Drosophila larval chemotaxis: A practical experiment for the introduction of young children to science.

López-Matas, Mariana[1,2], Moraea Phillips[1,2], and Bàrbara Negre[1,2,3]. [1]EMBL/CRG Research Unit in Systems Biology, Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain; [2]Universitat Pompeu Fabra (UPF), Barcelona, Spain; [3]Corresponding author: bnegre@gmail.com.

Abstract

The importance of introducing children to science and developing their interest in the subject is widely recognised. Here we adapt a well-established chemotaxis assay as an experiment for school children. The students observe the behavior of fruit-fly larvae in response to an attractive odor (*e.g.*, banana). We have performed this experiment successfully with children between the ages of 3 and 9. We also suggest additional steps as optional challenges for older students. A detailed protocol and list of materials are provided.

Presenting the Concept

Drosophila larvae demonstrate robust orientation behavior towards light and odors (Mast, 1911; Sokolowski, 1986; Sawin-McCormack *et al*., 1995; Cobb, 1999). Several assays have been developed to use larvae as a model for the investigation of sensory processing (Louis *et al.*, 2012). Here we present an adaptation of the "petri-dish assay" (Aceves-Piña and Quinn, 1979; Louis *et al.*, 2012) that can be effectively carried out by young children.

Larvae increase their size by approximately 200 times before reaching adulthood, for which they need to feed constantly (Robertson, 1963). Olfaction is thought to play a key role in the localization of food sources. When exposed to an attractive odor source, larvae quickly migrate towards higher odor concentrations. We make use of this behavior to introduce children to the use of *Drosophila* in science and to hypothesis testing.

Activity

We briefly explain the ecology of *Drosophila* and its life cycle (with figures and a food-vial containing larvae at various stages). We ask students about their expectations as to how the larvae find food. *Drosophila* larvae detect light but "do not properly see"; do they use their nose? The children smell the flask with the odor, can they recognise it? As they might find it difficult to recognise the odor, when using banana odor (isoamyl-acetate), having a ripe banana the children can smell and see will help them recognise the odor. The experiment will test whether *Drosophila* larvae like the smell of banana.

We give an overview of the outline of the experiment, followed by step by step instructions and the handing out of material, as they need it. If there is not enough material for students to work alone the experiment can be carried out in pairs.

Each child –or pair of children– is given a vial of larvae in food medium and a tube containing 10 ml of 15% sucrose solution. They pour the sucrose solution into the vial of larvae and leave it to rest for a few minutes, until most larvae have floated to the surface. While they are waiting, empty petri dishes can be handed out. Once the larvae have surfaced, they will pour the larvae in the petri dishes. Make sure they open the petri dish before pouring! Using a paintbrush the larvae can be picked from the liquid (sucrose solution transferred with the larvae from the vial) and transferred to a second petri dish containing agarose. They should transfer as little as possible of the sugar solution with the paintbrush. At this point a drop (10 ul) of test odor is placed close to one edge of the inner surface of the lid (placing the droplet inside a transparent reinforcement ring stuck to the plastic will stop it spreading). The lid is replaced –with the odor droplet

suspended above the agarose surface– and a timer can be set. After a few minutes most larvae will be very close to the odor source, sometimes even having crawled up onto the lid and immersing themselves in it.

The experiment can be complemented with the observation of the different developmental stages (vial with adults, larvae, and pupae) and adults (petri dish with frozen flies). Students can observe them by eye or with a magnifying glass or stereomicroscope. It is also an opportunity to learn how flies live in a laboratory. The activity ends with a short discussion of what they have done and seen and a round of questions encouraged by the supervisor.

We have performed this activity with children aged 3 to 9. They greet with enthusiasm hands-on experiences like this one. For younger children (3-4 years old) we try to have 1 supervisor for every 5 children. With this supervision the experiment can be performed in 45 minutes. If they are to work more independently, we recommend scheduling at least one hour.

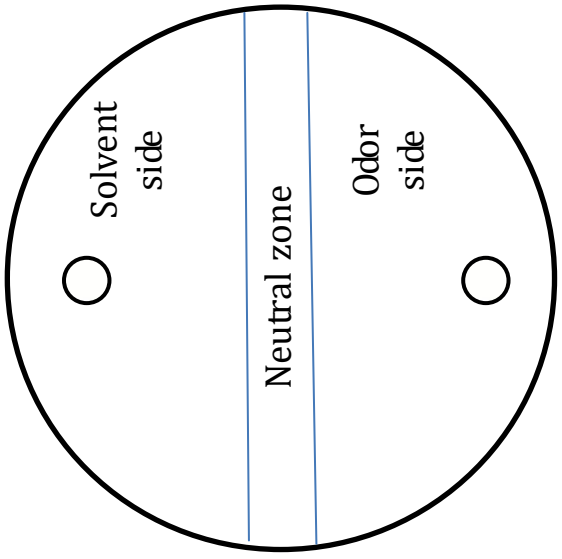
Didactic Possibilities

This activity can also be adapted for older students. We briefly introduce some possibilities to complement the experiment and make it more challenging. This also provides an opportunity to introduce the data collection concepts and the practical application of maths in experiments.

The object of this experiment is to make simple observations of larval behavior, but much more can be learnt. For example, how much do larvae like the odor? Do they prefer one odor to another? Can larvae be repelled by an odor? To answer questions like these we can introduce the use of quantification, comparison of samples, and the use of controls. Some suggestions are indicated below:

Quantification

- *How can we quantify the attraction of the larvae?* We can calculate an attraction index (ATTRAC). This involves marking out beneath the plate two lateral zones and a small neutral zone in the center (Scherer *et al.*, 2003).



The odor is placed on one side of the lid (odor-side) and a drop of paraffin oil on the other (solvent-side) as an odorless control. We spread the larvae over the neutral zone and leave them to roam. After 5 minutes, we count the number of larvae in each zone.

ATTRAC = (number of animals on the odor side
- number of animals on the solvent side)
/ (total number of animals in the plate)

This normalized score ranges from +1 (complete attraction) to -1 (complete repulsion). As the response may change over the duration of the assay, count always at the same time point. 5 minutes is enough time to allow the animals to roam without becoming habituated.

- *How do we know larvae are responding to the odor and not something else in the experimental set up?* We can do a control experiment: analyse the behavior of the larvae when exposed to the same stimulus on either side of the plate (the same odor on both sides or solvent on both sides). In this experiment we should obtain an ATTRAC = 0. If we get a different value, other factors are affecting the behavior of the larvae (maybe light or temperature).

Can we observe different behaviors?

- *Comparison of different concentrations.* How strong does an odor need to be for larvae to detect it? Do they behave differently at different concentrations? We can test a battery of concentrations to see how behavior (and the attraction index) changes.

- *Comparison of different odors.* Are larvae more attracted to odor X than to odor Y? We can compare the individual attraction indices of each odor, or we can do a direct comparison and calculate a preference index. In this case the experiment is carried out with two different odors on either side of the plate. The preference index (PREF) is calculated as the ATTRAC but substituting the solvent by odor 2. In this case a score of +1 indicates complete preference for odor 1, while a score of -1 indicates complete preference for odor 2.

  PREF = (number of animals on odor 1 side
  - number of animals on odor 2 side)
  / (total number of animals in the plate)

- *Comparison of different* Drosophila *species.* Different species have different food preferences and thus behave differently towards attractive odors. We can compare the "attractiveness" of a particular odor in different *Drosophila* species by calculating the attraction index of each species to that odor at any particular concentration. These comparisons can be used to introduce concepts like ecological differences and niche specification.

How do the larvae respond to the odor?

- *How do the larvae detect the odor?* Observe the anatomy of larvae and/or adults with a stereomicroscope or images. What sensory organs do they have? (see for example Gerber and Stocker, 2007, for a description of larval anatomy). Relate these observations to their behavior.

- *How do the larvae move in response to the odor?* Observe the larvae with a magnifying glass or stereomicroscope as they move around the agar plate. Record detailed observations of their behavior and movement. Which part of the body moves the most? Do you observe different head movements? How could this be involved in following an odor trail? (see Gomez-Marin *et al.*, 2011, for a description of larval movements during chemotaxis).

Material

For each experiment (1 or 2 children): 1 vial with 5-6 day old larvae, 1 empty petri dish, 1 petri dish containing 10 ml of 1% agarose (reinforcement rings for the lid are optional), 1 tube with 10 ml of 15% sucrose, 1 small paint-brush, a small container of water to clean the brush.
Additional material to be handled by the supervisors: banana odor (isoamyl acetate, CAS: 123-92-2), paraffin oil, pipette. The odor is best stored in a glass vial with a Teflon cap, but an eppendorf tube is fine for a few days.

Alternative odors: propyl butyrate (CAS: 105-66-8), pineapple odor (ethyl butyrate, CAS: 105-54-4), nail polish remover (ethyl acetate, CAS: 141-78-6).

Preparation of Material

Larvae: Flies are allowed to lay eggs in small food vials for 24 hours. After oviposition, flies are removed and egg-containing vials incubated at 22°C on a 12h-12h light cycle. (These growing conditions are recommended but not essential for the success of the experiment). In 5-6 days larvae reach the 3rd instar stage of development. They are easier to handle than the smaller larvae and also show a robust chemotaxis response. If wandering larvae start to appear on the walls of the food vial remove them with a paintbrush before the experiment, since at this stage they no longer forage and may behave differently towards the odor.

Odors: In this assay we pipetted 10 ul of a 1/40 dilution of isoamyl acetate in paraffin oil. (You can also use a dropper; in this case do the preparation tests with the dropper also). As the response may change depending on species (or even between different strains of the same species) it is advisable to carry out a test beforehand with a battery of dilutions, select the dilution that gives the clearest response. If the odor is too weak, larvae will take longer to respond; if it is too strong, the gradient may not be steep enough for the larvae to detect its direction – which can also result in a low attraction index. Testing of different concentrations can also be carried out by the students as a preparatory experiment.

More details about alternative set ups and tips on this assay can be found in Louis *et al.* (2012).

Acknowledgments: We are grateful to the schools CEIP Pins del Vallès (and the parents association initiative PINScience) and CEIP Diputació for allowing us to perform this science workshop, and for asking us to repeat it on several occasions. We would like to thank the Matthieu Louis and Johannes Jaeger laboratories and the Communication department at the Centre de Regulació Genòmica (Barcelona, Spain) for their support of outreach activities. We thank Matthieu Louis, Josefa González, and Elena Casacuberta for comments on the manuscript. We also thank the Louis laboratory for the development of the assay described here with funding from the European training network: FLiACT (http://www.fliact.eu/).

References: Aceves-Piña, E.O., and W.G. Quinn 1979, Science 206: 93-96; Cobb, M., 1999, Biol. Rev. 74: 425–459; Gerber, B., and R.F. Stocker 2007, Chem. Senses 32: 65-89; Gomez-Marin, A., G.J. Stephens, and M. Louis 2011, Nature communications 2: 441; Mast, S.O., 1911, *Light and the Behavior of Organisms*. Vol xi, 1st edition. Wiley, New York, p. 410; Louis, M., M. Phillips, M. Lopez-Matas, and S. Sprecher 2012, Behavioral analysis of navigation behaviors in the *Drosophila* larva. In: *Neuromethods*. Springer Science and Business Media, LLC; Robertson, F.W., 1963, Genet. Res. 4: 74–92; Sawin-McCormack, E.P., M.B. Sokolowski, and A.R. Campos 1995, Journal of Neurogenetics 10: 119-135; Scherer, S., R.F. Stocker, and B. Gerber 2003, Learn. Mem. 10(3): 217-25; Sokolowski, M.B., 1986, The relationship between third instar larval behavior and age. In: *Evolutionary Genetics of Invertebrate Behavior*. (Huettel, Milton Davis, ed.). Plenum Press.

Genetic drift leading to fixation of the *bw*^{1} neutral allele of *Drosophila melanogaster*.

Santangelo, Constance R., Kathryn A. Knackstedt, Henry B. Thorpe, Jennifer M. Kiser, Michael A. Balinski, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403.

We have previously observed a reduction in heterozygosity over time due to genetic drift for two neutral alleles (*bw*^{1} and *bw*^{75}) of the brown locus of *Drosophila melanogaster* in the presence of the scarlet (*st*) mutation (Clendenin *et al.*, 2014; Woodruff and Boulton, 2011). We also screened for losses and fixations of the two neutral alleles over time and observed one fixation for the *bw*^{75} allele (Clendenin, 2014). Yet, it was