RockEDU
SCIENCE OUTREACH

Drosophila
husbandry & study in the classroom

THE ROCKEFELLER UNIVERSITY

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Created by A. Ryba with RockEDU Science Outreach at The Rockefeller University, 2022

GENERAL "SAFETY" OVERVIEW

Drosophila melanogaster is one of many species of vinegar fly, harmless insects that eat microorganisms on decaying fruit. *D. melanogaster* are found around the world as human commensals (animals that have adapted to live close to human society).

However, flies breed **rapidly**. To avoid growing an annoying colony in a place you don't want it, handle flies only when you can be sure that they will remain contained (see p. 6 for handling methods).

Colonies grown in small vials will live about 3 weeks and those grown in larger bottles will live about 1 month before they run out of food. Containers can be disposed in normal waste, when necessary.

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WHERE TO GET FLIES

If you're not interested in trapping wild flies to culture in the classroom, there are multiple places that sell cultures at a reasonable price. Remember, it only takes a few flies and a bit of time to have a big colony!

Drosophila melanogaster are readily available for purchase online, thanks to their popularity as a "model" organism in lab. Carolina Biological sells wild-type (non-mutant) flies, along with fly lines that carry various visible mutations for genetics experiments. There are many other sites on the internet that will ship you living flies, but use caution when purchasing: some cultures can come infected with parasitic mites, microscopic creatures that outcompete flies for food and gradually make cultures sick.

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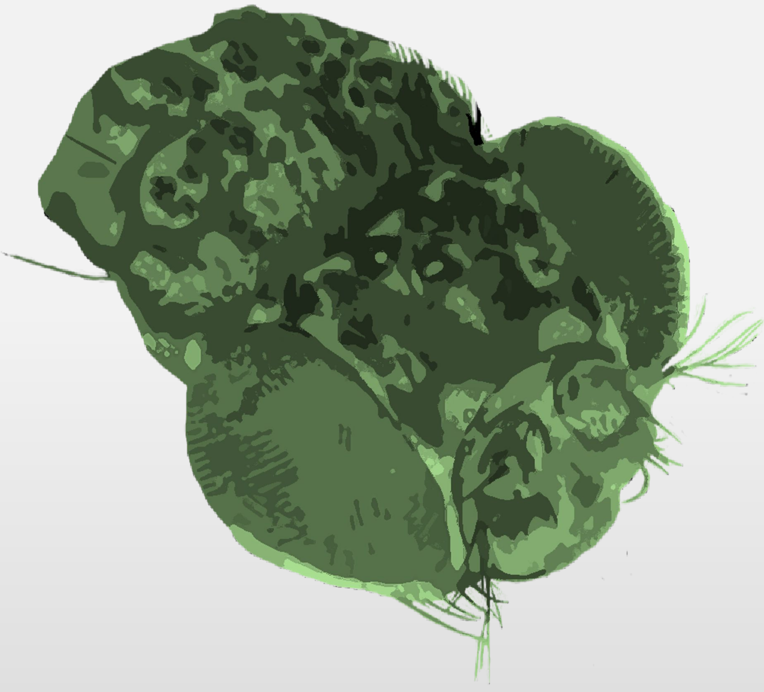
WHERE TO GET FLIES

The National *Drosophila* Stock Center at Cornell University supplies over 250 species of flies, if you're interested in more exotic species.

And the Bloomington *Drosophila* Stock Center is a researcher's go-to source for transgenic flies.



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FLY CONTAINMENT

The **fly morgue** and a **vinegar trap** will help manage escapees:

Set up a large, easy to access container of alcohol (generally 70% ethanol) to dispose of any loose flies used in experiments etc. The alcohol prevents bacteria from growing, so if you can get it, it's preferable to water. (The morgue in the picture has a small funnel in the top for ease of access.)

Flies ***love*** apple cider vinegar. Set up small jars of vinegar covered loosely with plastic wrap (just crumple it slightly and lay it over the trap without sealing it) to trap escapees.

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STORING FLIES

You'll want to culture flies in clear containers containing about 1.5 cm of food media and plugged with something porous (cotton is easiest).

Find plastic vials and/or bottles designed for fly culture at online lab supply stores (or on Amazon ;)) Or use old spice jars/baby food/takeout containers that have been thoroughly cleaned and sterilized.

A healthy *Drosophila* culture

Find custom plugs here/here or rip off bits of cotton wool (buy rolls). Even large cotton balls work! If your container is wide, you can cover it with cheesecloth or a similar porous fabric and secure it with a rubber band.

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FLY FOOD

As larvae grow, they burrow through a food substrate eating as they go. (See Life Cycle on p. 5 for details.) There a number of options for fly food, depending on your time and resources.

Two recipes are included as downloadable resources. One is very easy to prepare in a classroom and the other is a more standard recipe used in many fly labs. Keep in mind you might end up making hundreds of vials of food (depending on your needs), so some planning and pre-preparing goes a long way.

Banana food – requires only bananas and active dry yeast

Agar/cornmeal food– requires cooking, but contains antibacterial agents to prevent smells.

You can also buy pre-mixed food here.

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ENVIRONMENT
RAISING HAPPY FLIES

In lab, flies are usually kept in an incubator that maintains a constant temperature of **25°C** and relative humidity of **58%**.

They get **12 hours of light and 12 hours of dark** per day. The timing of the light/dark cycle sets their circadian rhythm.

In the classroom, keeping such constant conditions won't be possible – and isn't necessary. However, because flies can be sensitive to environmental fluctuations, having students take careful notes on temperature, humidity, and light conditions can be useful.

(See Logging Data on p. 18 for more details.)

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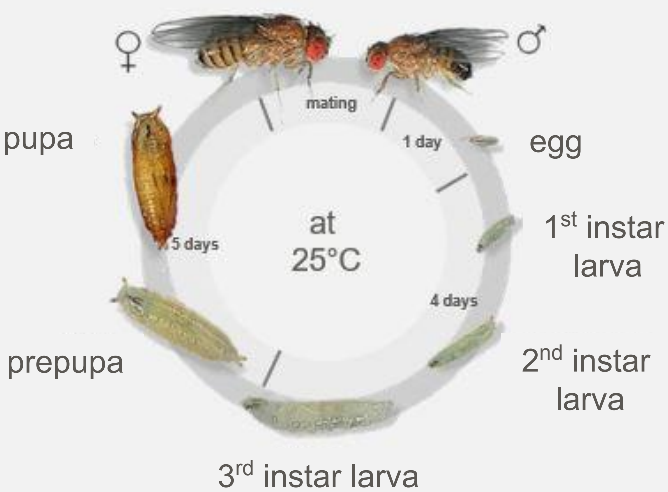
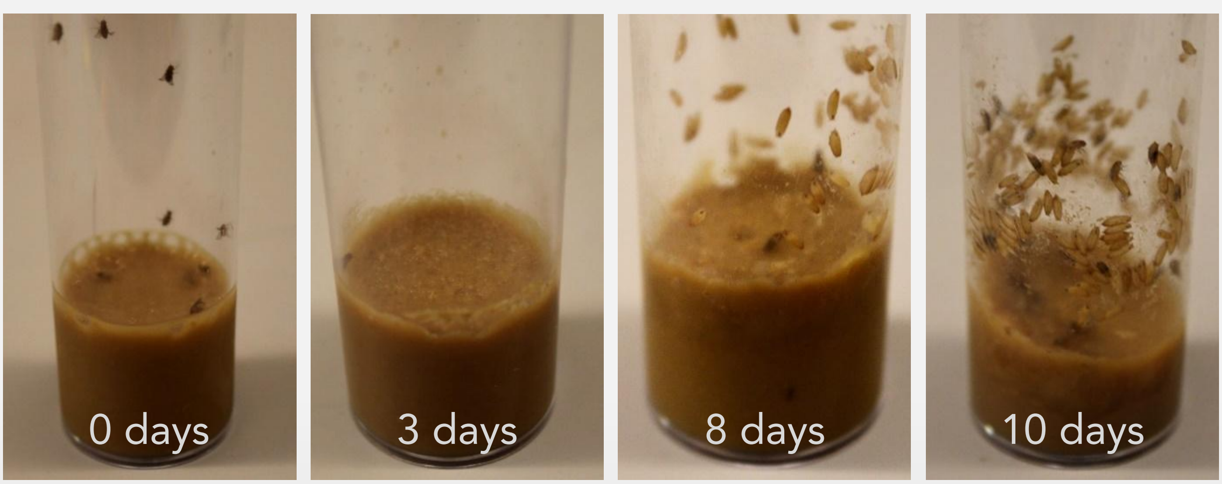
LIFE CYCLE OF A FLY



Flies undergo **complete metamorphosis**: adults lay eggs, which hatch into larvae. Larvae crawl out of their food media, pupate, and eclose as adults.

The timing of the cycle depends on temperature. At 25°C, flies have a **generation time** (egg to adult) of 10 days. At warmer temperatures, this cycle is faster; at cooler temperatures it's much slower.



Food looks scuffed a few days after a cross is set, as larvae hatch and eat. Larvae crawl up the walls and pupate around day 6. On day 10, pupae eclose as adults. After 15-20 days (depending on the amount of food), the cross gets old and should be disposed of.

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GENERAL WORKFLOW

The fly's life cycle suggests an ~10 day cycle of work: every week and a half a new generation of flies will be ready for use.

To keep things clean and to prevent an explosion of flies, you should pay attention to crosses. Here's an example to give you an idea of the flow of fly work.

DAY 0 (Friday) - place flies on fresh food (for small containers use ~5 females and 5 males, for large bottles use ~10 of each sex)

DAY 10 (Monday) – a new generation of flies will eclose. More flies in this generation will eclose every day this week. To prevent overcrowding, take flies out periodically this week and dispose of them or use them for experiments.

DAY 14 (Friday) – if you want a week's break, set new crosses and dispose of all original crosses.

PACE – do you prefer continuous access to flies? Then set new crosses every couple days and keep old crosses active until new ones are ready.

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BASICS OF “FLY PUSHING”

“Fly pushing” refers to the everyday work of fly husbandry – sexing, setting crosses, etc. It’s so called because fly researchers spend a lot of time pushing flies around under a low-magnification microscope. To handle flies easily, we anesthetize them on a pad that pumps out carbon dioxide. Researchers spend a lot of time setting and maintaining crosses – flies breed rapidly!

Soft bristled paint brushes are and efficient but delicate way to manipulate flies. Fine-tipped forceps can also be useful for picking animals up by the wings.

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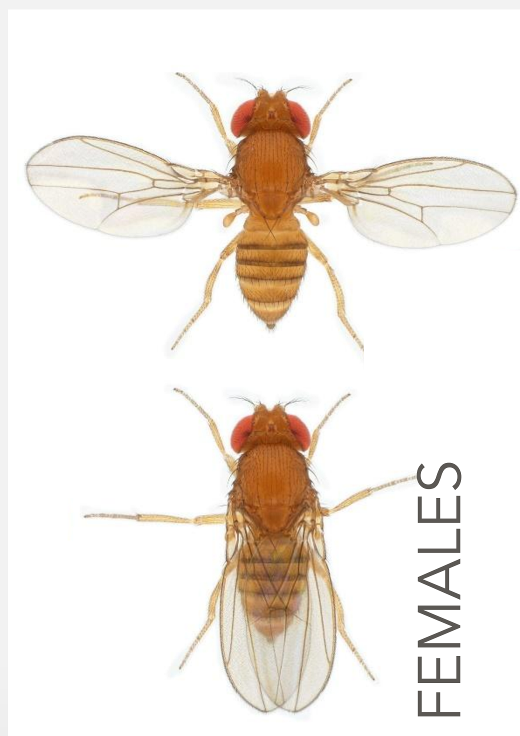
BASICS OF "FLY PUSHING"

Sexing flies – It's possible to distinguish male and female flies (even without a microscope). This skill can be very useful when setting up mating schemes between specific animals or for isolating sexes in behavior experiments.



Male flies have a blunt abdomen that's generally dark, in contrast to the pointy, lighter abdomen of females. Males also have appendage (claspers) on the end of the abdomen and sex combs on the forelegs. Females lack both.

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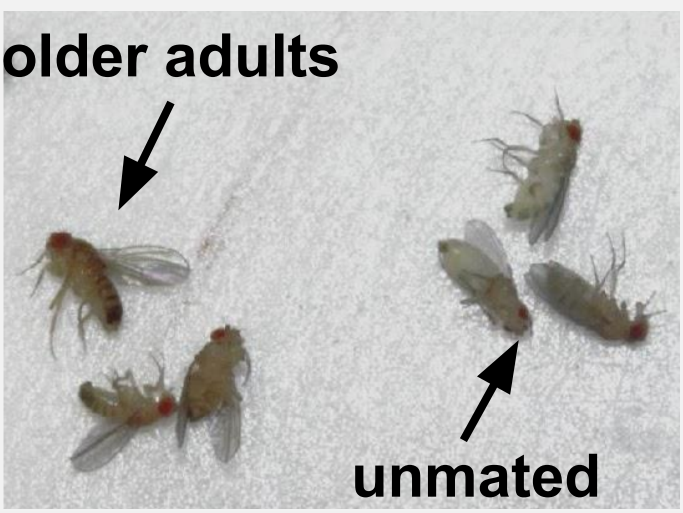
BASICS OF "FLY PUSHING"

Unmated flies – When dealing with fly genetics, it's important to be able to predict the genotype of offspring with certainty. The first step is to know exactly who is mating with whom. Flies are able to mate ~6 hours after the eclose as adults, so to be of the parents in a cross you need to isolate breeding pairs/groups onto new food before then.



male female

Unmated flies are distinguished by their slightly puffy appearance, the fact that they have hardly melanized (so are lighter in color), and the presence of a food spot in the lower abdomen known as a meconium.

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Carbon dioxide anesthesia

Carbon dioxide (CO_2) is a potent fly anesthetic. It's easy and safe for classroom use, and will knock flies out for minutes at a time. Alka seltzer tablets kick off CO_2 when placed in water, as does baking soda in vinegar. Included is a downloadable describing how to capture the gas to use as a cheap, easily deployed anesthetic.

You can watch a video of the procedure on the RockEDU website here.

Purchasing FlyNap

You can also use FlyNap supplied by Carolina Biological to anesthetize flies. (Unlike ether, it's deemed safe for classroom use, but it has a strong smell.) FlyNap comes as a kit, and knocks flies out very effectively for upwards of half an hour. It's more expensive than baking soda, but perhaps less fussy.

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LOGGING DATA

Careful data logging helps students monitor the health of their fly cultures. Students should choose one place to keep a lab notebook and set aside time to write in it daily. Lab notes record work and thought processes in real time, making it easier to iterate on methods that need tweaking. Careful notes also allow others to follow up should they want to. A few guidelines for note taking:

1. Include identifying information with each entry (e.g. date, cross name/number, etc.).
2. It's fine to make changes, but keep the previous version visible.
3. Below is a template, but feel free to modify based on your class's needs.
4. Keep students accountable. Perhaps they record data in a shared document periodically or submit notes for occasional review.

Date	Temperature	Relative humidity	Number of pupae	Notes
6/14/2021	25°C	49%	5	Lights on: 8 AM Lights off: 8 PM Air pressure: 29.8

A template for husbandry notes

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LOGGING DATA

A battery powered temperature and humidity sensor can be useful for logging environmental data to help care for fly cultures. These devices are readily available on Amazon. The sensor keeps a running record of the ambient temperature and relative humidity. You can switch between Farenheight and Centigrade by pressing the button on top. Generally, lab science records temperature in °C.

E.g. – the sensor shown above is available from Amazon for $14.

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ITERATION

Flies have a rapid generation time - just 10 days. This gives science classes a great opportunity to iterate on initial husbandry attempts.

Students should be able to collect about a week of data on their original crosses before the new generation of flies ecloses. The fast generation time means that experiments are easy to pilot: students get used to the rhythm of husbandry and will be able to tweak anything that isn't quite working for them. Science often involves this sort of iteration!

That is, students shouldn't stress too much if things don't work out the first time: a few mistakes and modifications are part of the process – especially when working with live animals.

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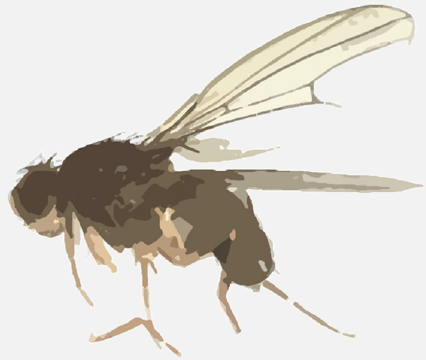
NOTES ON "FAILURE"



- Working with live animals increases the chances of unexpected things happening – e.g. crosses that don't take, escaped flies, animals that don't behave as expected...Sometimes these complications are born of mistakes. These mistakes needn't be seen as "failures", however! In fact, learning to become comfortable with uncertainty is one of the most important steps toward thinking like a scientist!

- Re-frame mistakes as additional data. They give students a better understanding of the limits of their system and show them what to modify going forward.

- Science is <u>full</u> of failures. Huge discoveries have come from screw-ups, and beautiful theories often contain fundamental errors. Encourage students to use inconsistencies and imperfections to uncover a new way of thinking. Complications reflect the intricacy of the natural world, rather than a weakness in science.

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NOTES ON “FAILURE”

- Sometimes, a “failed” experiment isn’t a mistake at all. Uncertainty is inherent to science, particularly when working with living creatures. Documenting the process and building on what you’ve learned is always enough.

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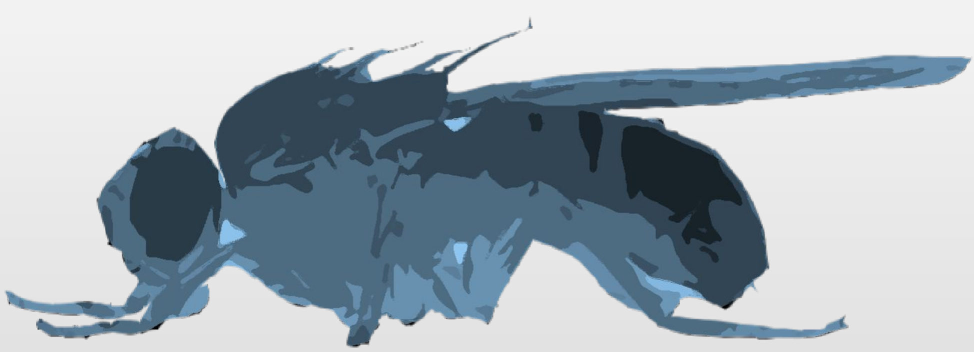
Observation & documentation:

Working with living organisms is an opportunity for students to practice two fundamentally important skills in science – observation and documentation. In addition to keeping careful track of environmental conditions, encourage students to spend 5 minutes every day watching their fly cultures. Do they notice their flies doing something interesting? Encourage them to note their observations. These explorations form the start of scientific inspiration. Helping student develop a more open-ended approach to science could be a whole set of lessons in and of itself, if you choose.



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WHAT'S NEXT?

IDEAS FOR EXPERIMENTS

If your students are confident and are excited by their flies, consider having them formulate their own questions. Have them record everything: observations, questions, hypotheses... For a few days. THEN sit down and synthesize the notes. Is there a pattern to observed behaviors? Is there something worth following up on with a more specific question? Time and resources may not allow them to test the question, but this process of iteration and scientific exploration is a valuable exercise in and of itself.

Alternatively, flies can be used to supplement a set curriculum on all sorts of topics in biology. Check out some starting points outlined in the *Drosophila* collection on the RockEDU website.

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