Fruit fly as a model for alcoholism: Integration of laboratory pedagogy and student-directed research

Elaine R. Reynolds, David Sison, Taisha Jerez, Gabriel Eusebio, and John Drummond
Biology Department and Neuroscience Program, Lafayette College
reynolde@lafayette.edu, sisond@lafayette.edu, jerezt@lafayette.edu, eusebiog@lafayette.edu, drummondj@lafayette.edu

NEUROSCIENCE AT LAFAYETTE

Background

Drosophila melanogaster **is a good model system for examining genetic predisposition to alcoholism. Flies exhibit a characteristic behavior upon alcohol exposure that includes hyperactivity and then sedation. Flies also become tolerant to drug exposure, with one or multiple exposures or chronic exposure. The alcohol-based behaviors are robust and easy to illicit and analyze, ideal for introducing behavioral analysis to undergraduates. The fly system also has many tools amenable to genetic analysis that are easily manipulated by students. Pedagogies using the model and tools can be used to engage students in active learning in order to achieve student outcomes related to model systems, genomic analysis, use of computer tools, and data analysis.**

As part of laboratories in General Biology and Neurobiology, insertion mutations available as part of the Fly Genome Project are being screened for alterations in ethanol sedation behavior and tolerance. The project has involved about 400 laboratory students and three research students. These inserts disrupt specific identified genes; so once an insert has been identified that alters the behavior, it can be quickly contextualized using Flybase. Inserts that show altered behavior are confirmed and examined in more detail as part of a student-directed research program.

The screen thus far has yielded a range of defects and mutants. Mutants that are sensitive to alcohol appear at a higher rate in the insertion population than resistant mutants and altered sedation kinetics are more common than inserts that affect tolerance behavior.

Student Outcomes and Assessment

Implementation of this module in its current form occurred 2 years ago as part of an revamped Gen Bio lab curriculum. Assessment of the module is ongoing.

For these laboratories we defined a set of student outcomes. Since most of the outcomes are for a 100 level course, the expectations are modest. We looked for students to explain:

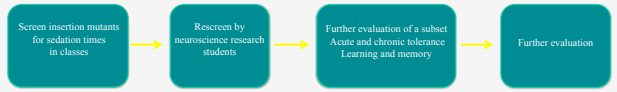
- **The role of model organisms**
- **Some basic genetic concepts**
- **Genome projects and data bases**
- **How behavior can be observed, quantified, and analyzed.**
- **How to use simple statistical tests to analyze and assess data.**

Student mastery of these outcomes were assessed through a lab report, performance on a lab exam, and performance on lecture exam on genetic concepts

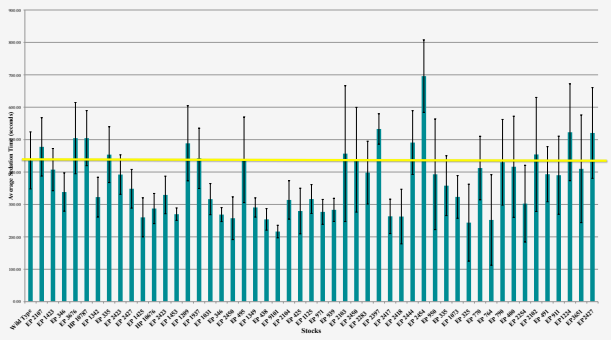
Effectiveness of the teaching module was assessed by comparing outcomes prior to introducing the module with outcomes after implementing the modules as well as student feedback on the modules.

For example with the same lecture instructor, the mean on the lecture exam rose from 79 to 81. While we saw no improvement on the overall lab exam, we are currently assessing student performance on specific questions pertaining to the lab.

Overview of the screen



Sedation data for strains in the secondary screen



Strain	Average	Std Dev	T-Test
Wild Type	435.70	87.95	
EP 2387	477.74	98.83	0.0180
EP 1423	407.38	65.17	0.0667
EP 346	338.30	59.18	0.0000
EP 3676	504.64	109.93	0.0086
HP 10787	504.58	85.83	0.0001
EP 1342	322.68	61.56	0.0000
EP 335	453.68	86.22	0.2956
EP 2423	392.20	60.83	0.0044
EP 2427	348.64	59.65	0.0000
EP 1425	268.12	60.47	0.0000
HP 10676	287.36	46.59	0.0000
EP 2423	329.40	58.04	0.0000
EP 1453	269.92	19.15	0.0000
EP 1269	488.60	116.82	0.0098
EP 1937	442.48	93.29	0.7038
EP 3811	316.34	48.07	0.0000
EP 346	268.72	21.57	0.0000
EP 2450	257.48	66.02	0.0000
EP 495	427.76	132.14	0.5252
EP 1349	298.70	29.66	0.0000
EP 438	253.96	33.13	0.0000
EP 9101	216.40	19.51	0.0000
EP 2104	313.92	59.39	0.0000
EP 425	279.70	70.61	0.0000
EP 1125	316.64	44.56	0.0000
EP 971	277.06	38.45	0.0000
EP 539	283.36	35.82	0.0000

Strain	Average	Std Dev	T-Test
Wild Type	530.95	253.93	
EP 2104	456.80	209.60	0.0702
EP 2450	438.04	161.49	0.0019
EP 2283	298.32	56.55	0.0000
EP 2397	532.87	47.02	0.0000
EP 2417	263.35	53.35	0.0000
EP 2418	262.66	84.41	0.0000
EP 2444	490.92	98.48	0.0000
EP 2454	499.93	111.88	0.0000
EP 950	393.01	176.76	0.0000
EP 335	257.94	92.34	0.0000
EP 1073	323.29	65.79	0.0000
EP 325	243.86	118.93	0.0000
EP 770	412.31	98.24	0.0000
EP 764	252.19	139.74	0.0000
EP 790	430.24	132.05	0.0002
EP 486	416.33	156.19	0.0001
EP 2254	302.52	118.53	0.0000
EP 2102	454.28	175.98	0.0142
EP 491	393.29	84.95	0.0000
EP 911	390.32	120.66	0.0344
EP1224	522.75	149.60	0.7836
EP651	409.91	166.10	0.0001
EP2427	520.59	139.88	0.7010

Significant p<0.05

Methods

The Berkeley Drosophila Genome Project has created an insertion into almost every gene in the genome. These insertion mutants can be screened for alcohol sedation phenotypes. All stocks were obtained at the Bloomington Stock Center. The location of the gene disrupted and its genetic and molecular characteristics where determined using FlyBase, the Drosophila data base
http://flybase.bio.indiana.edu/

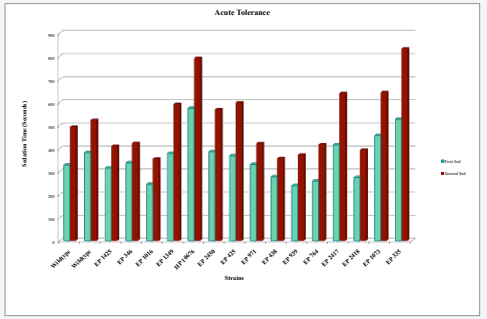
For sedation time and tolerance experiments

1. **Ten or so flies were put into a test tube**
2. **A cotton ball soaked with 95% EtOH was placed at the open end of the tube**
3. **Fly behavior was observed. The sedation time for each individual fly was recorded. The mean sedation time is the average sedation for any given number of individuals. In classroom experiments N=10-20 flies, In confirmation experiments N=50**
4. **Wildtype and insert mutants were statistically compared using t-test with a P< 0.05 considered significant**
5. **For tolerance, alcohol was reintroduced 1 hr after the first sedation. First and second sedations were statistically compared using t-test with a P< 0.05 considered significant**

Screen Summary

# of insert lines screened	**197**
# of positive classroom lines	**50**
# of positive/number screened	**27%**
# of confirmed positives	**43**
accuracy of classroom screen	**86%**
# of strains that are sensitive	**37**
# of strains that are resistant	**6**
Selected strain for future analysis	**16**

Acute tolerance data for selected strains



Strains	First Sed	Second Sed	T-Test
Wildtype	328.56	493.52	5.643E-15
Wildtype	382.88	523.20	2.938E-05
EP 1425	315.22	410.44	2.523E-07
EP 346	337.42	423.28	7.631E-06
EP 1016	244.96	355.80	1.087E-13
EP 1349	270.04	392.08	6.761E-23
HP 10676	574.14	791.92	2.725E-10
EP 2450	286.40	569.26	1.327E-08
EP 425	368.58	599.48	1.367E-18
EP 971	330.88	422.62	2.263E-06
EP 438	277.08	387.42	1.435E-05
EP 539	238.98	372.66	1.017E-11
EP 764	258.68	417.68	6.368E-08
EP 2417	415.88	629.68	3.578E-09
EP 2418	274.28	394.26	2.838E-04
EP 1073	456.08	643.80	6.828E-05
EP 335	526.28	822.80	1.218E-08

Conclusions and Work in Progress

Apparently there are many genes in the genome that can alter sedation rate. At best this can only be an initial screen for genes that could be a predisposing factor for alcoholism

When compared to the wildtype, the majority of the insertion strains displayed increased sensitivity to alcohol.

The initial screens done by the Intro to Biology and Neurobiology labs provided a useful foundation for determining which mutants should be chosen for further analysis.