

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Similar outcomes are observed in species exposed to similar selective regimes, but it is unclear how often the same mechanism of adaptive evolution is followed. *Adh* and *Pgm* encode metabolic enzymes and contain latitudinally structured, potentially adaptive variation in *D. melanogaster*. Here we present an analysis of selection affecting sequence variation in *Pgm* and *Adh* of *D. americana*, a species endemic to a large climate range that has been colonized by *D. melanogaster*. Unlike *D. melanogaster*, there is no evidence of selection on allozymes of ADH across the sampled range. This indicates that if there has been a similar adaptive response to climate in *D. americana*, it is not within the coding region of *Adh*. Instead, analyses reveal purifying selection on the *Adh* gene, especially within its intron sequences. Frequency spectra of derived unpreferred variants at synonymous sites indicate that these sites are affected by weak purifying selection, but the deviation from neutrality is less drastic than observed for derived variants in noncoding introns. This contrast further supports the notion that noncoding sites in *Drosophila* are often subject to stronger selection pressures than synonymous sites. At *Pgm*, segregating variants at a noncoding site upstream of the start codon appear to be associated with latitude. This site and the surrounding region are not particularly well-conserved and do not strongly resemble any known transcription factor binding sites. In order to evaluate the importance of the segregating variants, expression and selection analyses are required.
Microarray-based integrative genomics: From genome annotation to mapping mutations to genes

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Hybridization of cDNA to tiled genomic microarrays has allowed the empirical identification of all regions of genomic transcription for several genomes. Even though the Drosophila genome is one of the best annotated higher eukaryotic genomes, we have shown through tiling array experiments that a large number of transcripts have been missed using classic annotation strategies such as deep sequencing of cDNA libraries and computational predictions of genes. For example, we identified a large number of unannotated exons linked to known protein coding genes, in particular 5’ exons representing alternative transcription start sites. In addition, by identifying and then overlapping the location of novel 5’ exons with the location of known mutations that cause developmental defects or death, we were able to map mutations to the genes they affect. Similar to Drosophila, the human genome contains large numbers of novel exons associated with protein-coding genes. Thus, in collaboration with human geneticists at the University of Iowa Carver College of Medicine, we are now employing the tiling array strategy we developed in flies to hunt for disease-causing mutations in humans.
Identifying EMS mutants by whole genome sequencing

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In a previous screen for female meiotic mutants, we identified an allele of the ald gene that is silenced in cis a local Doc element insertion. This silencing is specific to the germline. To understand the mechanism underlying this germline specific cis silencing, we have conducted an EMS screen to identify suppressors that relieve this silencing. We have recovered seven mutants. To identify the lesions that relieve this silencing, we have taken a whole genome sequencing approach using the Solexa/Illumina platform. This approach allows simultaneous identification of both candidate lesions and SNP markers that can be used for mapping. We will present an analysis of this approach that identified five candidate lesions for one of the mutants. Further characterization of these lesions is now underway.

Data Blast: Karyosome as Death Star:
A conflict hypothesis for germ line development

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A primary challenge for the female germline is transmit both resources and genetic information to the next generation. However, as many others have pointed out, genetic parasites can proliferate within the germline at the expense of the progeny. Thus, preventing the proliferation of genetic parasites is expected to be a core function of the germline. I will present a framework for understanding Drosophila oogenesis within this context and propose a general hypothesis for understanding certain aspects of germline development.
An amino-terminal region of class 2 DYRKs is essential for autophosphorylation of the molecule and for protein kinase enzymatic activity

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The Drosophila minibrain and smi35A genes encode protein kinases from the DYRK family, MNB/DYRK1A and DYRK2, respectively. These proteins are dual-specificity kinases that autophosphorylate an essential tyrosine residue in the molecules own catalytic region but phosphorylate exogenous substrates exclusively on serine and threonine residues. The DYRK family is an ancient group with family members identified in organisms ranging from trypanosomes and yeast to humans. DYRKs can be sub-divided into Class1 (nuclear) and class2 (cytoplasmic) subgroups. Both subgroups share a conserved central kinase domain, and contain variable N- and C-terminal regions.

To better understand the structure and function of the DYRK molecule, our lab conducted deletion studies where we removed the majority of the non-catalytic N- and C-terminal regions from MNB/DYRK1A (class 1) and DYRK2 (class 2). We found that removal of the C-terminus from either MNB or DYRK2 did not affect tyrosine autophosphorylation and subsequent activation of the protein’s kinase domain. The N-terminus of MNB could also be removed without affecting autophosphorylation. In contrast, removal of the N-terminus of DYRK2 eliminated tyrosine autophosphorylation and kinase activity. This latter result was unexpected as the deletion leaves the kinase domain intact.

Based on this result, we compared the amino acid sequences of DYRKs from a wide variety of organisms. This analysis revealed that class 2 DYRKs contain a region in the N-terminus that is conserved across species and that is not found in class 1 DYRKs. Deletion of, or mutation of individual conserved residues within, the region was found to eliminate the ability of the molecule to autophosphorylate and to become active. Further work has shown that this region is essential for autophosphorylation/activation of Drosophila, human, worm and Trypanosome class 2 DYRKs suggesting that this is an ancient mechanism.
**Drosophila Lamin C linked to hormone signaling**

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The inner side of the nuclear envelope is lined with a network of intermediate filaments called lamins, which provides structural support for the nucleus, organizes the genome by making contacts with chromatin and transmits signals from the cytoplasm. Lamins are classified to A- or B-types depending on their structure and expression profile. In humans, mutations in A-type lamins give rise to a collection of diseases that exhibit tissue-specific defects, such as Emery-Dreifuss muscular dystrophy. We are using Drosophila as a model to identify tissue-specific functions of A-type lamins in development, with implications for understanding human disease mechanisms. Transgenic flies expressing wild type and mutant versions of Lamin C that are analogous to human disease forms were generated. Effects of expressing mutant versions of Lamin C in specific tissues were analyzed. Expression of wild type Lamin C does not alter nuclear architecture or viability. Amino acid substitutions within the Lamin C rod domain cause nuclear Lamin C aggregation, yet support viability. In contrast, deletion of the head domain causes no apparent nuclear defects, but results in semi-lethality when expressed specifically in larval muscle. Adult “escapers” possess malformed legs that are remarkably similar to those produced in flies defective for ecdysone signaling. Consistent with this observation, the ecdysone-induced *E74* gene shows altered expression. The ability of Lamin C to directly regulate gene expression was tested by tethering Lamin C upstream of a reporter gene. Association of Lamin C with the reporter gene caused localization to the nuclear periphery and transcriptional repression. These studies demonstrate a developmental role for Lamin C in muscle, with implications for regulating gene expression through hormone signaling.
Identification of factors involved in heterochromatin spreading and gene silencing

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The genomes of higher eukaryotes are packaged into two major types of chromatin, euchromatin and heterochromatin, which differ in molecular properties. Heterochromatin possesses unique histone modifications and has the ability to spread along the chromatin fiber, silencing genes. The goal of our studies is to understand the molecular mechanisms of heterochromatin spreading. The process of spreading has been challenging to study due to repetitive DNA sequences within heterochromatin. To overcome this problem we have tethered HP1 to euchromatic sites, generating ectopic domains of heterochromatin. We have taken two approaches to identify factors involved in heterochromatin formation and spreading. First, we assayed for recruitment of known chromatin proteins to sites of tethered HP1, such as HP1-interacting protein (HIP) and known histone methytransferases. Second, we performed a genetic screen to identify chromosomal deficiencies that modify gene silencing at sites of ectopic heterochromatin. This screen identified ten chromosomal deletions that modify gene silencing by tethered HP1. Genes within these deficiencies likely encode new factors involved in heterochromatin spreading.
Comparative Analysis of the *D. mojavensis* Dot Chromosome

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Comparative analysis of the fourth (or dot) chromosome of *Drosophila melanogaster* and that of *Drosophila virilis* has shown that the dot of *D. melanogaster* appears predominantly heterochromatic while the dot of *D. virilis* appears to be mostly euchromatic. This small chromosome carries approximately 80 genes in *D. melanogaster*; most of these same genes are found on the dot in *D. virilis*. Initial analysis of *D. mojavensis* by immunofluorescent staining for heterochromatin protein 1 indicates that this dot chromosome is heterochromatic. This suggests that despite the closer evolutionary relationship between *D. virilis* and *D. mojavensis*, the *D. mojavensis* dot chromosome will be more similar to that of *D. melanogaster* in its chromatin packaging. Analysis of the dot chromosomes of *D. melanogaster* and *D. virilis* shows that, even with the contrast in chromatin packaging, one finds a similar repeat density in comparison to the long chromosome arms; relatively large introns due to the repeat density; as well as similar codon bias. Differences were observed in the type of repeats, and in particular in the distribution of (CA)n dinucleotides. Although a large number of the same genes are found on both species’ dot chromosomes, many are in a different order, requiring at least 33 rearrangements to align. From the comparison of these species, it appears that the heterochromatin formation depends on the type and location of repetitious elements, rather than simply the density of repeats. With the help of undergraduates in the Genomics Education Partnership, we have recently completed sequence improvement of the dot chromosome of *D. mojavensis*, bringing it to mouse genome standard (no more than one error per 1000 bp). With this data in hand, we are conducting similar studies in regards to repeat type and density as were done for *D. melanogaster* and *D. virilis*, allowing for a three-way examination of repeat density and distribution, and of gene location among the species. Information gathered from a *D. mojavensis* dot chromosome finished to mouse genome standard could lead to further understanding of the underlying mechanisms and requirements for heterochromatin formation.
Imprinting of the *Drosophila melanogaster* Y chromosome is a potent modifier of the roX1-roX2- male lethality.

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**Abstract:** Genomic imprinting underlies parent of origin differences in gene expression and chromosome behavior. Studies on imprinting from insects to mammals have revealed four classes of genomic-parental origin effects: those resulting in transcriptional silencing of a parental allele; non-random segregation; heterochromatinization or destruction of one parental chromosome; and differential epigenetic modifications that mark paternal and maternal chromosomes. To date imprinting in *Drosophila* has only been detected through its influence on position effect variegation (PEV). We have found that imprinting of the Y chromosome can influence dosage compensation in *Drosophila melanogaster* males. The non-coding roX (RNA on the X) RNAs recruit the MSL (male specific lethal) complex to the single male X chromosome. The MSL complex selectively binds along the length of the male X chromosome and modifies chromatin, resulting in increased expression of X-linked genes. This process, termed dosage compensation, is essential to balance X-linked and autosomal expression. roX1-roX2- males cannot dosage compensate and they die as third instar larvae. We have discovered that the Y chromosome is a potent modifier of the roX1-roX2- phenotype. A maternally derived Y chromosome suppresses roX1-roX2- lethality, while the normal paternal Y enhances lethality. We hypothesize that imprinting of the Y chromosome indirectly influences the ability of the MSL complex to localize to the X chromosome in the absence of unmutated roX RNAs.
The role of a Su(Hw) binding region in transcriptional regulation at the IA locus in Drosophila

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Insulators are DNA elements that constrain the action of enhancers and silencers to establish independent transcriptional domains. To understand mechanisms of insulator action, we are studying the Drosophila Suppressor of Hairy-wing [Su(Hw)] protein, the DNA binding component of the retroviral gypsy insulator. Su(Hw) binds hundreds of sites distributed throughout the genome that are related to the gypsy insulator. To define the role of these binding regions (BRs), we are studying IA-2, an element that has two Su(Hw) binding sites and acts as an insulator in transgene assays. IA-2 is located within the cytological location IA between the differentially regulated protein coding genes yellow (y) and achaete (ac).

Our recent characterization of the y-ac intergenic region uncovered a novel, developmentally regulated, non-coding RNA gene, named yar for y-ac intergenic RNA, placing IA-2 between y and yar. The IA genes are temporally expressed during embryogenesis where ac expression is followed by yar, followed by y. To define whether the IA-2 insulator contributes to the transcriptional independence of the IA genes, we used homologous recombination to generate flies deleted for IA-2 and tested whether the timing and/or levels of y, yar and ac RNA were altered. Loss of IA-2 reduces yar RNA, without changing y or ac, implying that IA-2 is an activator of yar transcription. Phylogenetic analyses showed that the yar promoter is the only intergenic region conserved over evolution.

To test whether yar expression contributes to temporal regulation of y or ac, homologous recombination was used to generate flies lacking the yar promoter. We found that loss of yar transcription had no effect on the timing or levels of y or ac mRNA, indicating that other intergenic elements establish independent transcription of IA genes.

Based on these data, we conclude that non-gypsy Su(Hw) BSs make multiple contributions to genome organization, including insulator and activator functions. Further, independent regulation of adjacent genes may involve multiple classes of regulatory elements.
"Enhancer" Movement as a Possible Evolutionary Mechanism

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Enhancers are non-coding DNA-elements that control the expression of genes in a time, place and quantity specific manner. They have been found to be located in the immediate 5’ upstream, 3’ downstream or the intron of a gene as well as at a relatively distant region in the genome. Conventionally, researchers use sequence conservation when looking for orthologous enhancers, where they also assume the location of the enhancer to be conserved in different species. We have discovered that the function of the “body enhancer” in the pigmentation gene yellow has been relocated between Drosophila species. Reporter gene studies revealed that the 5’ upstream element of yellow was sufficient to drive expression in the body of D. melanogaster. The orthologous region from D. virilis, however, failed to drive any expression in the body. Additional reporter gene studies showed that intron of D. virilis yellow is sufficient to drive expression in the body, confirming the functional movement of yellow “body enhancer” between D. melanogaster and D. virilis. Ultimately, our goal is to determine when and how this relocation occurred.

Asymmetry in the Evolution of Transcription Factor Binding Sites Affinity Between Two Closely Related Drosophila Species

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An enhancer is a regulatory piece of DNA that contains binding sites recognized and bound by transcription factors. Previously people have found these regulatory elements quite variable in sequence despite their functional conservation. To better understand enhancer evolution, we aligned sequences of ~100 regulatory regions containing ~800 transcription factor binding sites experimentally identified in Drosophila melanogaster to its close relative species, simulans. After polarizing the changes using Drosophila yakuba, we estimated the effect each change has on binding affinity using Position Weight Matrix. We unexpectedly found a striking asymmetry: among the 36 substitutions fixed on D. simulans lineage with estimated effect between [-1,1] the ratio of affinity-decreasing ones to affinity-increasing ones is 35:1, compared to 26:8 on D. melanogaster lineage. This pattern is not caused by PWM bias/quality problems. We suggest that turnover of binding sites may contribute to the asymmetry. However we think it’s premature to conclude that all the observed sequence changes are compensated via binding sites turnover and are phenotypically inconsequential. The possibility of enhancer function divergence remains for further experimental studies.
The Effects of Repetitious Element 1360 on Heterochromatin Formation in Drosophila melanogaster

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Eukaryotic genomes are packaged into two different states, euchromatin and heterochromatin. Heterochromatin is associated with the centromeres, telomeres, regions of high repeat density, and other species-specific regions, such as the fourth chromosome of Drosophila melanogaster. When a normally euchromatic gene is placed in or near a heterochromatic region, it can be stochastically packaged as heterochromatin in some cells, resulting in a variegating phenotype known as position effect variegation (PEV). Previous studies from the Elgin lab have suggested that repetitious element 1360 may act as a target for heterochromatin formation in the genome of Drosophila melanogaster (Sun et al. 2004. Mol Cell Biol; Haynes et al. 2006. Curr. Biol.). To directly test if 1360 is capable of supporting heterochromatin formation, constructs were designed containing either one or four copies of 1360, flanked by FRT sites, upstream of an hsp70-white reporter. The reporters were mobilized via transposase, and new insertions with either a red or variegating phenotype were recovered. With one copy of 1360, 4% of recovered inserts exhibit PEV and with four copies of 1360, 12% of recovered inserts exhibit PEV. Previous mobilizations of an hsp70-white reporter lacking the 1360 element resulted in a 1% recovery rate of flies exhibiting PEV (Wallrath & Elgin. 1995. Genes Dev.). Interestingly, the percentage of variegating insertions recovered on the fourth chromosome has not increased. Rather, a higher fraction of the variegating inserts are now on the second and third chromosomes. Mapping of the variegating insertions showed that some (but not all) were located in normally euchromatic regions of the chromosomes arms. To test the dependency of the variegating phenotype on the presence of 1360, this portion of the construct was removed by FLP using FLP-FRT recombination. Surprisingly, only a subset of sites was dependent upon 1360 for the variegating phenotype, indicating an unappreciated complexity in heterochromatin. We are further investigating the genomic location of both sensitive and insensitive sites to determine factors that may play a role in 1360 dependency.
Elucidating the evolutionary mechanisms of pigmentation differences between Drosophila species.

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Many different phenotypes have evolved both within and among species. Despite the knowledge about phenotypic differences, the underlying developmental or genetic mechanisms responsible for these phenotypic changes are not yet well understood. Drosophila pigmentation presents an excellent model for gaining insight into this question through myriad patterns across many species. Two such species are D. americana and D. novamexicana. D. americana is a very dark pigmented species found east of the Rocky Mountains and D. novamexicana has a very light yellow pigmentation and is found in the southwestern United States. The Drosophila pigmentation genes ebony and tan have previously been found to be associated with this drastic pigmentation divergence. Ebony is required for yellow pigment formation and its expression has been shown to be much higher in the lighter D. novamexicana. Since Tan expression promotes darker pigment formation, it is hypothesized that D. americana would show higher Tan expression levels. However, in situ hybridization and RT-PCR data shows tan mRNA expression to be similar between these two species. Functional or differential developmental mechanisms in tan may be playing a role in this recent pigmentation divergence.

Artificial selection for divergent egg size in wild Drosophila melanogaster

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Early development of Drosophila melanogaster embryos proceeds through 13 nearly-synchronous cycles of DNA replication and nuclear division in a common syncitium. After mitotic cycle 13, cellularization occurs at the periphery of the embryo and subsequent mitotic cycles are no longer synchronous. The assumption has been that cell number at cleavage cycle 14 is constant regardless of embryo size. Yet, there is considerable genetic variation for embryo size within D. melanogaster strains and this variation is very likely adaptive. In order to examine the relationship between cell number, cell density and embryo size we used replicate lines of D. melanogaster artificially selected for large and small egg volumes. These lines exhibit a mean increase of nearly 40% in large-egg lines relative to small-egg lines. Embryos were identified as cycle 14 (stage 5) after in situ staining for expression of giant (gt) and even-skipped (eve) with Sytox Green used as the nuclear stain after the techniques of Lott et al. (2007). We then used confocal microscopy to obtain images to test the assumption of constant cell number at the cellular blastoderm stage and compare cell number and density across embryos. We used the same technique to compare these results to lab strains of D. melanogaster showing genetic variation for egg size.
Sexual Antagonism, Adaptation to Climate, and the Evolution of Neo-Sex Chromosomes in *Drosophila americana*

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An evolutionary conflict occurs when alternate genotypes have selective interactions with sex, so that a genotype advantageous to females is deleterious to males, and vice-versa. This conflict is resolved if sexually antagonistic loci exhibit sex-biased inheritance, as on sex chromosomes of species with chromosomal sex-determination. This maximizes benefits of antagonistic alleles in the correct sex and minimizes costs in the incorrect sex (Rice 1984). Sex chromosomes harbor disproportionately many sexually antagonistic loci (Gibson et al 2002), although the mechanism(s) by which these loci are concentrated on sex chromosomes and precise contributions of these loci to divergence between X/Y remains unclear.

*Drosophila americana* is polymorphic for a fusion between the X and 4th (Muller’s B) chromosomes. Population frequency of sex-linked chromosome 4 is positively correlated with latitude, indicating selective interaction between climate and sex on loci within this chromosome arm. An inversion (In(4)a) within some fused 4th chromosomes strengthens sex-linkage through reduced recombination. We aim to characterize the interaction between sex, sex-linkage of 4 and In(4)a, and climate by contrasting phenotypes related to survival and reproduction under extreme climate conditions between the sexes, karyotypes, and populations collected from across the full latitudinal range of *D. americana*.

Our data reveal variation in adult starvation resistance between sexes, and between northern and southern iso-female lines, variation in pupal cold resistance among iso-female lines, and variation in patterns of ovary growth and development in virgins in response to light and temperature cues among iso-female lines and outbred populations. Continued studies will robustly determine the extent of this variation attributable to alleles segregating on autosomal or sex-linked 4th chromosomes, illustrating the role of sexual antagonism in driving the evolution of neo-sex chromosomes in *Drosophila americana*. 
Analysis of Glutamate receptor transcript in the Drosophila neuromuscular junction

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mRNA regulation is increasingly being identified as an important mechanism underlying protein targeting in various polarized cell types. Local translation of synaptic proteins has been known to be a factor in synaptic plasticity. We are studying possible role of mRNA regulation in the related process of synapse formation, using the Drosophila neuromuscular junction (NMJ) as a model system.

It has been established that presynaptic innervation is required as a trigger for several synaptogenic processes, like the production and targeting of Glutamate receptors to the nascent synapse. However, not much is known about how this occurs, and how glutamate receptors are continually added to a growing synapse. We are studying the mRNA of Glutamate receptor subunits to uncover possible regulatory processes at the transcript level. Other studies in our lab have revealed the regulation of transcript abundance by known mRNA regulatory mechanisms in larval muscle cells. Currently, we are using Fluorescent In Situ Hybridization to visualize the mRNA. It appears that the subunits GluRIIA and GluRIIB are distributed in the muscle with a punctate distribution, which suggests that they may be aggregates of mRNA. These puncta appear both in the early first and late third instar larvae.

We are co-immunostaining for known components of RNA granules and RNA associated proteins to determine whether these mRNA aggregations are associated with any of these proteins. We also can visualize GluRIIA mRNA by means of the MS2-MCPGFP system, in which, by expressing recombinant GlurIIA mRNA with RNA stem loops and co-expressing stem loop binding MCP-GFP fusion protein, we are able to tag GluRIIA mRNA with GFP and directly visualize them. GluRIIA-MS2-MCPGFP aggregates seem to form under some conditions, in this system. We will utilize this method of visualization to further test colocalization of known RNA regulatory proteins with these aggregates.
Atg1 positively regulates glutamate cluster formation at the Drosophila NMJ

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Chemical transmission in the central nervous system (CNS) relies on specialized structures called synapses. Synaptic communication is dependent upon the spatially correct formation of presynaptic terminals and the localization of postsynaptic receptors. The development and assembly of glutamatergic synapses is of particular importance because the majority of excitatory transmission in the CNS occurs via ionotropic glutamate receptors. My preliminary data indicates that the autophagy-specific gene 1 (Atg1) is necessary for the formation of glutamate receptor (GluR) clusters in Drosophila at the neuromuscular junction. Animals lacking a functional atg1 gene exhibit a reduction in GluR cluster size. This mutation specifically affects GluRs as there is no observed difference in other synaptic proteins examined. Future experiments will test whether atg1 affects GluR cluster formation by signaling through known pathways that regulate cell growth and whether mutations in atg1 affect the expression or localization of GluRs.

A reverse genetics screen for genes involved in regulating Drosophila sleep

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Sleep is a highly conserved behavior that is required for proper health and cognition in all animals studied to date, yet a function for sleep has not been described. Drosophila melanogaster exhibits many of the hallmarks of mammalian sleep, including increased arousal threshold, consolidated immobility, and regulation by both circadian and homeostatic drive. Although great strides have been made in recent years towards understanding the neural circuitry and molecular pathways that regulate circadian rhythms in Drosophila, the regulation of sleep by the homeostatic drive is poorly understood. To better understand how sleep is regulated genetically, we have taken a reverse genetics approach to identify alleles that alter total sleep duration or sleep consolidation under 12h light:12h dark (LD) or constant darkness (DD) conditions. Taking a candidate approach, we focused on genes involved in intracellular signaling, neuronal signaling and excitability, and genes previously identified with circadian- or sleep-related expression patterns. We have screened through ~1200 pre-existing transposon insertion alleles, covering ~1000 genes. To overcome the potential confound of genetic background variability, each allele was examined over a deficiency from 1 of 2 isogenic collections: DrosDel and Exelixis, and outliers shifted ≥ 2 standard deviations from the population average in 3 separate experiments were selected as hits. This approach has allowed us to identify 30 genes with promising roles in sleep regulation. Included in these hits are multiple ion channels, 2 neuropeptide receptors, factors involved in several cell signaling pathways, metabolic and stress-response genes, and several genes of no known function.
**BS mutant-like behavior in three wild Drosophila species**

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Under mechanical or temperature stress, the laboratory mutant Drosophila melanogaster bang-sensitive mutants will show a loss of movement control followed by an uncoordinated wing flipping during recovery. Recordings from the indirect flight muscle dorsal longitudinal muscle (DLM) show a repertoire of initial discharge, giant fiber failure, delayed discharge and recover at a lower threshold after intense electrical convulse across the brain in the bang-sensitive mutants compared to wild melanogaster lines. Using the same behavioral and physiological criteria, three wild drosophila species, robusta, funebris and macrospina are also proved to show very similar bang-sensitive behavior. Because they exist in nature as very successful species for a long time, this phenomenon implies that bang-sensitive behavior may be closely related to the environmental niche and may have important biological advantages.

**A Forward Genetic Screen for Mutations that Affect Postsynaptic Glutamate Receptor Clustering at the Drosophila Neuromuscular Junction**

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Glutamatergic synaptic communication is responsible for a majority of the fast synaptic transmission in the mammalian central nervous system (CNS). As such, glutamatergic synaptic transmission is involved in a number of processes including learning and memory, epilepsy, and neurodegeneration. Therefore, the processes regulating glutamate receptor (GluR) expression and localization is of great interest. We are currently attempting to uncover novel mechanisms that regulate *Drosophila* GluR cluster formation by screening transposon insertion mutants generated by the Berkeley *Drosophila* Gene Disruption Project. We have found that approximately one third of these mutant lines exhibit quantitative differences in GluR immunolabeling. Because GluRs are used by the animal for locomotion, we have assayed the movement of those lines with aberrant receptors and found that a small percentage also exhibit movement defects. This study has the potential to identify new genes and/or processes that regulate GluR clustering. Future experiments will attempt to uncover the mechanism(s) of GluR regulation by those genes identified in this screen.
Regulation of glutamate receptor subunit availability by microRNAs

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MicroRNAs are small noncoding RNAs thought to regulate production of many metazoan proteins. Unfortunately, almost all microRNA-target interactions are currently predicted using still-evolving and generally untested bioinformatic algorithms, making it difficult to determine whether microRNAs regulate any particular target unless one already knows which microRNAs are likely to do so. Some microRNA-dependent processes can be identified by their dependence on dicer, a ribonuclease required for microRNA synthesis. Unfortunately, elimination of dicer leads to early developmental arrest and severe morphological anomalies, making this approach impractical for study of late-stage developmental processes (such as synaptogenesis). Here, we show that this problem can be circumvented in Drosophila by tissue-specific expression of dicer1 RNAi, and use this approach to show that glutamate receptor subunit abundance is suppressed by microRNAs to regulate receptor assembly. Using these results, we then fine-tuned existing bioinformatic parameters to predict specific microRNAs likely to interact with glutamate receptor subunit transcripts, and then validated one of these predictions: MiR-284.

Trophic and transmitter roles for serotonin at the same neural circuit

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Correct differentiation and positioning of individual synapses during development is fundamental to the proper functioning of neuronal circuits. While classical transmitters such as serotonin (5-HT) appear to play a critical role in neurogenesis in addition to their functions as signaling molecules in the nervous system, this process is not well understood. Using transgenic tools, we show that that constitutive disruption of neuronal 5-HT synthesis during central nervous system (CNS) development in the larval stage of the fruit fly, Drosophila melanogaster, resulted in aberrant 5-HT projections to the gut. In all species tested, including Drosophila, feeding is enhanced when 5-HT synthesis is disrupted in mature animals, but constitutive knockdown of neuronal 5-HT synthesis causes a decrease in larval feeding. When control animals are given 5-HTP, the immediate precursor to 5-HT and the product of the tryptophan hydroxylation reaction, feeding is depressed, as expected. However, when animals with decreased developmental neuronal 5-HT levels are given 5-HTP as larvae, feeding is significantly enhanced, demonstrating an abnormal behavioral response consistent with its impaired circuitry. This work confirms that 5-HT acts as a trophic factor in CNS development, with direct effects on neuronal branching; it also demonstrates that 5-HT can not correctly modulate a behavior if the synaptic circuitry is improperly developed.
Effects of social isolation on neuromuscular excitability and aggressive behaviors in Drosophila: Altered responses in mutants affecting cellular oxidation state

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Social deprivation is known to trigger a variety of behavioral and physiological modifications in animal species but the underlying genetic and cellular mechanisms are not fully understood. As we described previously, adult female flies reared in isolation show increased frequency of aggressive behaviors than those reared in group. Here we report that isolated rearing also caused markedly enhanced activity-dependent facilitation of synaptic transmission at larval neuromuscular junctions. Group-reared larvae displayed gradual synaptic facilitation upon repetitive nerve stimulation at low external Ca2+ levels. In contrast, a large fraction of isolation-reared larvae showed a sudden, explosive jump in the magnitude of neuromuscular transmission that was correlated with supernumerary firing of motor axons. We found that mutations of two genes, Hyperkinetic (Hk) and glutathione S-transferase-S1 (gstS1) alter the response to social isolation in Drosophila. Hk and gstS1 mutants displayed increased adult female aggression and larval neuromuscular hyperexcitability even when reared in group. Significantly, these mutant phenotypes were not obviously enhanced further by isolated rearing. Products of these two genes have been implicated in reactive oxygen species (ROS) metabolism and we have previously shown in these mutants increased ROS levels at neuromuscular junctions. Our data suggest that altered cellular ROS levels can exert pleiotropic effects on cellular processes that underlie neuronal and behavioral responses to social isolation stress, representing an important form of neural plasticity.
PHENOTYPES OF MUTATIONS IN NERVANA 3, A BETA SUBUNIT OF Na/K ATPASE, IN DROSOPHILA

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The plasma membrane-localized Drosophila Na/K ATPase is dependent on the beta subunit for its transport to the plasma membrane and for regulating its activity. Two beta subunits have been characterized in detail, nervana 1 and nervana 2, which are both expressed broadly in the nervous system and epithelia. In situ hybridization shows that a third gene, nervana 3 (nrv3), is expressed in a subset of CNS neurons and, peripherally, in chordotonal organ neurons. This expression pattern suggested the possibility that nrv3 could be important for hearing, mediated by the antennal chordotonal array called Johnston’s organ. We currently have three nrv3 mutant alleles, two of which (nrv3^{15} and nrv3^{47}) were created by imprecise excision of the NP6215 Gal4 insertion, located near the 5’ end of nrv3 gene. The third nrv3 mutant allele, nrv3^{f04395}, is a piggyBac insertion just 5’ of the fourth coding exon. All nrv3 alleles result in homozygous lethality at early larval stage. Our immunohistochemical studies in late stage embryos show that the alpha subunit localizes to the plasma membrane of CNS neurons, while Nrv3 localizes to the PNS, especially the lateral pentascolopidial organ (Ich5), as well as a subset of the CNS. These data agree with our in situ hybridization studies. Finally, the NP6215 Gal4 insertion drives reporter expression in a similar pattern. This driver also expresses in some of the adult PNS, including chordotonal organs, suggesting that nrv3 has the potential to play a role in fly hearing. Experiments are in progress to characterize the details of the larval lethal phenotype, to rescue the lethal mutant phenotype using the UAS/Gal4 system, as well as to construct mosaic animals using the MARCM technique with a view to testing auditory function of nrv3 in Johnston’s organ. A transgenic fly line with inducible UAS-RNAi (nrv3) construct has been crossed with different tissue specific Gal4 drivers which knocks down the mRNA expression levels of nrv3 in the appropriate tissue. Knocking down nrv3 expression in the nervous system resulted in severe adult uncoordination and early mortality in the experimental animals. Experiments are also in progress to test the auditory function of such flies using electrophysiology.
**Modulation of behavior by dopamine and juvenile hormone**

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In vertebrates, steroid hormones play a role in differentiation of neural networks. In *Drosophila*, juvenile hormone (JH) has a direct effect on the modeling of developing neural circuitry. Metabolism of JH has been shown to be controlled, at least in part, by biogenic amines, specifically dopamine (DA). In both mammals and *Drosophila*, dopamine modulates locomotor behavior, female sexual receptivity, and ovarian development. Our studies focus on elucidating the specific interaction between JH and neuronal DA using transgenic lines to manipulate presynaptic DA levels. We show that these interactions are age- and sex-dependent, suggesting that JH may act as a functional correlate of the gonadotropic hormone estrogen.

**The vertebrate Wnt Inhibitory Factor-1 regulates Hedgehog activity during fly wing development**

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In simple terms, morphogen is a secreted ligand whose signaling potential depends on its local concentration. This means that cell’s responsiveness to a morphogen depends on its position relative to the site of morphogen production. Typically, cells near the morphogen source show maximum response, while the ones at distance respond moderately. In a fly, and also higher organisms, such behavior is apparent in tissues that respond to Hedgehog morphogen (Hh). We are trying to understand how Hh activity is maintained at proper range during *Drosophila* wing development. In an earlier study we characterized *Drosophila* shifted (shf) gene and showed that without it Hh diffusion is very limited. However, the vertebrate Shf ortholog, the Wnt Inhibitory Factor-1 (WIF-1), has no known role in helping Hh activity. Instead, human WIF-1 binds and inhibits morphogens of Wnt family, including *Drosophila* Wingless (Wg). We tested for any possible functional conservation between zebrafish WIF-1 (zWIF-1) and fly Shf. First we found that while zWIF-1 is a potent Wg inhibitor it can still rescue some, but not all, Hh activity in shf mutants. Such pro-Hh function of the vertebrate protein can be much improved when we substitute its distal part for a similar one found in Shf protein. In fact, the zWIF-1/Shf chimera can restore shf mutant phenotype to nearly wild-type. Next, we are planning on finding out which aspects of zWIF-1 function are used to promote Hh activity.
**Isolation and Characterization of SIN3 Complexes in *Drosophila***

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SIN3 is involved in transcription regulation, and more specifically, transcription repression. It serves as a scaffold for the assembly of its corepressor complex, which represses transcription through the action of the histone deacetylase RPD3. The SIN3 complex has no DNA binding activity of its own, but is recruited to DNA by various DNA binding factors and other corepressors. An understanding of the proteins that interact with SIN3 is essential for determining which genes are regulated by SIN3, and for fully characterizing the role of SIN3 in growth and development. HA-tagged SIN3 was thus immunoprecipitated from Drosophila tissue culture cells in order to identify potential complex members. Our data indicate that different alternatively spliced isoforms of SIN3 form unique histone deacetylase complexes. These results support findings in yeast systems in which distinct SIN3 complexes are formed that localize to either the promoter or the coding sequence of a gene. Our results suggest that multi-cellular organisms may also have multiple distinct SIN3 complexes. Knowing the identity of the proteins in these complexes will aid us in determining whether metazoans also require distinct histone deacetylase activities at different regions of a gene.

**Spatial regulation of achaete via global activation and repression by Hairy and Delta***

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During vertebrate and invertebrate development, organs and tissues must be precisely patterned and periodic proneural gene expression is an early and essential event in neuronal patterning. On the surface of the *Drosophila* leg, a group of small mechanosensory bristles, called microchaetae, are organized in a series of longitudinal rows around the leg circumference. This orderly arrangement of the leg bristles provide a simple model system with which to investigate the molecular mechanisms involved in the precise proneural gene expression. In the prepupal leg, the proneural gene, *achaete*(ac), is expressed in longitudinal stripes which comprise the leg microchaete primordia. We have found that Hairy and Delta function concertedly and non redundantly to define periodic ac expression. This process involves broad and late activation of ac expression and refinement in response to a prepattern of repression, which is established by Hairy and Delta. These findings have allowed us to formulate a general model for generation of periodic bristle patterns in the adult leg and this model is supported by the analysis of an enhancer that specifically directs ac expression in the leg microchaete proneural fields. This enhancer contains a small activation element, which directs broad expression of ac along the circumference of prepupal legs, and two repression elements, one which is Dl/N-responsive and the other which is Hairy-responsive.
Food processing in Drosophila (or “Seal a Meal” redux)

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The peritrophic membrane (PM) forms a sleeve-like lining that separates food from cells within the digestive tract of many invertebrates including Drosophila. Even though it was first described almost 250 years ago, little is known about its composition or biological function. We first encountered the PM in studies of the larval digestive tract of Drosophila spectrin mutants. After failing to observe any effects in larval feeding experiments with a variety of chemicals, we ultimately fed them with a solution of 1% sodium dodecyl sulfate in 0.2% Bromphenol blue tracking dye. Ingestion of a large amount of this harsh detergent solution had no apparent effect on the ability of larvae to mature to adulthood. We followed this up with a number of further observations. Newly hatched larvae fed with a yeast suspension undergo rapid excretion of many times their body length of PM within a few hours. This early excretion event does not occur in the absence of food. The isolated PM is almost transparent by light microscopy and is resistant to all chemical treatments tested. The PM is more difficult to detect in older larvae, especially when grown on agar medium. We found that it can be stabilized and more easily monitored by incubating larvae in a solution of bromochloroindolyl phosphate (BCIP), a soluble phosphatase substrate that produces an insoluble blue product. The PM appears to move like a conveyor that could move food through the digestive tract. However, measurements of the rate of PM production using BCIP indicate that it moves about 7.5-fold slower than the maximal rate of food passage through larvae (which is probably powered by peristalsis). Several candidate gene products have been identified as components of the PM in other insects, although they only account for a small fraction of its total mass. A number of related gene products have been uncovered by the Drosophila genome project. We characterized the expression pattern of four Gal4 lines from public stock centers. All of the lines exhibit significant Gal4 expression in cells of the cardia that were previously suggested to secrete the adult PM (King, 1988). Thus these lines are well-suited for further dominant negative and RNAi-based approaches aimed at unravelling PM structure and biology.
Echinoid (Ed) and Friend of Echinoid (Fred) are highly homologous cell adhesion proteins that regulate EGFR and Notch signaling cascades, crucial pathways for transmitting information between cells during development. Echinoid has been shown to undergo homophilic interactions and loss of ed causes multiple R8 photoreceptor cells in the Drosophila retinal development. Fred is highly similar to Echinoid in structure: 70% of its extracellular domain is identical, yet little is known about its function. Here, we will first show that Ed and Fred undergo homophilic and heterophilic interactions that mediate cell adhesion in vitro. Second, we will show that Ed and Fred co-localize at the membrane in the morphogenetic furrow in eye imaginal discs and that the multiple R8 phenotype seen in ed mutants is enhanced by mutations in fred. Third, we will present a possible regulatory mechanism for Ed and Fred: ectodomain shedding. Finally, we will propose how the interaction between Ed and Fred regulates EGFR and Notch signaling pathways during the Drosophila retinal development.
Regulation of the Sex combs reduced gene within the transverse row bristle primordia of legs in the first thoracic segment

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The Drosophila adult has one pair of legs on each of its three thoracic segments (the T1-T3 segments). Although they are homologous, legs from different segments have distinct morphological features. For example, on T2 legs, a group of small mechanosensory bristles (mCs) are organized into a series of longitudinal rows (L-rows) along the circumference of the tibial and tarsal segments. mCs are also organized into L-rows on the tibia and tarsus of T1 legs. However, at specific positions along the circumference and proximal/distal (P/D) axes of the T1 leg tibia and tarsus, the L-rows are replaced by a group of mCs organized into a series of transverse rows (T-rows). Our studies indicate that the pattern of T-row bristles on T1 legs is established as a result of Hox gene modulation of the pathway for patterning the L-row mC bristles. In T1 prepupal legs, Scr is expressed at high levels within the T-row primordia. We have found that Scr modifies the mC pattern on T1 legs, partly via repression of Delta, a key regulator of leg mC patterning, specifically within the T-row primordia. Our model for T-row patterning suggests that a central step in this process is establishment of spatially defined Scr expression within defined domains of the leg primordium in response to the global regulators of leg development. Hence, the mechanisms that generate morphological diversity among the legs will require an understanding of the regulation of Scr in the T-row primordium. We will present our studies on regulation of Scr by genes known to pattern the leg along its circumference and along the P/D axis. This analysis will also provide the opportunity to determine the genetic and molecular mechanisms through which patterning information along the leg circumference and P/D axis is integrated, a process about which not much is known.
Control of *Hdc* expression: initial results from reporter-gene fusion studies

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Histamine is a biogenic amine that is used as a neurotransmitter by photoreceptors and possibly by central histaminergic neurons. Histidine decarboxylase (HDC) is the enzyme that synthesizes histamine, using histidine as a substrate. Mutations in the *Hdc* gene have been identified which result in functional blindness (Burg et al., 1993). Additional mutant phenotypes have since been identified, such as temperature preference, which may reflect the action of histamine as a central neurotransmitter (Hong et al., 2006). We are interested in understanding how tissue-specific expression of *Hdc* is controlled. Previous deletion analysis of an *Hdc* transgene, shown to completely rescue the *Hdc* mutant phenotype, indicated that specific regions 5' to the ORF of the *Hdc* gene were necessary for expression in the central brain and photoreceptors (Burg and Pak, 1995). A unique 5' end of the *Hdc* transcript was identified, suggesting that the tissue-specific expression may be controlled by different transcriptional promoters. The genomic region containing these putative promoters was fused to eGFP in order to determine if this 5' region is sufficient for normal *Hdc* expression. A 4.3 kb genomic XhoI-NcoI fragment was fused to the enhanced green fluorescent protein (eGFP) in the pGreen Pelican transformation vector. The region used included the first intron, which is part of the 5'UTR of the *Hdc* transcript (Boozer and Burg, pers. commun.) We recently completed the initial analysis of the pHdc-eGFP transformant flies, and determined that the pattern of eGFP expression is significantly different from that expected for *Hdc*. The results suggest that 4.3 kb 5' portion of the *Hdc* locus, while necessary for full expression, is not by itself sufficient to direct expression in all tissues that normally express the *Hdc* gene.

Using fruit fly to dissect honeybee worker division of labor

-Brain gene expression markers of honeybee behavioral/social state (hive working vs. foraging) may be involved in behavioral output of the circadian clock

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It has been shown that hundreds of genes in the brain are strongly associated with the behavioral stage of the honeybee worker (hive working vs. foraging). There are multiple differences in behavior changes with the switch of social role. For example, the nurses are arrhythmic while the foragers have precise internal clock. We investigate, in the current research, that if the genes in fruit fly homologous to the brain gene expression markers of honeybee behavioral state (hive working vs. foraging), are behavioral-related. And if yes, are they affecting the rhythmic behavior. By measuring locomotion behavior of flies with single gene knocked-down by RNA interference, we identified at least 2 genes (homologous to genes highly expressed in honeybee hive worker brains), fax (failed axon connection) and BM-40-SPARC, are related with the dawn/dusk locomotion activity of the fruit fly. These results may be analogous to honeybee observations: these genes are down-regulated in foraging bees (which are hyperactive during peak foraging periods) and knock-down of these genes in flies causes hyperactivity but only at specific times of day. Measurements of the behavioral difference in phototaxis and foraging strategy are also ongoing in the laboratory. We believe this line of research can not only help to discover more behavioral related genes, but also can lead to deeper understanding of how complex (e.g. social) behaviors are assembled from simple behavioral components.
Biomonitoring of Environmental toxicants using UAS-GAL4 Transgenic *Drosophila melanogaster*

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UAS-GAL4 Transgenic *Drosophila melanogaster* having hsp70- lacZ genes was examined for the first time to monitor toxicity of two most extensively used organophosphate insecticides, Methyl parathion and Monocrotophos in agriculture. Different concentrations of these insecticides were fed to third instar larvae of transgenic *Drosophila* strain for different time intervals and expression of hsp70-lacZ was examined in relation to larval mortality by β-galactosidase activity and vital dye staining. Our results showed that both insecticides used at LC₅₀ and other concentrations after various time intervals was concurrent with extensive tissue damage as indicated by trypan blue staining. The present study suggests that both toxicants pose cytotoxic potential to *Drosophila*. The use of UAS-GAL4 Transgenic *Drosophila melanogaster* having hsp70-lacZ genes for rapid, effective and sensitive measurement of cytotoxicity will be discussed.