

Do not just copy these down for the abstract! These are the learning objectives, not the actual experiment objectives!

LAB 03 – Animal Behavior

Objectives:

- To learn how to properly compose an AP lab report.
- To observe an organism's behavior and to design an experiment to investigate their responses to environmental variables.

Introduction:

Drosophila melanogaster is an organism that has been studied in the scientific community for more than a century. Thomas Hunt Morgan began using *Drosophila melanogaster* for genetic studies in 1907. The common fruit fly lives throughout the world and feeds on the fungi of rotting fruit. It is a small fly, and one could question why so much time and effort have been directed to this organism. It is about the size of President Roosevelt's nose on a dime, but despite its small size, the fly is packed with interesting physical and behavioral characteristics. Its genome has been sequenced, its physical characteristics have been charted and mutated, its meiotic processes and development have been investigated, and its behavior has been the source of many experiments. Because of its scientific usefulness, *Drosophila* is a model research organism. Its name is based on observations about the fly; the fly follows circadian rhythms that include sleeping during the dark and emerging as an adult from a pupa in the early morning. This latter behavior gave rise to the *Drosophila* genus name, which means "lover of dew." The explanation for the species name *melanogaster* should be clear after observing the fly's physical features. It has a black stomach. No doubt the dew-loving, black-bellied fly will continue to make contributions to the scientific community and to student projects.

This investigation explores the environmental choices that fruit flies make. A choice chamber is designed to give fruit flies two choices during any one test, although students could also think about how to build an apparatus that would give fruit flies more than two choices. Adult fruit flies are attracted to substances that offer food or an environment in which to lay eggs and develop larvae. Typically those environments are rotting or fermenting fruit – hence the name "fruit fly". Adult fruit flies are attracted to bright light, and their larvae move away from bright light. Adult fruit flies also demonstrate a negative geotaxis; they climb up in their chambers or vials against gravity. Movement toward a substance is a **positive taxis**. Consistent movement or orientation away from a substance is a **negative taxis**. In most cases, the experiments done in the choice chamber will be chemotactic experiments, as indicated by the number of flies that collect on one end of the chamber or another in response to a chemical stimulus. At some point, students may wish to investigate if the chemotactic response is greater than a geotactic or phototactic response. The flies could also exhibit a behavior that is not oriented toward or away from the stimulus; rather, the stimulus elicits a "change of rate" of a response. Such behavior would be considered a kinesis.

Although some activities in this investigation are open inquiry, the components in the beginning are structured inquiry, allowing for students to learn and practice key skills before they move on to more independent research.

You will investigate fruit fly movement using a choice chamber that exposes the flies to different substances that you insert into the choice chamber. Because flies are very common in households (in fact, fruit flies live almost everywhere that humans live), think about using foods or condiments that might result in a positive or a negative chemotactic response from the flies. As the old saying goes, "You attract more flies with honey than vinegar!" Let's see if this is true... Your group will be responsible for bringing in these items for the investigation.

Here are a few questions that your group should think about before starting your prelab:

- What foods or condiments do you think would attract or repel flies? Why?
- Do fruit flies exhibit a response to light or to gravity?
- How can you alter the chamber to investigate those variables?

Procedure:

Handling *Drosophila* Cultures, Tossing Flies, and a Control Run

1. Using established *Drosophila melanogaster* cultures, carefully toss between 10 – 30 living flies (we are using the apterous variant) into an empty vial. Be sure to plug the vial as soon as you add the flies. Do not anesthetize the flies before this or any of the behavior experiments – don't worry, you'll get to use FlyNap in the *Drosophila* Genetics Lab later on!
 - **Tossing Flies:** When flies are 'tossed' they are tapped into an empty vial. It might be a good idea to take the existing vial and place it in the refrigerator for a minute or two to slow the movement of the flies – *Drosophila* are ectotherms, so their metabolic rates are directly linked to the ambient temperature. Tap a culture vial (push the vial down on a solid surface several times) on the table to move the flies to the bottom of the vial. Quickly remove the foam or cotton top and invert an empty vial over the top of the culture vial. Invert the vials so that the culture vial is on the top and the empty vial is on the bottom, and tap the flies into the empty container by tapping it on a solid surface several times. Be sure to hold the vials tightly to keep them together. You must then separate the vials and cap each separately. Do not try to isolate every fly from the original culture. It is difficult to separate flies, and you may lose a fly or two in the process.
2. Prepare a choice chamber by taking a clear plastic straw and with a marker, evenly divide it into two labeled sections: I – for substance A and II – for distilled water (or a second substance, depending on your experimental design).
3. Take two pieces of cotton and dip in distilled water. Do NOT saturate the cotton – if liquid starts to run down the side of the choice chamber, it will trap the flies. We want the cotton to fit snugly in the plastic straw – we don't want any escapees!
4. Place one of the cotton pieces into one end of the choice chamber. Attach a funnel to the other side of the choice chamber. Carefully, take your vial of about 10 – 30 flies and carefully toss them into the straw. The flies should 'fall' to the cotton plug at the other side of the choice chamber.
5. Quickly, remove the funnel and fit the other distilled water cotton swab into the other side of the choice chamber. This will be our control group run. Since there is no substance except for water on either side of the chamber, there should be no preference exhibited by the fruit flies. They should be relatively equally distributed in both regions.

6. Lay this control group down on a white surface (for easier observation) on a flat (think about why!) surface. It should be easy to see the labels on the choice chamber along with the distribution of flies.
7. Give the flies 5 minutes of undisturbed time, and then count (it would be a good idea to take a picture with your phone to 'capture the moment') the number of flies at each section of the chamber. Create a table to record the number of flies you observe. Again, at this point, there ***should*** be no preference. Carry out a chi-square analysis to reject or not to reject the NULL HYPOTHESIS that there is no preference. (This is good chi-square practice and also you need to "show" that there is no preference!)

Testing Different Chemical Stimuli

8. Begin to test each substance you are including in your investigation. Place a few drops of one substance on the end of a new cotton swab (or extra cotton attachment). Remove the cotton swab in Section II and replace with your substance.
9. Lay this experimental group down on a white surface (for easier observation) on a flat surface
10. Give the flies 5 minutes of undisturbed time, and then count the number of flies at each section of the chamber. Create a table to record the number of flies you observed at this time. Carry out a chi-square analysis to test if the NULL HYPOTHESIS is rejected or not.
11. Repeat steps 8 – 10 for each substance you are testing. If you are finding that there is a positive taxis for multiple substances, then you should compare these side-by-side.
12. Follow directions given to 'dispose' of the flies. They might be used again tomorrow, or they might wind up in the morgue...

Hints For This Lab and its Write-Up:

- **Make sure you do appropriate background research for this investigation.** DO NOT just use the information in the Introduction section of this lab. Cite all sources completely and correctly.
- LARGE SAMPLE SIZE would help... Figure out how to do this. There is more than one way!
- Your hypothesis about substance preference is **NOT** the same as the NULL HYPOTHESIS that you will use in the chi-square analysis... make sure you understand this!
- Quantify the results and express them graphically. Your lab write-up should have the graphs (Excel) of all trials. Chi-square tables can be included in an appendix section if you do not want to muddle your results section, but you **MUST** incorporate the values obtained from these in the results. **USE STATISTICS APPROPRIATELY!**
- Your DISCUSSION section does not just restate the results. Review your "How to Write a Lab Report" in your binder.
- I encourage you to start the write-up ASAP. You can get help from me or have me look over a section as you complete them. Just don't wait until the day before – because then, the answer will definitely be "NO!"