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An active learning approach to teach aspects of human dietary health using fruit flies as a model

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We present a module to investigate the relationship between diet, development and behavior using the common laboratory model, *Drosophila melanogaster*. Module activities can be implemented as standalone units or as a sequence allowing for investigation of complex concepts and flexibility in implementation. Building on the extensive knowledge of *D. melanogaster* life cycle, physiology, genetics and behavior allows for various types of questions to be investigated including questions of human-related diseases such as diabetes and metabolic syndromes. Activities can be individualized through alteration of diet type or acquisition of different *D. melanogaster* stock lines. The interdisciplinary nature of the investigations provides opportunities for diverse content or to implement activities as a course-based undergraduate research experience. In addition, authentic research questions related to diet and health in a model invertebrate organism can be developed for presentations at scientific meetings. We provide pedagogical support in the form of laboratory protocols, video tutorials and supplemental information to adapt activities to different classroom budgets.

Keywords: Drosophila melanogaster, human health, physiology, CURE

Introduction

Aspects of diet and health are introduced in biology classrooms through the topics of metabolic needs and homeostasis. These topics are important for undergraduates to understand, but they can be difficult for students to relate the biological basis of health to individual, real-world applications. In a social media dominated world, the amount of information about 'diet' and current fad-diet trends are both overwhelming and biological misinforming. The conversation of diet is commonly framed in relation to socio-ecological factors and metabolic syndrome, with specific relation to stroke, heart disease and obesity. Yet dietary changes have been used to treat disease for over two-thousand years (Todhunter 1965). For example, the Ketogenic diet – high fat, low protein– has been used for a treatment for epilepsy beginning in the 1920s (Wheless 2008). Our objective was to develop a simplified teaching module that explores the relationship between diet and physiological mechanisms.

This module highlights the relationship between diet, development and behavior using *Drosophila melanogaster* (fruit flies) as a model. Fruit flies are a commonly used model organism in research to understand biological principles and became well recognized as a model for studying genetics (Rubin and Lewis 2000; Morgan 1910). Using a project-based learning approach and building on the tremendous amount of knowledge about the life cycle, physiology, genetics and behavior of *Drosophila*, this module provides students the opportunity to investigate questions concerning chronic health of their interest. This module has been

implemented in both high school and undergraduate classrooms.

Module Description

This teaching module is composed of an integrative series of activities designed to explore the influence of diet on growth and behavior, neural mechanisms, fecundity, survival, and population dynamics. The activities can be run individually as stand-alone units or as a series. Here we discuss two of the activities: (1) influence of diet on the development of *D. melanogaster* and (2) the effects of diet on behavior. Given the focus on human dietary health, we choose to focus on three specific diets, high fat (Ketogenic), high protein, and high sugar. We suggest that students work in groups of two or three, where each group then serves as a replicate for the classroom data.

Activity One – Diet & Development

For this activity students develop and test hypotheses about how diet can influence growth by tracking larval development. Larvae from the fly colony are provided to students, where they are then placed into tubes of each experimental diet and one control diet. Students can then measure time to pupation by checking development every twenty-four to seventy-two hours (twenty-four hours is preferred given the fast development time). Time to eclosion – emergent from the pupal case – can also be measured, by marking and numbering the location of each larva once they have pupated. Data can be represented as the total number of pupae as a percent of the number of larvae included, or as the time to eclosion by diet. This data can be graphed by diet type, or a Pearson's chi-square test can be conducted for additional analysis. Students can then discuss their hypotheses in relation to their, and the classroom, results, and discuss the physiological mechanisms that may be affected by diet.

Activity Two – Diet & Behavior

For the second activity, students develop and test hypotheses of how diet can influence behavior using a behavioral assay. This activity provides students with a whole organism view of the influence of diet on underlying physiological mechanisms. Prior to the activity, students should review larval anatomy and behaviors (Appendix A), developing hypotheses about how each diet type may influence one or more behaviors. Students can then create an ethogram (catalog of behaviors) to use during their assay. This provides a standardized procedure to quantify qualitative data. See Appendix A for an example ethogram.

After larvae have grown on experimental diets for a minimum of twenty-four hours, individual larvae are then transferred to a petri dish to be assayed. Using a pencil, students will stimulate each body segment of an individual larvae then record its behavior. The frequency of each individual behavior can be graphed by diet type. While our focus is on human dietary health, introducing this activity through discussion of anti-predatory behaviors provides a foundation to incorporate discussion of evolutionary mechanisms and more ecological, non-human, related concepts.

Time Requirement

Each individual activity can be completed in a two-to-three-hour laboratory time but can also be tailored for individual classroom needs. The greatest time commitment is in establishing a fly colony, which we suggest should be done approximately two weeks in advance. Information on how to establish a fly colony, including creating fly media and diets can be found in Appendix B. If conducting these activities in sequence, we suggest students prepare two experimental and two control tubes during the first experiment.

A class can be divided for students to take on various tasks and then share out their findings with the rest of the class. This can be readily accomplished in an informal manner or have the groups present a formal report to the class with accompanying literature research.

Engaging the Students

With the growing focus in lesson plans to engage students in experimental design and problem-based learning, this module highlights many approaches for the students to ask questions how diet affects health by using relatively easy to implement exercises which the students can alter depending on their curiosity. Such open-ended inquiry will help foster critical thinking skills, primary literature research and experimental design and draw conclusions from the evidence to explain the observations. Allowing the students, the freedom to modify the exercises and follow through on their questions builds a sense of scientific identity (Staub et al. 2016; Esparza et al., 2020).

Student Outline

Objectives

1. To explore the role of diet in physiological and developmental processes
2. Generate informed hypotheses regarding the effects of diet on the development and behavior of *Drosophila melanogaster*
3. Design and conduct experiments to test hypotheses

Background Information

Diet and Health

The general notion is "energy in" must be balanced with the "energy out" for the developmental and adult life requirements of organisms to be in a healthy state. Excess "energy in" can led to storage of the energy in the form of fat or surplus circulating levels of substances which can have harmful consequences, such has high lipids and sugar content in the cardiovascular circulation. Therefore, metabolism and diet type are keys factors in the energy homeostatic balance. And we use different diet types for both medical treatments for purely cosmetic reasons. For example, body builders commonly use a high protein – low carbohydrate diet to build and maintain lean muscle mass. Yet diet can also be used to control our cholesterol or blood sugar levels, and a ketogenic diet (high-fat, adequate-protein, low-carbohydrate diet) is also being used to treat epilepsy. We can investigate the effects of diet on physiological processes, mimicking the different factors observed in human conditions, by altering the diets of *Drosophila*.

Life cycle of Drosophila

After males fertilize the eggs and the female lays them on a substrate, the larvae will emerge in 1 day when maintained at room temperature (21^{o}C or 70^{o}F). Larvae develop through three stages which are easily identified by morphological changes in their mouth hooks used for feeding (Figure 1). In the late 3rd instar stage, the larvae crawl out of the food and find a place to become a pupa. After about 7 days the pupa emerge as adult fruit flies. The adults typically live for two to three weeks depending on the crowding and environmental conditions.

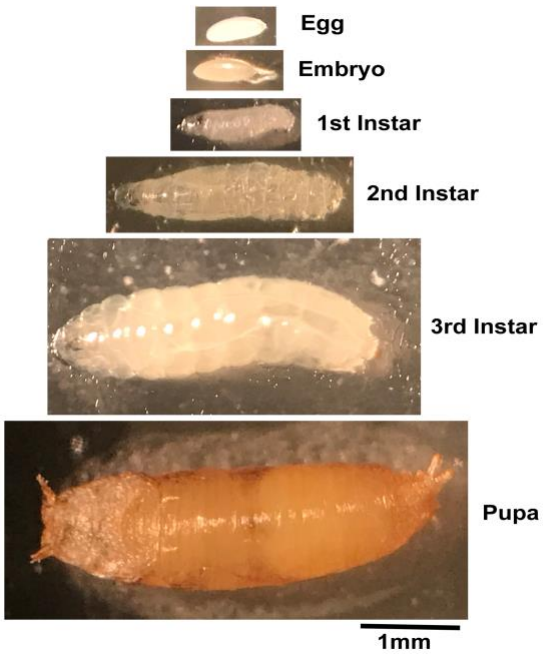

Figure 1. Development life stages of Drosophila.

Diet Options

1) *High Fructose* - A condition which is of increasing prevalence in the USA and other industrial nations is that of metabolic syndrome. This condition results in an increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels, increasing the risk for heart disease, diabetes, and stroke. To mimic some of these factors in *Drosophila* we can use a high sugar diet with alpha-fructose, a simple sugar.
2) *High Fat (Ketogenic)* - A diet high in fat can also result in metabolic syndrome. Therefore, to mimic these factors in *Drosophila* we can use a high fat diet by using various amounts of 100% coconut oil.
3) *High Protein* – To mimic a diet high in protein, like what a body builder may use, we can use synthetic soybean extract as a protein source.

Choose a diet from the list above and develop a hypothesis regarding how that diet may affect the development of *Drosophila*. State your hypothesis:

How might the diet influence the behavior of the larva? State your hypothesis:

Now let's set up the experiment to test your hypotheses.

Experiment One – Influence of Diet on the development of *Drosophila*

To test the effects of diet on larval development, we will compare larvae grown on a high fat and high sugar diet. Here we will set up replicates of each tube. We can use a one of the replicates to investigate the effects of diet on behavior (experiment two).

Materials

- 2 tubes of 10% high fat larval diet
- 2 tubes of 10% high sugar larval diet
- 2 tubes of standard fly media
- 1 dish of 2nd or 3rd instar larvae
- Paint brush/forceps (to move flies)
- Permanent marker

Procedure

1. Place 10 larvae (1st or 2nd instar larvae) into each of the high fat and high sugar larval tubes
2. Be sure to note what instar they are and how many of each you include in the tube
3. Allow larvae to burrow into food. Check every twenty-four hours for changes in instar stage
4. Once pupated, mark and index the location of all pupae using a sharpie as shown in Figure 2 (next page). Note date and time pupa form in notebook.
5. Place tubes in a light-controlled environment at 22 degrees Celsius for 24 hours
6. Check every 24 hours to determine number of individuals that pupate
7. Note any changes in pupation and survival in your notebook.

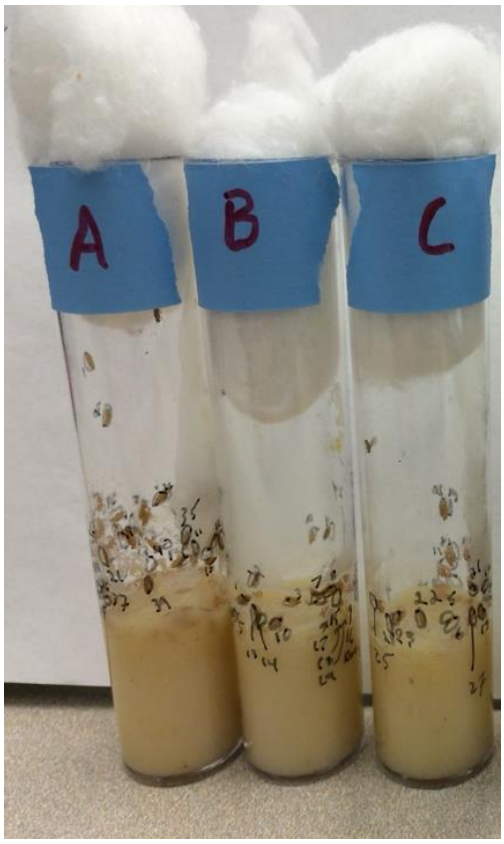

Figure 2: Marked and indexed pupa in different diet types. Diets were labeled using a lettering system, and all larvae were circled and marked using a permanent marker.

What is your dependent and independent variable? What is your control?

What was the percent survival based on diet type? Do you accept or reject your hypothesis?

Experiment Two – Effects of Diet on Behavior

Next let's investigate the effects of diet on behavior. But first we need to understand different behaviors of *Drosophila* larvae. Larvae respond to external stimuli in a variety of ways. These behavioral responses are adaptive and act as an anti-predator defense (Robertson et al. 2013). Prior to proceeding review the different behaviors that larvae can exhibit (see Appendix A).

Develop a hypothesis to explain how each diet might influence larval behavior. State your hypothesis:

Materials

From experiment one:

- 1 tube of 10% high fat larval diet from experiment one
- 1 tube of 10% high sugar larval diet
- 1 tube of standard fly media
- Tweezers or forceps to move larva
- Petri dish
- Pencil
- Stopwatch

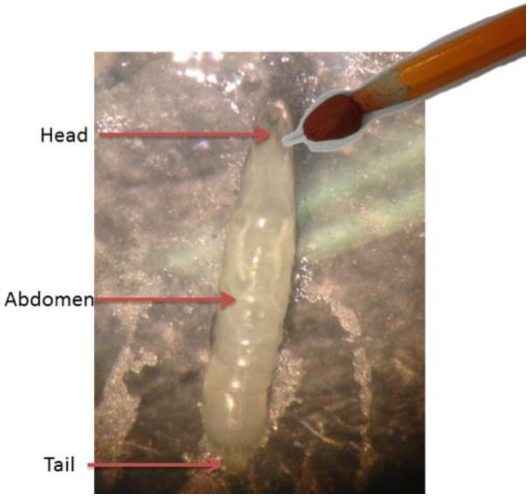

Figure 3. Illustration of body segments on larvae and points of contact.

Procedure

1. Using tweezers or a small paint brush remove a single larva and place it on a petri dish
2. Identify the stage of the larva
3. Allow larvae to acclimate for 15 secs prior to beginning the trial
4. Count the number of peristaltic waves for 15 seconds
5. Stimulate the larvae at the abdomen on the right side on the larva's body segment as shown in Figure 3 (right)
6. Record the behavioral response
7. Wait 15 seconds
8. Repeat steps 5-6 for each additional body segment
9. Conduct stimulus test on a minimum of 10 larvae per diet type and control

Note: You may stimulate larvae on either body size, but keep it consistent throughout!

When all stimulus tests are finished, calculate the fraction of each behavioral response by diet type. Do you accept or reject your hypothesis?

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Timmeren SV, Diepenbrock LM, Bertone MA, Burrack HJ, Isaacs R. 2017. A Filter Method for Improved Monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) Larvae in Fruit. Journal of Integrated Pest Management. 8(1): https://doi.org/10.1093/jipm/pmx019

Materials

Our goal is for college level programs of all budget types to be able to use this model. We have provided information on all necessary materials and supplies including where materials can be purchased and alternative options in both Appendix B and on the module website (http://web.as.uky.edu/Biology/faculty/cooper/ABLE-2021/ABLE-2021 metabolic%20syndrome%20in%20flies/Home-aspects%20of%20human%20dietary%20health-Drosophila%20model-ABLE%202021.htm). This includes information on starting and maintaining a fly colony, conducting various behavioral measures, how to measure developmental time from larva to pupa, and how to build a microscope.

Fly Growth

The life cycle of *Drosophila melanogaster* is simple and predictable. Females lay up to 500 eggs on fermenting fruit. The male fertilizes the eggs, and in 24 to 30 hours the larvae emerge. Larvae are short, segmented, and whitish yellow in color and crawl around, feeding on food and gorging themselves. Larvae progress through three distinct stages, as denoted by changes in their mouth hooks (small, black hooks at the anterior of the larvae). Toward the end of their larval stage, fruit flies begin climbing the walls of their enclosure, eventually becoming immobile as they transition into the pupa stage. At this time, they turn dark brown in color and form a hard outer shell. They will remain in this state for about a week, after which time they emerge as adult fruit flies. An adult female fruit fly can begin to mate about two days after emerging.

The two activities presented here are set up sequentially, therefore setting up a duplicate vial will allow one to fully pupate and the other to be tested for behavioral differences. Eggs, first instar or second instar larvae can be used to set up the experiments. Growth from egg to third instar larva takes approximately five days (Figure 1), and larval stage can be determined by anatomical and behavioral differences. Adult flies can be exposed to each food type allowing them to lay eggs, then eggs can be moved to hatch on different food types. This would require a minimum of a four-hour time window. This approach makes it difficult to know the number of viable larvae one is starting out with. However, one could use control food and compare relative to the controls. For more precise measures of survival (experiment one), we strongly suggest moving first or second instars into various types of food. It is easy to damage the larvae, so care is required when transferring them. We suggest having a fly colony started on traditional media to make it easier to set up the activity and provide flexibility in timing. A procedural outline for establishing a fly colony and preparing individual vials for the activities can be found outlined in the Appendix B.

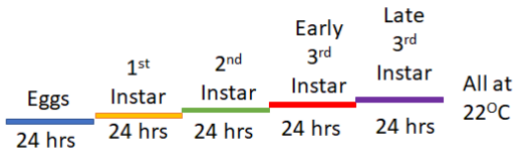

Figure 1. Developmental timeline of Drosophila at 22 degrees Celsius.

Food Preparation & Fly Diets

To maintain adult flies for breeding and rearing, a cornmeal-molasses-agar media can be made. A complete ingredient list and instructions can be found for the media in the Appendix B. Various additions can be included to this standard media to investigate the effects of different diet types. For example, if one wants to examine essential amino acids in a diet, different amounts of amino acids could be used and in combinations. Different *Drosophila* lines with mutations are available to investigate defects in amino acid transport and enzymes used in metabolism (St Clair et al., 2017; Sasamura et al, 2013). Flies can also be requested from one of the authors (Robin Cooper).

To focus on diets related to human health one, or more, of three different diets can be used for this activity: (1) high fructose, (2) high protein (soybean extract), and (3) high fat (coconut oil). These additions can be included in the standard media in 5%, 10%, 20%, 40% per weight of diet. A higher fat diet than 40% is difficult to use as the fat does not mix well with the food. Standard fly media food can be used and mixed to these percentages based on wet weight of the readymade food. This is the easiest approach in our experience. Do be careful and keep the food moist and not allow it to dry out. Adults may stick to the food or the side of a tube if their wings are wet and touch the surface, so some care is needed to avoid this issue.

Notes for the Instructor

We developed the module to allow for flexibility in content investigated by building on the

same two foundational modules. Therefore, the module can be implemented as consecutive units, as it's presented here, or as stand-alone single units. The behavioral assays in locomotion and diet we present relate to precise neural control of the musculature which is readily relatable to human performance, how diet effects neural function and cellular metabolism for energy production. Specifically, the research in how the ketogenic diet alters neural function for control of epilepsy relates to the basic understanding of synaptic transmission (Rogawski et al., 2016). Yet the ease of the modules allows variation in specificity, so it can be tailored to classroom needs. Additional concepts can be explored using these two modules as building blocks. These concepts include survival of the adults and population dynamics (Oh and Oh 2011, Potter et al. 2016, Pulver et al. 2011); the effects of a ketogenic diet on behavior and function related to treatment of epilepsy (Boison 2017); and using heart rate as a bioassay for health of the larvae exposed to various diets (Spindler 2005, Potter et al. 2019). Various stages of the larvae can be used for investigation into these questions. The development from larvae to adults can also be addressed by varying diets.

The techniques and tools introduced to conduct this module are also open ended in that the participants will see that they are made up of very basic inexpensive materials and new instrumental designs are possible for the behaviors measured. The idea of coming up with novel approaches in measuring behaviors and developing ethograms can be encouraged for a class to encourage teamwork. Additional information on integrating these additional activities can also be found at the module's website.

This module, and others in the series, have been implemented in several undergraduate classroom. Supplemental videos, available on the module website, feature undergraduates explaining many of the concepts or techniques. These videos can be used as supplements to classroom learning or as pre-laboratory activities.

For additional engagement regarding the context of the activity, a pre-lab assignment where students investigate the health status of their local community could be implemented. Using the CDC website (https://www.cdc.gov/healthyyouth/data/yrbs/index.htm), students can examine the rates of cardiovascular disease for example. This can spark interest in the topic and help provide more specific background for students to help frame the concepts for students.

We performed an informal survey on how the students felt about these activities. They appreciated the variety of different approaches which were taken and the relative ease in obtaining data that they could then present at local undergraduate research conferences. In addition, they were able to divide the overall project into subgroups with some focusing on development and others on behaviors.

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About the Authors

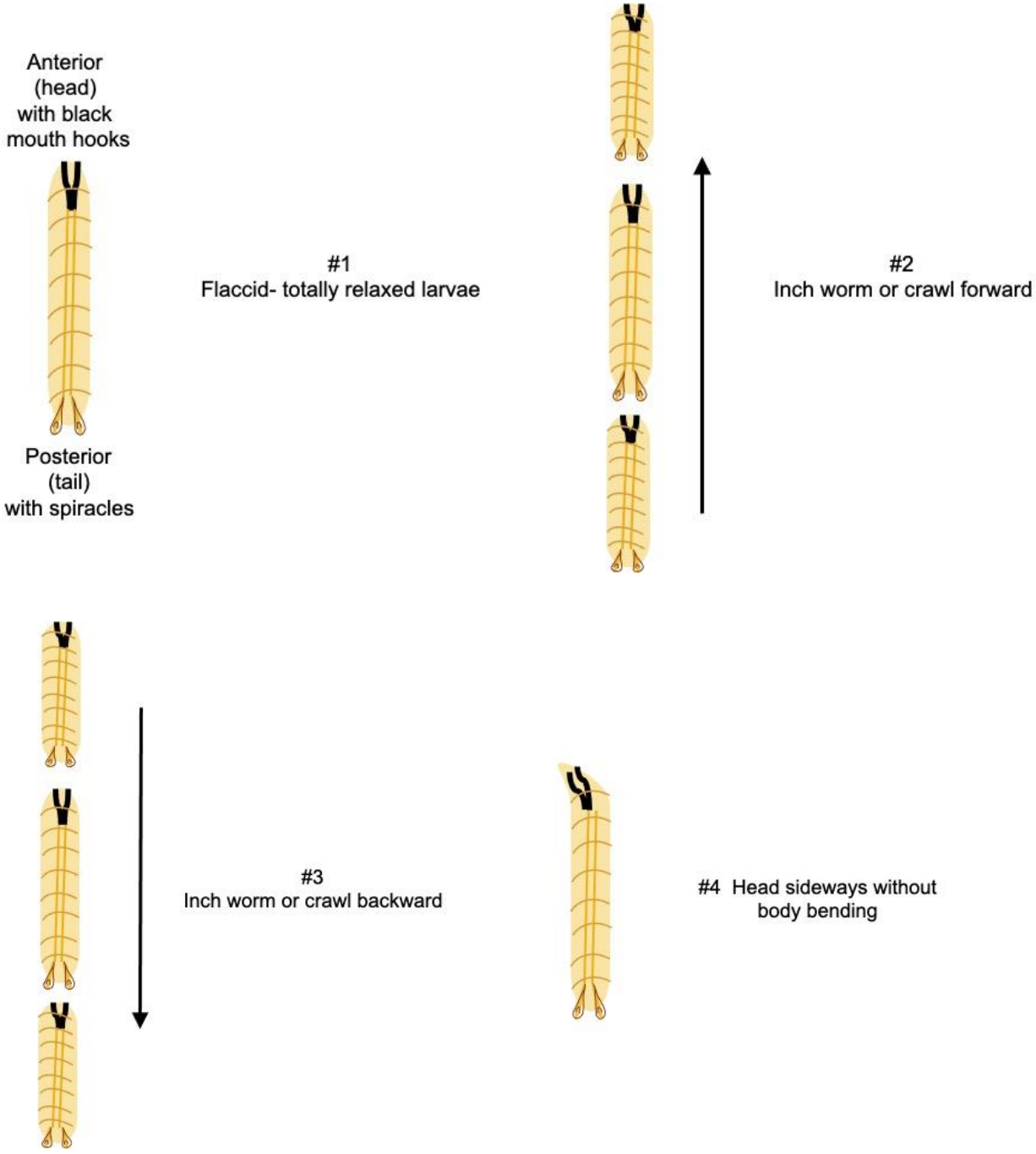
Brittany L. Slabach is a vertebrate ecologist. She received her B.A. from College of the Atlantic in 2009, her M.Sc. from Tufts University in 2012 and her Ph.D. in Ecology and Evolution from the University of Kentucky in 2018. She is currently a Visiting Assistant Professor at Trinity University where she teaches in the introductory biology series and upper division courses in ecology.

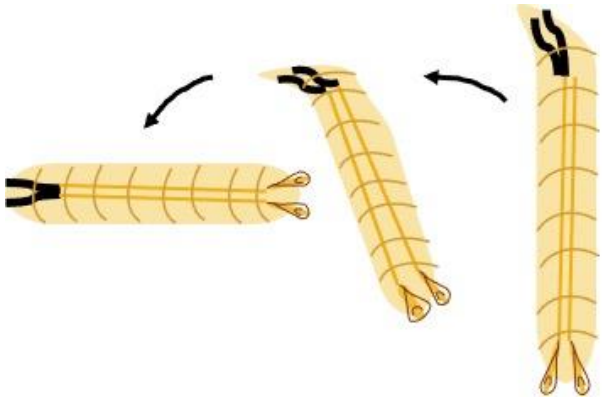
Robin Cooper is an instructor of animal physiology and neurophysiology at the University of Kentucky. He received a B.S. from Texas Tech in 1983 and a Ph.D. in Physiology from Texas Tech Medical School in 1989. He has been at the University of Kentucky since 1996.

Appendix A

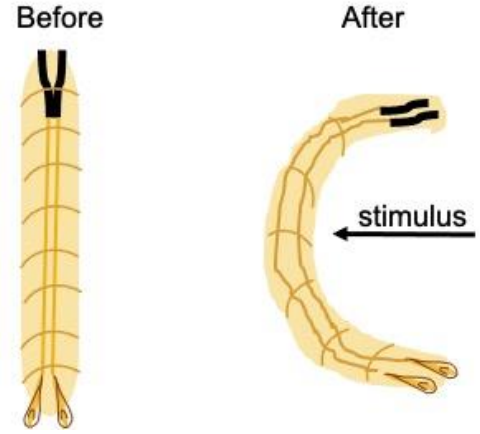
Larval Behaviors

Videos of individual behaviors can be found on the module's website.

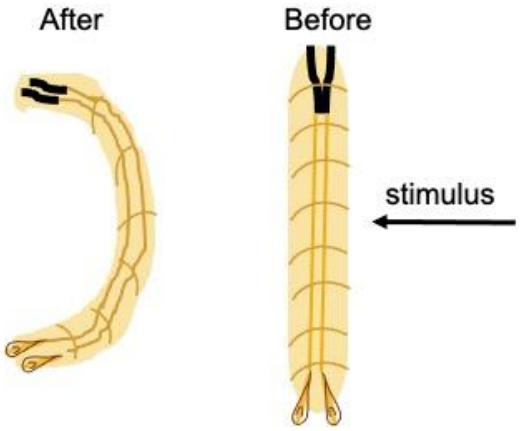




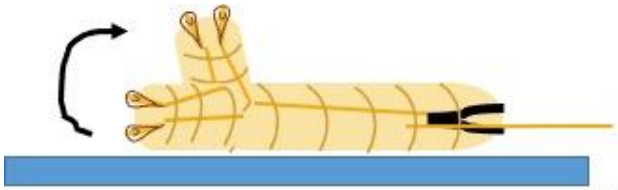
#5
Head sideways
and body moves
that direction



#6
Full C-bend toward stimulus



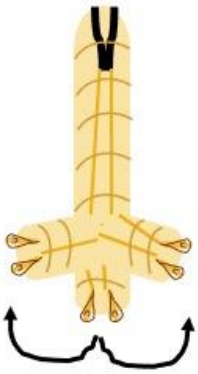
#7
Full C-bend toward stimulus



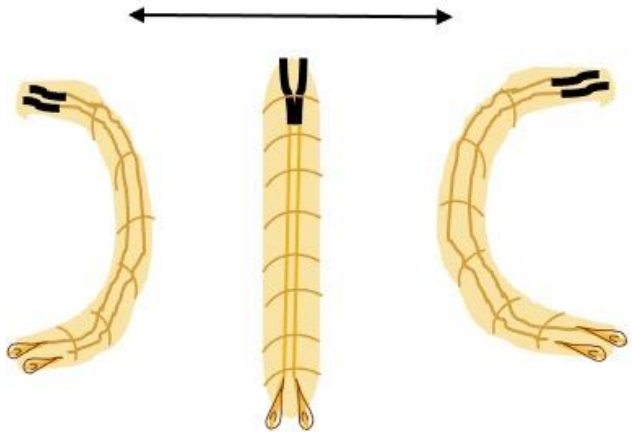
#8
Tail raised off substrate over body

#9
Head raised off substrate over body

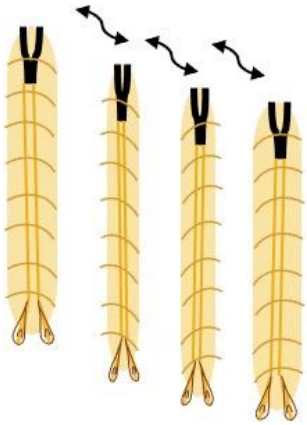
#10
Head wag back and forth



#11
Tail wag



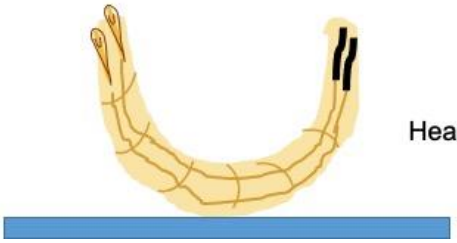
#12
Body back and forth with C-bends



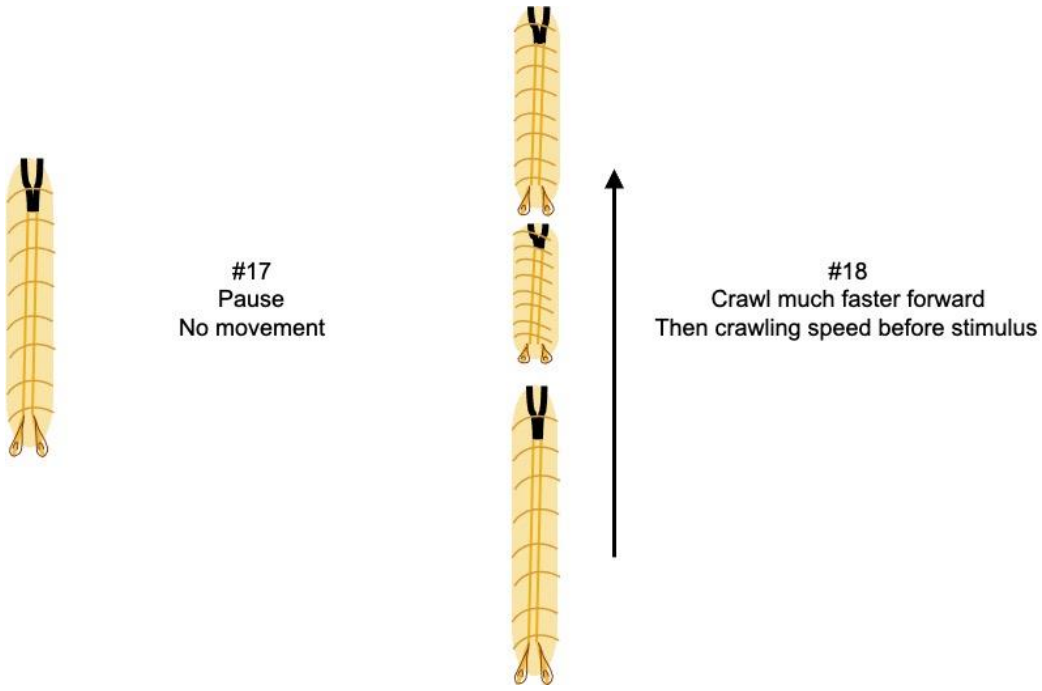
#13
Rolling

#14
Tail bends sideways without body bending

#15
Tail tuck under body



#16
Head and tail rise off surface



Behavioral Ethogram

An ethogram is a category of behaviors. We suggest students review larval behaviors and focus on those that are specific to their hypothesis. Creating an ethogram of their focal behaviors, like that below, will provide a standardized way for students to quantify behaviors.

Behavior	Head	Abdomen	Tail
Reverse	2		
Head raise	2		
Pause	1	2	
C-bend	5	2	1
No Reaction		7	9
Turn	1		
Head Retract		1	
Roll			
Tail Flip			

Appendix B

Standard Fly Media

All items can be purchased from Sigma Aldrich and fly media can be purchased ready to use from Archon Scientific. Additional information on where to purchase ingredients and for making your own fly media can also be found at the Indiana University Bloomington Drosophila Stock Center website: https://bdsc.indiana.edu/information/recipes/bloomfood.html

Ingredients:
- 420 mL water
- 4.5 gm agar
- 60 mL of unsulfured molasses
- 49 gm cornmeal (any kind)
- 6.5 gm brewer's yeast
- 145 mL cold water
- 3.4 mL of propionic acid (acts as a mold inhibitor)

Mix 420 mL of water and agar, bringing the mixture to a boil for about 3–5 minutes. Add unsulfured molasses and heat to boiling again. Mix cornmeal, brewer's yeast, and cold water in a separate container until all lumps are removed. Add cornmeal-yeast mixture to molasses-agar mixture. Boil mixture for 5 minutes, stirring constantly. Cool mixture to 60°C. Add propionic acid. Pour culture medium 1-inch deep into sterile culture jars with sterile plugs. Add a sprinkle of active baker's yeast (from a saltshaker) to each jar before adding flies.

Additional Diet Options

To create additional diet options including high fructose, high protein, or high fat diets, soybean extract or coconut oil can be used. These additions can be purchased online or at your local grocery store. These diets can be created using standard media in 5%, 10%, 20%, 40% per weight of diet. Standard fly media food can be used and mixed to these percentages based on wet weight of the readymade food. This is the easiest approach in our experience. It should be noted that to use a higher fat diet than 40% is difficult and can result in suffocation of larvae.

Fly Colony

Drosophila can be housed in vials partially filled with fly media. The larvae of *D. melanogaster* can be grown in the laboratory within a plastic cage. A plastic beaker and petri dish can be used to create the cage (see movie on website or visit https://youtu.be/vJ7ZV0hxM5g). Petri dishes wish 1% apple juice agar (1/2 filled) bottom with some standard food pressed against one side of the dish to hold it in place so it will not be dislodged when turned over when switching out the dishes. A small hole is cut in the bottom of the plastic beaker small enough that cotton plug can cover the hole. (Note: Use of a soldering iron makes it easy to cut out a hole without the plastic cracking. Don't breathe the fumes from the melting plastic).

A colony can be started by including 50 males and females. Flies can also be ordered through the Indiana University Blooming Stock Center (https://bdsc.indiana.edu) or can be requested from one of the authors (Robin Cooper). Having adults in this container for 3 days prior to egg pulse will ensure a good amount of freshly collected eggs. Each day for 3 days, preferably first thing in the morning, replace the apple juice agar dish with a fresh dish. On 3rd day replace the dish for a fresh one and allow the adults to lay eggs for 4 hours. Remove this dish and mark 4-hour egg pulse with time of collection. Repeat again for another 4 hours and label "hours of collection time on the dish and 4-hour egg pulse".

Use a dish with food to keep the adults alive for the next day so collections can be repeated. The dishes with the eggs cannot have a petri dish lid as the CO_2 will build up and the embryos and larvae will die. We use lids with fine netting glued to the lids and then place the lids on the dishes. The dish can be left for a day like this if one is collecting 1st instars. If one wants to collect second or third instars, then the agar and food will dry out if left without checking for moisture. What works is using a larger petri dish with paper around the smaller dish and a lid placed to the side as not to trap the CO_2. For additional information on how to knock out flies see movie (https://youtu.be/sGP_5ByY4NM).

After 24 hours at 22-24 degree Celsius the egg dish will start to show the hatching and first instars can be collected. These larvae if allowed to continue to develop will yield second instars (day two), and third instars (day three). Early third instars will remain in the food and late third instar (day four) will be wandering and stop eating as they find a place to pupate. Depending on the time to dedicate to this exercise instars can be removed at the desired stage and placed in the food of choice. Once the colony has started, you can transfer the instars into a long tube containing the experimental food. Continue to repeat this procedure for the different foods to be examined.

Apple juice plates: Used for colony growth and crawling behaviors

Ingredients:

- 10.1 g Agar (general lab agar)
- 330 mL of water
- 11.1 g of table sugar
- 111 mL of Apple juice (juice, not drink flavor)
- 0.66 g1 p-Hydroxybenzoic acid methyl ester, Methyl paraben, NIPAGIN - (SIGMA, catalog # H3647-100G, CAS Number 99-76-3)

Mix agar with water and bring to a boil. Be sure bubbles are occurring to dissolve all the agar. Turn off heat but keep on stirrer. Add sugar and apple juice to mix fully. If going to keep for a few weeks add preservative. Pour into the plastic Petrie dish and cover. Then place in zip lock bag and put in refrigerator.

Microscope

Watching the larvae crawl and responses to touch, one can use a stereomicroscope (a standard dissecting microscope) with at total 20x magnification (such as using an eye piece 5x and zoom at 4x). An observer can then record all behavioral responses to touch or number of inch worm movements in a set period. If one does not have a dissecting scope, one can use simple and inexpensive approaches to magnify the larvae. We made a movie to show various approaches one can use (https://www.youtube.com/watch?v=wTSynwmyHBQ).

[1] Be careful to not breath this in! Use proper PPE when handling.

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