Teaching behavioral genetics using *Drosophila* larval phototaxis.

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Introduction

This exercise will teach students about behavior, the genetic basis of behavior, and neuronal development. The exercise involves a distinctive *Drosophila melanogaster* larval behavior, negative phototaxis, quantified in a simple Petri dish arena assay. Variation in this behavior is demonstrated in a simple assay, easily accomplished during a 3-hour lab session, in which students collect larvae to be tested, construct the assay arenas, and collect data.

Larval *D. melanogaster* generally display negative phototaxis – that is they are repelled by light (Lilly and Carlson, 1990). In third instar larvae, this behavior gradually diminishes as the larvae leave the food medium in search of a pupation site and immediately prior to pupation, larvae respond indifferently to light (Godoy-Herrera *et al*., 1992). Larvae detect light using Bolwig's organ, consisting of an anteriorly located cluster of 12 photoreceptors (Hassan *et al*., 2000; reviewed in Friedrich, 2008). Four of the 12 photoreceptors become the adult eyelet, these future adult receptors express the rhodopsin 6 gene that is also active in 8 photoreceptors of Bolwig's organ (Sprecher and Desplan, 2008). A characteristic head swinging behavior may mediate the negative phototaxis behavior. If so, this behavior suggests that the larvae detect differences in light intensity through unequal stimulation of photoreceptor cell clusters and use this stimulation to orient away from the light stimulus (Busto, Iyengar and Campos, 1999; Scantlebury, Sajic and Campos, 2007).

There are a number of mutations in *D. melanogaster* that have been shown to affect larval phototaxis behavior. β3-tubulin is expressed in *Drosophila* embryos and functions in development of Bolwig's organ and nerve; larvae with mutations of the β3-tubulin gene show defects in phototaxis (Dettman *et al*., 2001). Busto, Iyengar, and Campos (1999) presented evidence that mutants for either *neither inactivation nor afterpotential C* (*nina c*) and *no-receptor potential A* (*norp A*) display defects in phototaxis. *Nina C* mutant adults have reduced rhabdomeres due to retinal degeneration and defects in phototransduction (Porter *et al*., 1992), and *norp A* protein is expressed in all adult photoreceptors and encodes phospholipase C (Schneuwly *et al*., 1991). Stocks of these three mutations are available through the Bloomington Stock Center (http://flystocks.bio.indiana.edu/). Alternatively, for a more involved experiment, students could be charged with their own crosses and stocks to create mutants via *P*-element excision (*e.g*., of the e03267 line in the Harvard Exelixis Collection with a *P*-element inserted near the *norp-A* gene), making this specific teaching lab the culmination of a semester long project.

Materials and Methods

Behavioral assay procedures and protocols are similar to those described by Lilly and Carlson (1990). Details are given below.

Fly Preparation

Approximately 10 days before the lab session, adult flies should be transferred to new food to lay eggs. Approximately an hour before the lab, adults should be removed from stock vials, leaving

only larvae. This timing ensures that very few larvae will be late 3rd instar or entering the pupal stage (*i.e.*, relatively light-insensitive), decreasing error associated with the assay, and maximizing the number of larvae collected from each vial. Four or five vials of each genotype per lab group should be sufficient to collect 3 sets of approximately 50 larvae of the genotypes to be assayed. The number of larvae per test plate could be lowered to 10 or 20 larvae to save time during the lab.

Assay Plate Preparation

Behavioral assays are conducted using agarose plate "arenas". Before students begin collecting their larvae they should start to boil 2 beakers of water for their agarose. Alternatively, to save time, plates can be prepared prior to the lab by the instructor. Figure 1 is a schematic diagram showing how the test plates are constructed. To make the test plates, equal numbers of dyed and clear agarose plates are made; about 30mL of 1% agarose is needed for each plate. To make the darkened agarose approximately 10 drops each of red, blue, and green food colouring are added to 200 ml 1% molten agarose. Once the agarose is hardened plates are cut into four equal sections, removing two diametrically opposed sections and replacing them with the opposite coloration of agarose. After switching out the sections approximately 10 ml of clear 1% agarose is poured on top of the test plates so as to ensure that no crevices or bumps exist, otherwise larvae will burrow into spaces in the agarose. Allow the test plates to cool to room temperature before testing.

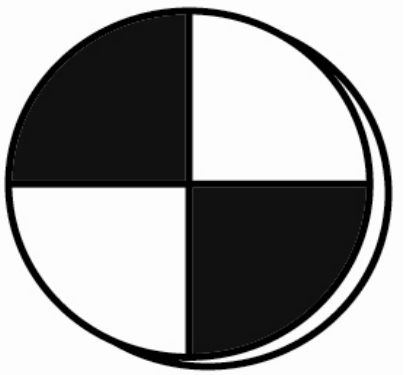

Figure 1. The Larval Phototaxis Assay is constructed from darkened and regular clear agarose. For each assay, approximately 50 larvae are placed onto each plate. Plates are then placed on a light box and larvae are allowed to migrate for 5 minutes.

Collection of Larvae

Early third instar larvae are removed from the culture by adding 1-2 mL of water to the medium and lightly agitating the surface with a paintbrush; this water and culture mixture is then poured into a Petri dish or weigh boat and third instar larvae can be carefully collected using tweezers or a paintbrush. Dissection microscopes with black stages are very helpful for this step. Collected larvae are placed into a pool of clean water to rinse off any remaining food particles. After being rinsed they are placed into another pool of water in a covered Petri dish where they remain until testing.

Table 1. Data table with typical results for wildtype and mutant larva with defects in larval phototaxis.

Trial 1	Wildtype	Mutant
Dark	48	28
Clear	2	22
Total	50	50
D-C	46	6
RI=(D-C)/ (D+C)	46/50 = 0.92	6/50 = 0.12

Testing Larval Phototaxis

Approximately 50 larvae of a given genotype are placed in the centre of the test plate that is then placed, without its cover, on a light box in a dark room for 5 minutes. Plates are then scored for the number of larvae on the dark and clear portions of the plate and a response index (RI) is calculated. The RI is the number of larvae on the dark portions (D) minus the number on the clear portions (C) with this quantity divided by the total (D+C), i.e., RI = (D-C)/(D+C). See Table 1 for a typical results table that students would fill in during lab.

Each genotype should be assayed in triplicate so students can determine the mean RI and standard deviation. In more advanced courses, students can perform a simple one-way ANOVA and

subsequent Tukey's HSD to determine significant differences for the data they have collected during this lab.

Conclusion

The above protocol offers instructors a dynamic teaching exercise that can be altered to accommodate students at a variety of levels, from an introductory genetics course, to an advanced behavioral or developmental genetics course. This teaching protocol gives students an opportunity to gain experience working with *Drosophila*, a model species widely used in the study of behavior, development, and genetics. The protocol provides students with hands-on experience in the acquisition of scientific data, from the basic level of sample collection and preparation, to the more advanced data analysis and presentation. The protocol also provides the opportunity for direct experience assaying for mutant phenotypes and in more involved versions the possibility of incorporating actual mutagenesis experiments with this behavioral assay.

References: Busto, M., B. Iyengar, and A.R. Campos 1999, J. Neurosci. 19: 3337-3344; Dettman, R.W., F.R. Turner, D. Hoyle and E.C. Raff 2001, Genet. 158: 253-263; Friedrich, M., 2008, BioEssays 30: 980-993; Godoy-Herrera, R. Alarcon, M. Caceres, H. Loyola, I.I. Navarrete, and J.L. Vega 1992, Revista Chilena de Historia Natural 65: 91-101; Hassan, J., M. Busto, B. Iyengar, and A.R. Campos 2000, Behav. Genet. 30: 59-69; Lilly, M., and J. Carlson 1990, Genet. 124: 293-302; Scantlebury, N., R. Sajic, and A.R. Campos 2007, Behav. Genet. 37: 513-524; Schneuwly, S., M.G. Burg, C. Lending, M.H. Perdew, and W.L. Pak 1991, J. Biochem. 266: 24314-24319; Sprecher, S.G., and C. Desplan 2008, Nature 454: 533-537; Porter, J.A., J.L. Hicks, D.S. Williams, and C. Montell 1992, J. Cell Biol. 116: 683-693.

Drosophila adult eye model to teach Scanning Electron Microscopy in an undergraduate cell biology laboratory.

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Abstract

We have devised an undergraduate laboratory exercise to study tissue morphology using fruit fly, *Drosophila melanogaster*, as the model organism. *Drosophila* can be reared in a cost effective manner in a short period of time. This experiment was a part of the undergraduate curriculum of the cell biology laboratory course aimed to demonstrate the use of **S**canning **E**lectron **M**icroscopy (**SEM**) technique to study the morphology of adult eye of *Drosophila*. The adult eye of *Drosophila* is a compound eye, which comprises of 800 unit eyes, and serves as an excellent model for SEM studies. We used flies that were mutant for *Lobe* (*L*), *eyeless* (*ey*), and *pannier* (*pnr*) for our studies. The mutant flies exhibit different morphologies of the adult eye. We employed a modified protocol, which reduces sample preparation steps and makes it practically feasible to complete the protocol in