Drosophila Workers Unite!
A laboratory manual for working with Drosophila

By Michele Markstein
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Title and Description of Primary Image: Drosophila Workers Unite! This homage to “Rosie the Riveter” celebrates workers, women, and the model organism Drosophila melanogaster.

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This book is dedicated to all my students—past, present, and future.
Acknowledgements

I am grateful to my graduate and postdoctoral mentors, former labmates, and the students in my own lab for making this book possible. In particular, I would like to thank Kristopher Kolbert, a former undergraduate in my laboratory, who designed the Drosophila Rosy-the-Riveter protagonist of the book and produced all of the drawn illustrations. Kristopher convinced me to include a section on hidden figures in the chapter on “Drosophila researchers then and now.” I am also indebted to Jonathan DiRusso, who as an undergraduate in my lab developed our protocol for imaging adult flies (without shadows!) and produced the majority of photographs in this book. I would also like thank Sonia Hall for encouraging me to transform the original version of this manual, which I wrote to accompany a laboratory course, into a full-fledged manual for all Drosophila beginners. Lastly, I am indebted to colleagues for their insightful comments on drafts of this manual including: Laura Quilter, Sonia Hall, Gregory Davis, Michael Dietrich, Pamela Geyer, Norbert Perrimon, and Michael Levine.

Author’s Note

This book explains the tricks of the trade of working with Drosophila. Normally these tricks are passed down from researcher to researcher, not learned from a book. In fact, I never intended to write about the basics of doing fly work. However, over the last six years, this book basically wrote itself while I trained over 120 students in laboratory courses and over 80 students in my research laboratory. Drosophila Workers Unite! is intended to be a staple for all fly labs.

Are you new to Drosophila?

Then this book is for you!

Drosophila Workers Unite! will teach you how to work with flies in the lab, from putting them to sleep to designing and setting up genetic crosses. If you read the first few chapters before your first day in lab, you will thoroughly impress your teacher! The book starts with pointers on how to find your way around a fly lab and ends with online resources that you can use whether you are a beginner or an expert. In addition, you can learn about the five Nobel prizes awarded for fly research and pick up some facts about the first fly researchers that your lab head and/or instructor probably doesn’t know.

Note to teachers: If you are teaching a unit on Drosophila, this book will help your students hit the ground running. It explains why Drosophila is a powerful model organism, beginning with a discussion of the Drosophila genome and available genetic tools. This book will also help you teach students how to distinguish females from males, how to set up genetic crosses, how to recognize common dominant markers, and how to use the Gal4-UAS gene expression system. Unique to manuals on fly pushing, this book also discusses Nobel Prizes awarded for Drosophila research, as well as early “hidden figures” in Drosophila research who developed many of the tools that we use today, including the CyO balancer and S2 tissue culture cells.
# Table of Contents

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1. Drosophila: Great and Mighty!</td>
<td>p.6</td>
</tr>
<tr>
<td>Chapter 2. The Fly Life Cycle: Pros and Cons &amp; How to Maintain It</td>
<td>p.10</td>
</tr>
<tr>
<td>Chapter 3. Introduction to CO2 and Fly Pushing</td>
<td>p.20</td>
</tr>
<tr>
<td>Chapter 4. How to set up fly crosses</td>
<td>p.28</td>
</tr>
<tr>
<td>Chapter 5. Drosophila Chromosomes, Genetic Symbols, and Genotypes</td>
<td>p.38</td>
</tr>
<tr>
<td>Chapter 6. Genetic Markers</td>
<td>p.42</td>
</tr>
<tr>
<td>Chapter 7. Drosophila Balancers</td>
<td>p.56</td>
</tr>
<tr>
<td>Chapter 8. The Gal4-UAS Gene Expression System</td>
<td>p.60</td>
</tr>
<tr>
<td>Chapter 9. Drosophila Nobel Prizes</td>
<td>p.70</td>
</tr>
<tr>
<td>Chapter 10. Drosophila Researchers: Then and Now</td>
<td>p.76</td>
</tr>
<tr>
<td>Chapter 11. Resources for Students and Teachers</td>
<td>p.92</td>
</tr>
<tr>
<td>Chapter 12. Major Drosophila Stock Centers</td>
<td>p.94</td>
</tr>
<tr>
<td>References</td>
<td>p.96</td>
</tr>
</tbody>
</table>
1. Drosophila: Great and Mighty!

It may surprise you to learn that Drosophila researchers often forget that fruit flies are insects. Indeed, we often think of Drosophila as simplified humans or inexpensive mice. This is because on a molecular level, Drosophila are surprisingly similar to us!

The molecular similarities between Drosophila and humans came into sharp focus when their full genome sequences were published at the turn of the century (Figure 1.1; Adams et al., 2000; Lander et al., 2001; Venter et al., 2001). It turns out that Drosophila has almost as many genes as we do—14,000 genes compared to our 21,000 genes. Even more impressive, over 65% of human disease-associated genes have a correlate (homolog) in Drosophila (Ugur et al., 2016). This means that you can use Drosophila to learn about many of the genes and genetic pathways that function in humans.

What makes Drosophila great and mighty is that it enables us to do experiments that would not be feasible in humans, or most other model organisms. What kinds of experiments? Experiments that we want to perform in living animals (“in vivo”) on a massive scale, such as a genetic screen to uncover all the genes involved with how cells communicate. Experiments where we want to turn specific genes on in specific cells, at specific times, and at specific levels. Experiments where we want to learn about how embryos develop, or how animals age, or how we learn, for example. Experiments where we want to test the limits of genetic engineering.

Drosophila biology reflects many facets of human biology from the mechanics of cell division and cell polarity to developmental biology, behavior, and disease. The protagonist in this book is Rosy (one of the first mutants discovered) and an homage to the inspirational Rosie the Riveter.
The complete sequence of the fly genome was published in the Journal Science in 2000. The entire issue is devoted to understanding what the sequence means in relation to the development, physiology, evolution, and similarity to humans, of Drosophila.

In Drosophila, we can do all of these kinds of experiments in living animals on a massive scale.

Textbooks like to say that what makes a model organism great is that it has a short life cycle, is small, and is easy to grow in a lab. These details are somewhat boring and are neither necessary nor sufficient to make a model organism great. Drosophila has all these features, but so do countless other small animals.

What Drosophila has, that your run-of-the-mill fast reproducing, small and friendly animal does not have, is a long history of well-funded research. Drosophila has been cultured in the lab for over 100 years with decades of support from the Carnegie Institute, the National Institutes of Health (NIH), the National Science Foundation (NSF), the Howard Hughes Medical Institute (HHMI), as well as countless smaller foundations including the March of Dimes, the American Heart Association, and the American Cancer Society.

Over the course of the last 100+ years, researchers have built genetic tools that make doing genetics in Drosophila easier and more powerful than any other multicellular animal on earth (with the exception of the roundworm C. elegans, which is admittedly equally awesome). These tools include:

1. Visible “markers” that make it possible to track the inheritance of any linked gene.
2. Balancer chromosomes, which make it possible to keep stocks with lethal mutations.
3. The Gal4-UAS system which makes it possible to express transgenes in any tissue at any time in the life cycle of the fly.
There are admittedly some drawbacks to working with Drosophila. First, they are alive. Second, they like to mate even when you don’t want them to. Third, they cannot be stored cryogenically. What this means is that you have to take care of them.

Adult flies have one major mission in life, which is to make as many offspring as time permits. At the standard so-called “room temperature” of 25°C, adult flies develop from fertilized eggs in 9–12 days. This is great because it means you can get lots of flies fast. But it also means that you have to stay on your toes, keeping flies happy and making sure they don’t get overcrowded.

To compensate for the fact that flies are constantly reproducing, you will have to transfer flies to new vials or bottles to keep them from overpopulating and destroying themselves within the confines of their homes. Every Drosophila researcher occasionally forgets to transfer flies in a timely fashion, resulting in an “overpopulation catastrophe”. If you experience this, the good news is you’ll be very careful in the future to transfer your flies frequently!

Topics covered in this Chapter:

1. Fly Life Stages
2. Fly Life Cycle
4. How to Keep Your Own Stock Collection
5. Quick guide to flipping flies
**Fly Life Stages**

Although Drosophila researchers often see the fly as a simplified human, it is indeed much more than that. With flies you can study 4 completely different life forms, depending on the stage you decide to study: embryos, larvae, pupae, and adults (Figure 2.1).

Fly eggs can be easily collected by the hundreds to thousands to study embryogenesis. In fact, the 1995 Nobel Prize in Physiology or Medicine was awarded to three Drosophila researchers for their work using Drosophila embryos to dissect the genetics of how a fertilized egg develops into a multicellular animal: Dr. Christiane Nüsslein-Volhard, Dr. Eric Wieschaus, and Dr. Edward Lewis. If you are interested in cell and organismal growth, larvae are an excellent choice to study, as they undergo tremendous growth in just a few days. On the other hand, if metamorphosis is your favorite subject, you can study pupae which undergo radical metamorphosis involving massive cell death and the growth of new body parts from specialized cells, housed in 19 structures called imaginal discs. Adult flies are also a popular choice to study, as their molecular and cellular biology, physiology, and behavior provide insights into human biological processes and diseases. For example, the discovery of innate immunity in humans owes it origins to research in Drosophila. The scientist who discovered the molecular basis of innate immunity in Drosophila, Dr. Jules Hoffman, was awarded the 2011 Nobel Prize in Physiology or Medicine.

**Fly Life Cycle**

Regardless of which stage of the fly you work on, you will need to have an idea of the complete fly life cycle to plan your experiments.

If you want to work with eggs and embryos, plan on doing experiments within minutes to hours after eggs are laid by their mothers. If you want to work with larvae (also called instars), you have a choice of three stages, called L1, L2, and L3. You can collect L1 larvae about 24 hours after eggs are laid. However, L1 and L2 larvae are very small and can be difficult to study. L3 larvae are the most frequently used in experimental studies: they are the largest and most robust. With a pair of #5 forceps, you can easily transfer L3 larvae from vial to vial, and with some practice, you can dissect imaginal discs out of them. Expect L3 larvae to crawl out of the food about 5 days after eggs are laid. Once an L3 crawls out of the food, it will crawl as a larva for 12-16 hours and then pupate.
During the first 12 hours of pupation the pupa is called a “pre-pupa” and can be distinguished from the other pupal stages because it is translucent white. Pupae darken as they age, transitioning from white, to caramel, to dark brown. You can gauge when a pupa is about to hatch based on how dark it is.

In Figure 2.2 you can see pupae at different stages, including dark pupae (e.g., #2), which will hatch in the next 24 hours. About a day before a pupa hatches, you can see adult structures through its pupal case: the wings appear as two black structures running along the length of the pupal case, and the eyes appear as two prominent spots in the head region. When a fly hatches out of the pupal case (referred to as eclosion), it leaves behind its empty pupal case, which you can spot by looking closely at the walls of old vials or bottles. In total, a pupa requires about 5 days to develop and hatch (eclose) into an adult fly.

Standard Temperatures for Rearing Flies

The rate of fly development from egg to adult is described in this manual for the standard temperature of 25°C, often called "room temperature." You can speed up fly development by raising progeny at 29°C, where it takes only 7-9 days for a fertilized egg to develop into an adult. However, flies are generally less healthy at 29°C, so do this only if you need to. You can also slow down fly development by raising flies at cooler temperatures. For example, if you raise flies at 18°C, it typically takes two weeks for a fertilized egg to develop into an adult. 18°C is ideal for keeping stocks because it means that you can flip the vials less often than at 25°C. Most fly labs have incubators dedicated to each of these standard temperatures (Figure 2.3).
How to Keep Your Own Stock Collection

Most fly labs keep a fly "stock collection" which consists of two vials (copies) of every fly stock used in the lab. The flies in the stock collection are typically not used in experiments directly. Instead, they are simply maintained and available when a researcher needs a copy of a stock. In most labs, the stock collection is maintained by a designated individual with lots of experience raising flies. If you want a stock from the collection, this person will flip the flies from the stock collection to a new vial and give it to you to keep for your experiments. This method of handing out copies of stocks helps to ensure that the stocks in the collection stay pure.

The key to keeping any stock collection is to flip the flies frequently so that they do not get overcrowded. This means that you should transfer your stocks to new vials on a regular schedule. Most labs maintain their stocks at cool temperatures in the range of 16°C-18°C, because flies grow more slowly at these cooler temperatures, thereby giving you several weeks before having to transfer them to new vials. If you are lucky enough to have access to an 18°C incubator, you can keep your stocks healthy by flipping them to new vials on a monthly schedule. However, if you keep your stocks at room temperature or 25°C, you will need to transfer your stocks to new vials every two weeks.

Guide to flipping flies

Figure 2.4
How to flip flies. Before flipping your first stock of cherished flies, you should practice with a vial of flies from the trash. Review the steps to the left and ask an experienced fly pusher to guide you.
Stock Maintenance

Keep each stock in two separate vials, side by side in an 18°C incubator as follows:

Day 1: Flip your flies to a new vial, Vial #1

Day 2: About 16-24 hours later, flip flies from Vial #1 to a new vial, Vial #2.

If you follow this method, you will have your stock in two vials. Vial #1 looks empty, but it is not! It has all the fertilized eggs that were laid in the 16-24 hour window that the adults were present in the vial. Vial #2 has the parents of the fertilized eggs that are in Vial #1. See Figure 2.5.

After adults have lived in Vial #2 for an entire month, the vial will likely be overcrowded with adult flies and the food will likely appear “soupy,” which is a side-effect of having too many larvae churning the food. If you look closely at the adults they will likely have battered wings as a result of age and overcrowding. You may wonder then, what the point is of keeping Vial #2 around? It is simply a backup vial. For example, Vial #2 can come in handy when you want adults before they have hatched from Vial #1.

Tip: 18°C is the preferred temperature for maintaining your stocks of flies because at 18°C they develop slower and live longer!
3. Introduction to CO₂ & Fly Pushing

Every lab that works with flies must have a way to temporarily put flies to sleep so that researchers can examine them, count them, and set them up in genetic crosses. These skills are collectively called “fly pushing.”

There are many ways to put flies to sleep. In the olden days, researchers used ether vapors to put flies to sleep. However, this practice has largely been replaced with safer methods that include ice, FlyNap (triethylamine), and carbon dioxide gas (CO₂).

CO₂ is the most commonly used method in research labs because it is highly effective and easy to work with. This manual focuses on how to use CO₂.

**Note:** It is essential to ask someone for help before working with your lab’s CO₂ tank and regulator. The pressure inside a CO₂ tank is enormous and dangerous if not handled correctly. Again, do not work with the CO₂ tank until trained by someone in your lab!

**Topics covered in this Chapter:**
1. CO₂ tanks, needles, and flypads
2. Getting the right flow of CO₂
3. Getting CO₂ into fly vials
4. What if flies start to wake up?
**CO₂ tanks, needles, and flypads**

A full tank of CO₂ has a PSI (pounds per square inch) of 750. You may have heard that if a tank were to fall over without a safety cap it would shoot through the wall like a torpedo. This is not a myth! For a real-live view, see demonstration by *MythBusters*. To avoid catastrophes make sure your tank is fully secured, as shown in Figure 3.1.

![Figure 3.1 Working Under Pressure](image1.png)

CO₂ tanks are under enormous pressure and must be secured with a belt (as shown) or a boot.

To regulate the amount of gas released from the CO₂ tank, three control devices are used: a regulator that enables gas to flow from the tank itself, a quarter turn valve that enables gas to flow at your fly work station, and a clamp to control to flow of CO₂ through the needle at your fly work station. Typically, the tank regulator (Figure 3.2) is set to release CO₂ at about 10 pounds per square inch (PSI), Once the gas is turned on at the regulator, you can control the flow of CO₂ with the valve at your fly station.

**Figure 3.2 CO₂ Regulator**

Tank regulators typically have two gauges: one shows the pressure in the tank and the other shows the pressure flowing from the tank to the fly stations. The gauge on the right shows tank pressure is 500 PSI and the guage on the left shows pressure flowing to the fly stations is 10 PSI.
Getting the right flow of \( \text{CO}_2 \) at your station

Most commonly, you will have a "quarter-turn" valve at your microscope station (Figure 3.3). Do not open it all the way because it will likely allow too much pressure locally and will burst apart the tubing. As the tubing bursts, it may knock over the morgue or other glassware leading to an unpleasant start to your day in the fly room.

You should turn the valve slowly until you have "just enough" \( \text{CO}_2 \) flowing from the needle. Get someone in your lab to demonstrate what "just enough" \( \text{CO}_2 \) is for your system. Typically people use the sound of \( \text{CO}_2 \) flowing from the needle, or the feel \( \text{CO}_2 \) flowing from the needle to estimate the right amount of pressure. Of course, the final test is how the flies behave. If they start to wake up during your work, you probably need to turn the valve a little further open.

\( \text{CO}_2 \) at your fly station typically has two outlets: (1) a fly pad and (2) a needle (Figure 3.4).

Use the needle to put the flies in vials and bottles. As shown in Figure 3.4, hold the vial or bottle upside down while gassing the flies with \( \text{CO}_2 \) so that the flies fall asleep on the cotton rather than in the food. Once you put flies to sleep in a vial or bottle, you can gently tap them onto the fly pad. The fly pad is engineered to distribute flowing \( \text{CO}_2 \) to the flies, thereby keeping the flies asleep.

Remember to hold the vial upside down when putting flies to sleep. This is to ensure that they fall asleep on the cotton, not the food!

Paintbrushes are the most commonly used tool to move flies around on the fly pad. Some people prefer to use feathers or even forceps. Whichever you use, remember your job is to be a "gentle giant."

**Figure 3.3**

Quarter Turn Valve When the valve handle is perpendicular to you, it is fully closed, and when it is pointing at you, it is fully open. Typically, it’s a bad idea to open the valve all the way. Turn the handle slowly and stop once you have a “good flow" of \( \text{CO}_2 \) from the needle.

**Figure 3.4**

Microscope & Fly Station Fly stations deliver \( \text{CO}_2 \) to the fly pad and a needle. The fly pad is used for sorting flies and the needle is used to put flies to sleep in vials (as shown) and bottles.
What if flies start to wake up?
First, do not panic. Depending on how awake they are, there are different steps you can take. Hopefully you are calmly reading this in advance of any wake-up catastrophes.

What if flies start to twitch!
If flies start twitching on your pad, make sure that CO₂ is not still flowing to your needle. If it is, clamp it shut to direct more CO₂ to your flypad. Also, cup your hands over the flies to concentrate the CO₂ around them. If this doesn't help, check that the tank still has gas!

What if flies start walking!
If flies start to walk, you're in trouble. As fast as possible, brush them into a vial! If they are walking off the pad, you must kill them. See Massive Wake Up Catastrophe!

Massive Wake Up Catastrophe
If flies are starting to stream off your fly pad, there's no other solution than to kill them all. Ask for help! But if no one's around, you'll have to do it yourself! Your choices are to crush them by hand (with or without paper towels) or to drown them with a handy squirt bottle of water or ethanol. If you use the drowning method, you will have to follow-up with paper towels to clean them off the bench top. If you use the crushing method you will have to follow up with ethanol, to clean up the mess. Don't forget to wash your hands after!

Getting CO₂ into vials
First open the CO₂ valve and adjust until you have a “good flow” of CO₂ from the needle. Then pick up the vial or bottle of flies and turn it upside down in your hand. Next, insert the CO₂ needle between the cotton plug and sidewall of the vial/bottle. Keep holding the vial/bottle upside down as the flies fall asleep onto the cotton plug. You can help them fall down faster by gently tapping the sides of the vial/bottle. Once it looks like all the flies are sleeping, wait an extra 5 seconds before pulling out the needle, to ensure that the flies are deep asleep when you transfer them to the fly pad.

Keep the vial/bottle upside down throughout the process. Once they are asleep, gently remove the cotton plug and tap the flies onto the fly pad. Then put the cotton back in the vial/bottle. We recommend that you keep the vial/bottle on its side so that any remaining flies have a chance to wake up without getting stuck in the food at the bottom of the vial/bottle. Remember, do not put more flies on the pad than you can sort in 10 minutes!
4. How to Set Up Fly Crosses

Setting up fly crosses is the bread-and-butter of being a fly geneticist. As long as you know how to identify females of one genetic stock and males of another, you can combine them in a vial and get fertilized eggs. This process is called "crossing flies."

To cross flies, you will need to know how to identify females and males. The process of identifying the sex of flies is called "sexing flies." In this chapter we will teach you how to sex flies and set them up in fly crosses.

Topics covered in this Chapter:

1. Sexing flies
2. The importance of virgin females
3. Collecting virgins
4. The clock method
5. Clearing vials and bottles
6. Visual virgins
7. Collecting virgins using a genetic trick
8. Specifics of using heat-shock inducible Hid
9. Setting up crosses
**Sexing flies**

Sexing flies means telling the difference between females and males. It's easy. Turn them on their back and look at their genitals (Figure 4.1). Female genitalia are pale in comparison to male genitalia. Males can also be identified by dark bristles, called sex combs, on their front pair of legs. It's challenging to spot the sex combs but gratifying once you do. So, give it a try! However, don't rely on sex combs to identify male flies because it takes too long. Instead, get used to looking at fly genitalia to identify females and males.

**The importance of virgin females**

Fly genetics has been successful over the last 100 years because flies are easy to cross and their progeny have many phenotypes you can track. But before you begin, you need to know something about fly sex that your parents may not have told you: female flies like to control the fertilization of their eggs by storing sperm in an organ called the spermatheca. Each time a female mates, she stores the sperm in her spermatheca which she then uses to fertilize her eggs. This means that for you to set up meaningful crosses, you need to use virgin females. Using virgin females is the only way to ensure that the offspring from the crosses you set up are from your crosses and not from earlier matings that the females set up themselves.

**Collecting virgins**

There are three ways to collect virgin females. Most researchers use the “clock method,” which is based on the fact that females cannot mate for several hours after they hatch (eclose) from the pupal case. The clock method enables you to collect large numbers of virgin females, but takes careful planning. Another commonly used method is the “visual method,” which enables you to collect the youngest of the virgin females based on their appearance, which for the first hour after hatching is visually distinct from typical adult flies. This method is a convenient way to collect virgins without any planning on your part. But it is not a good method to use when you need a lot of virgins because “visual virgins” represent only the youngest of all the virgin flies in your vial. The third method requires that you use special fly stocks that kill off all the males before they have a chance to mate with the females—in these stocks, all the females remain virgin because there are no males for them to mate with.
**The clock method**

Luckily for us, when female flies hatch at 25°C, they cannot mate for the first 8 hours of their adult life. So, if you collect female flies in the first 8 hours of their adult life, they will not have mated and you set them up in crosses that you completely control.

How do you know if a fly is 8 hours old or younger? The trick is simply to use “clearing” and timing: if you clear a vial or bottle of all adults in the morning and then return 8 hours later in the afternoon, any adults in the vial/bottle must be 8 hours old or younger.

If you think about it, the clock method could have you running into lab every 8 hours to collect virgin females. Luckily, you can avoid this simply by having your flies hatch at 18°C rather than 25°C. This is because at 18°C fly development slows down, stretching the window that newly eclosed females remain virgin from 8 hours to 18 hours. This means that you can “clear” a vial or bottle of all the adults, and when you come back the next day, within 18 hours, all the new adult females will be virgins. This is great because it means you can collect lots of virgins and still have dinner and a full night of sleep. Typically, researchers clear vials and bottles at 4 PM and collect virgins the next day before 10 AM (Figure 4.2).

**Clearing vials and bottles**

For the timing method to work, it is essential that you master the art of “clearing” vials. Clearing is a polite word for killing all the adults. The most ethical way to do this is to put the flies to sleep with CO2 before dumping them into the morgue. After dumping out the sleeping adults, look carefully in the vial/bottle and along the walls, to make sure there are no lingering adults remaining. If there are, tap them out, or use a disposable wooden dowel to push them into the food (which is another way of killing them).

**Visual virgins**

When flies hatch, they have a distinct “newly hatched” white puffy appearance for their first hour of life that makes them easy to spot. Females with a newly hatched appearance must therefore be virgins. However, males that just hatched also have a newly hatched appearance, so be sure to look carefully at the genitals of newly hatched flies so that you select only the females when collecting virgins.

Another visual marker that distinguishes young flies is a dark abdominal spot, which is typically present in flies for their first 2-3 hours after hatching. The dark spot is the fly equivalent of meconium, sometimes called flyconium, and is the remnants of the fly larval gut inside the intestine of the adult. Flyconium is typically expelled within the first 3 hours of an adult fly’s life. If you forgot to clear your vial or bottle, you can still collect the youngest of the virgins because of their distinct appearance.

---

**Figure 4.2**

The Clock Method of Collecting Virgins

If you use this method you will likely keep the vials/bottles at 18 degrees and collect flies on an 18 hour schedule, making sure the vials are clear of all adults at 10 AM and 6 PM.
Collecting virgins using a genetic trick

There is an easy genetic method to get virgin females that doesn’t involve running around 8-hour and 18-hour clocks to collect them. The method is to use a stock with male flies that are genetically engineered to die before they hatch. This kind of stock only gives rise to females, which in the absence of their male brothers, remain virgins indefinitely. This allows you to collect virgins at your leisure to set them up in genetic crosses.

If you think about it, a stock of flies must have both females and males to make new generation after generation of progeny. So how can a stock persist if all the males are engineered to die? The trick is that they are engineered to die only under certain conditions, such as a “heat-shock” in which the fly vial/bottle is placed at 37°C for 1-2 hours. You should have at least two copies of the stock: one to keep at normal temperatures to maintain the stock, the other to subject to heat-shock to collect virgin females.

The stocks for this purpose have a special Y chromosome engineered with a lethal transgene, called “heat-shock inducible Hid.” The Hid transgene encodes a protein that activates programmed cell death (apoptosis). It is engineered under the control of a heat-shock promoter which activates gene expression when flies are placed at 37°C (see Figure 4.3). Since males are the only flies in the stock with a Y chromosome, when you place flies at 37°C, only the males die. Typically, the heat-shock is carried out in a vial of fly embryos, aged 0 to 24 hours old. The heat-shock causes all the male embryos to die while all the female embryos continue developing, eventually becoming larvae, then pupae, and finally adults. Since there are no males after the heat shock, the hatched females remain virgin indefinitely. You can get Hid fly stocks from the Bloomington Drosophila Stock Center.

**Figure 4.3**

Heat shocking in a water bath

Heat-shock experiments are most commonly done by submerging fly vials and bottles in a 37°C water bath. Be sure to keep the cotton tops above the water though!
**Specifics of using heat-shock inducible Hid**

The Hid transgene works best when it is expressed during the first 0-24 hours of the fly’s life, while it is still an embryo. To use this method, you need to get a collection of 0-24 hour fly embryos. Sounds hard, but it’s easier than it sounds! Simply put adult flies in a vial/bottle of freshly made fly food overnight. Label the vial/bottle “embryo collection.” When you come back the next day, flip the adults to a new vial/bottle. The vial/bottle labeled “embryo collection” now contains a collection of embryos that are 0-24 hours old. Put this vial/bottle in a 37°C incubator (preferably half-way submerged in a water bath) for 1-2 hours to activate expression of the Hid transgene (Figure 4.3). Then put the vial/bottle at 25°C to enable surviving females to develop. In about 10 days the adult flies—all female—will hatch. In rare cases you may see a few adult males too. These are called “escapers” because they escaped death. Luckily, male escapers tend to be sterile, so your females will very likely still be virgins.

**Setting up crosses**

Once you know how to put flies to sleep, and how to collect virgin females, it’s easy to set up fly crosses. You simply count out about 5-8 sleeping virgin females and 5-8 sleeping males and put them in a new vial where they will wake up, mate, and lay eggs. It is best to keep vials of sleeping flies on their sides; once the flies wake up you can right the vial. The mating flies are referred to as a “cross.”

When female and male flies are first introduced, they need a couple of days to become acquainted with each other. After that, the females will start laying lots of fertilized eggs every day. Therefore, it’s a good idea to flip your cross to new vials every one to two days after the initial “getting to know you” period. Flipping the cross to new vials every one to two days will ensure that the vials do not become overcrowded with progeny.

If you forget to flip your cross to new vials, the food will become oversaturated with larvae. Under these conditions, the larvae will not get sufficient food, they will develop poorly, and they will often be undersized or “minute.”

To figure out how many times to flip your cross, think about what fraction of the progeny will be the genotype that you want and how many flies you need with that genotype. On average, expect about 100 offspring from each vial of a healthy cross with 8 fertile females crossed to 8 fertile males.

Once you calculate how many progeny you need to collect, check with your mentor to make sure you have a good plan for raising and flipping the cross.

Note: Use young flies and fresh food to increase the number of progeny from your crosses.
5. Drosophila Chromosomes, Genetic Symbols, & Genotypes

Drosophila, like us, is a diploid organism. This means that it has two copies of each chromosome set: it gets a complete set of chromosomes from mom and a complete set of chromosomes from dad. In humans, a complete set (1n) consists of 23 chromosomes. Drosophila is simpler: a complete set (1n) consists of 4 chromosomes. The first pair are the sex chromosomes X and Y, and the other three pairs are autosomes, designated chromosome 2, 3, and 4.

Topics covered in this Chapter:

1. The 4 chromosomes: 1 pair of sex chromosome
2. Sex chromosomes
3. Writing genotypes
Drosophila Genotypes

Genotypes are written for each of the three major chromosomes: 1, 2, and 3. The 4th chromosome and the Y chromosome have so few genes, that they are usually ignored for the routine work of most fly geneticists.

Flies, like us, are diploid. They have two copies (homologs) of every chromosome. The genotype of the homologs is separated by a slash mark “/” and genotypes of different chromosome types are separated with a semicolon.

Females have the chromosomes: 
1/1 ; 2/2 ; 3/3

Males have the chromosomes: 
1/Y ; 2/2 ; 3/3

A completely normal fly is called "wild type" and is written as:
Female: +/+ ; +/+ ; +/+  
Male: +/Y ; +/+ ; +/+  

A fly that is homozygous for a loss of function mutation in the white (w) gene, which is on the X chromosome is written:

w-/w- ; +/+ ; +/+  

Since this above genotype is homozygous, it can be abbreviated:
w- ; + ; +

Fly geneticists often skip writing minus signs because they typically only highly mutant alleles. Thus, it is more common to write the genotype of a homozygous white mutant as:
w; +; +

A fly that is heterozygous for a mutation in white, is written:
w/+ ; +/+ ; +/+  

A male fly with a mutation on X is "hemizygous" for the mutation.

A male hemizygous for white is written:
w/Y ; +/+ ; +/+  

Note: there are many shortcuts for writing genotypes. Check with your instructors to learn the shortcuts used by your lab.
6. Genetic Markers

“Genetic markers” are visual traits that you can use to track flies with different genetic backgrounds. The most commonly used markers are dominant, meaning that you can see them in flies that have only one copy of the mutation causing the trait. In contrast, a recessive marker generally requires that the fly has two copies of the mutation causing it. The one exception is X-linked recessive markers: they can be visualized when the mutation is present in males because males have only one copy of the X chromosome.

The most commonly used markers affect traits that are easy to detect in adults, like eye color, wing shape, and bristle length. Researchers also use markers that can be detected earlier in the life cycle of the fly, such as “Tubby,” which results in short tubby pupae.

Researchers use markers to keep track of the inheritance of an entire chromosome or a specific mutation. By tracking a marker, researchers can deduce whether the fly has the specific “invisible” chromosome or mutation. For example, a researcher studying a gene that gets expressed in the fly stomach and affects digestion may find it useful to use markers that affect easy-to-detect phenotypes like eye color or wing shape to track the inheritance of the stomach-expressed gene, even though they are not studying eye color or wing shape.

Here we highlight five commonly used markers (see Figure 6.1):
- white+ —> red eyes
- Bar —> dimpled/narrow eyes
- Curly —> curly wings
- Stubble —> short bristles
- Tubby —> short tubby pupae
The “white plus” (w+) marker

w+ is the most commonly used marker in Drosophila. It is dominant and produces red eye color. (Figure 6.2)

How to detect the w+ marker

Any student can detect the w+ marker because eyes are among the most prominent features of fruit flies. You don’t need a dissecting microscope; you can see their large red eyes by looking at them through the vial, or on a piece of fruit when you find flies in your kitchen.

The white gene nomenclature: w vs. w+ vs. w-

w is the genetic abbreviation for the gene “white”. The wild type version (allele) of white is denoted “w+” and it results in flies with red eyes. The mutant version of white is denoted “w-”. Flies that have only the w- version of the white gene have white eyes.
How white got its name

You may wonder why the gene that normally gives flies red eyes is called white. It turns out that most genes are named for their mutant phenotype!

The white gene encodes information to make a transmembrane pump that transports pigment precursors that are required to make the red pigment you normally see in fly eyes. If a fly lacks a functioning copy of the white gene, it will be unable to transport pigment precursors and as a result the fly will have white eyes. On the other hand, if a fly has even just one functioning copy of the white gene, it will be able to transport pigment precursors, resulting in an eye that is red (or some shade of red as described below).

Discovery of the white gene led to many insights and a Nobel Prize in 1933. The white mutant was discovered by Thomas Hunt Morgan who was a renowned professor of developmental biology at Columbia University. He had been breeding flies in hundreds of glass milk bottles hoping to discover a spontaneous mutant or “sport.” After two years of searching unsuccessfully for a single mutant fly he was ready to give up (Kohler, 1994). But one lucky day in 1910, he spotted a fly with white eyes among hundreds of flies with red eyes (Figure 6.3). He named this fly “white” because it had white eyes. It was one of the first Drosophila mutants ever discovered and saved.

Morgan isolated the white eyed fly, a male, and crossed it to normal (wild type) red eyed females. All of the F1 progeny had red eyes, indicating that white was recessive to red. When Morgan crossed the F1 red eyed progeny with each other, he found that ¼ of the F2 offspring had white eyes, confirming that the white mutant was due to a recessive mutation. However, this was not a simple recessive mutation because all of the white eyed F2 flies were males (Morgan, 1910). This observation, plus results from additional crosses, led to the discovery of X chromosome-linkage and proof that genes are located on chromosomes.
The genetics of the white gene at its normal (endogenous) locus

The endogenous white gene is located on the X-chromosome and has the genetic symbol "w" (Figure 6.4).

A Drosophila female has two X chromosomes typically has one of the following genotypes and corresponding phenotypes:

- w-/w- = white eyes
- w+/w+ = red eyes
- w+/w- = paler red eyes *this is because the degree of pigmentation is sensitive to the dosage of the w+ gene product.

A Drosophila male has one X chromosome typically has one of the following genotypes:

- w-/Y = white eyes
- w+/Y = red eyes *these males will have red eyes that are equal in intensity to females that are w+/w+ due to a phenomenon called dosage compensation!

**w+ and transgenic flies**

The w+ marker is most commonly used marker to detect and track transgenic flies. Scientists make transgenic flies by engineering stretches of DNA in the lab and inserting the engineered DNA into the fly genome. The engineered DNA typically encodes two linked genes: the gene being studied (which is rarely possible to detect on its own) and a wild type copy of the white gene (w+). The engineered DNA is then inserted into a fly genome that lacks a normal copy of the white gene at its endogenous locus. Without the engineered DNA, this genome instructs the development of flies with white eyes because it lacks a functioning copy of white. However, a fly that inherits the engineered DNA will get a copy of w+ and therefore will have red eyes. In this instance, w+ serves as a marker gene that lets scientists know that the fly is carrying transgenic DNA.

This is a powerful trick that lets researchers track the inheritance of any engineered genes they wish to study: regardless of how hard it is to detect the engineered genes, by linking the engineered genes to w+, they can follow the genes through generations because the linked w+ transgene will give flies red eyes.

**The genetics of {w+} in a w-/w- background**

w+ is a useful marker to track transgenes as long as it is inserted into a genome that lacks a wild type copy of white. Flies that lack w+ at the endogenous locus have the genotypes w-/w- (female) and w-/Y (male), and are normally white eyed. Since since w+ is dominant to w-, flies that have a w+ linked transgene can be distinguished by their red eye color. The w+ transgene is often denoted with brackets, [w+], to distinguish it from the endogenous gene.
Shades of red
Interestingly, w+ is a "dosage sensitive gene". The intensity of red pigment produced by the transport activity of the w+ gene depends on how much w+ protein is produced. This means that flies with two copies of the w+ gene typically have darker red eyes than a fly with only one copy of w+ (Figure 6.5).

For example, females with a homozygous w+/w+ genotype have darker red eyes than females with a w+/w- heterozygous genotype. Heterozygous w+/w- females often have orange colored eyes as shown in Figure 6.5.

Male flies have only one copy of the X chromosome and therefore can have only one copy of w+ under normal non-transgenic circumstances. However, due to a process called dosage compensation, genes on the single X chromosome in males get expressed at double the rate, resulting in the same output of gene expression as the two X chromosomes in females. As a result, males with the hemizygous w+ genotype, w+/Y, have eyes that are as dark red as homozygous w+/w+ females!

The eye color that results from (w+) transgenes ranges from pale yellow to dark red. This is because the amount of gene expression of any gene, including transgenes, depends on their location in the genome. Transgenes that land in highly compacted regions called heterochromatin, tend to be poorly expressed. As a result, very little white protein is produced, resulting in eyes that are pale yellow to light red. Conversely, transgenes that land in open regions of the genome called euchromatin, are highly accessible to transcriptional machinery, resulting in high levels of white mRNA production. As a result, high levels of white protein are produced, resulting in eyes that are intensely red.

Summary on the use of the w+ marker
Most flies in a modern genetics labs are transgenic. They lack endogenous wild type copies of the white gene, but have red eyes because they carry a (w+) marked transgene. In these flies, w+ is simply a marker that lets researchers know the fly is carrying other transgenic DNA of interest.
Bar \((B^1)\) is named for its mutant phenotype, which results in flies with narrow eyes (Tice, 1914), as shown in Figure 6.6. Bar is abbreviated with a capital “B” to signify that the first mutant discovered in the Bar gene was dominant. (The white gene, on the other hand was discovered by a recessive mutation and hence the abbreviation for the white gene, “w,” is lowercase). Not all alleles of the Bar gene result in dominant phenotypes. However, the allele you are likely to encounter, \(B^1\), results in a dose-dependent dominant phenotype as follows that differ in females and males because it is X-linked.

In females
\[
\begin{align*}
B^1/B^+ &= \text{Subtle kidney shaped eyes} \\
B^1/B^1 &= \text{Narrow shaped eyes}
\end{align*}
\]

In males
\[
B^1/Y = \text{Narrow shaped eyes}
\]

The \(B^1\) allele is used to distinguish a special version of the X chromosome called the FM7 balancer, discussed in the next chapter.

Curly \((Cy^1)\) is named for its mutant phenotype, which results in flies with wings that curl upwards, as shown in Figure 6.7. Curly was discovered by Lenore Ward, who in 1923 published the observation that the \(Cy^1\) mutation causes two phenotypes: in one copy it causes the wings to curl upwards and in two copies it causes flies to die before they reach adulthood (Ward, 1923). Hence, \(Cy^1\) causes a dominant wing phenotype and is also a “recessive lethal.” Ward mapped \(Cy^1\) to the second chromosome and recognized that this could be a powerful marker for mapping genes to the second chromosome.

Curly is one of the most widely used markers in Drosophila. It is used to mark a special version of the second chromosome called the CyO (pronounced “Curly-O”) balancer. You can read more about this balancer in the next chapter.
**Stubble (Sb^1)** was discovered by Calvin Bridges in 1923 and is named for its thick short bristles on the back of the fly thorax (Bridges and Morgan, 1923), as shown in Figure 6.8.

The Sb^1 dominant bristle phenotype is hard to see without a dissecting microscope, but with proper lighting and magnification, it is easy to spot.

Sb^1 is used to track inheritance of mutations on the third chromosome. It is also commonly used as a marker for a special version of chromosome three, called the TM3 balancer. You can read more about this balancer in the next chapter.

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**Tubby (Tb^1)** was discovered by Charlotte Auerbach (Auerbach, 1943), who is widely credited for establishing that chemicals, such as mustard gas, can be as powerful as X-rays in creating heritable mutations (Beale, 1993).

The Tb^1 mutant is dominant and easy to spot in L3 larvae and pupae: it is short and "tubby" compared with wild type counterparts, which by comparison are long and lean, as shown in Figure 6.9. Although Tb^1 mutants are dominant during the L3 and pupal stages, they are undetectable at the adult stage. Therefore, if you are using this marker, you must plan to screen for it before Tb^1 flies hatch into adults.

The Tubby allele Tb^1 causes a dominant visible phenotype in the size and shape of larvae and pupae and is also a recessive lethal. Tb^1 is most often used as a marker for special versions of chromosome 3 called TM6, as described in the next chapter.
7. Drosophila Balancers

Balancers are special chromosomes that make Drosophila one of the most powerful genetic organisms on Earth. On the next page we provide an overview of the three essential features of balancers.

For more information about balancers, check out:


2. The Bloomington Indiana Stock Center Balancer Introduction Page: https://bdsc.indiana.edu/stocks/balancers/balancer_intro.html
Balancers have three essential features:

1. Balancers have at least one dominant “marker,” meaning a mutation that causes a dominant visible phenotype (reviewed in Chapter 6). For example, the most commonly used balancer for chromosome 2, named CyO, carries the dominant mutation Cy\(^1\), which produces flies with curly wings. If you are using CyO flies in a genetic cross, you can spot all the progeny that inherited the CyO balancer because they will have curly wings.

2. Balancers have a recessive lethal mutation. For example, the Cy\(^1\) allele is recessive lethal. As a result, flies cannot have two copies Cy\(^1\) and therefore cannot have two copies of the CyO balancer. Since balancer chromosomes have one or more recessive lethal mutations, flies must be heterozygous for each specific balancer they are carrying.

3. Balancers have multiple inversions that prevent the production of viable crossovers between the balancer and its non-balancer homolog during meiosis. Thus, flies with balancer chromosomes give rise to progeny that either have the balancer or the homologous chromosome of the balancer. Flies with balancer chromosomes do not give rise to progeny with recombinant chromosomes between the balancer and its homolog.

As a result of inversions, genes along a balancer chromosome are positioned in a different linear order than genes along the balancer’s non-balancer homologous chromosome (Figure 7.1). As a result, when crossing over occurs between a balancer and its non-balancer counterpart, the resulting recombinant chromosomes, called exchange chromatids, have the wrong genetic content, leading to inviable progeny.
8. The Gal4-UAS Gene Expression System

The Gal4-UAS gene expression system enables fly researchers to express any gene, in any tissue, at any time, in the development and lifetime of the fly. The way it works is simple and elegant.

To understand how the Gal4-UAS system works, let’s review the “Central Dogma” of molecular biology: information flows from DNA → RNA → protein. The Gal4-UAS system is a way to regulate the first arrow, the flow of information from DNA to RNA. This flow of information from DNA to RNA is called transcription. Transcription is mediated by interactions between proteins called “transcription factors” and a large multiprotein complex called RNA polymerase. RNA polymerase does the heavy lifting of transcription: it unzips the DNA helix and slides along one strand of the DNA, assembling RNA that is complementary to the DNA sequence. However, it cannot do all this work on its own. RNA polymerase relies on transcription factors to instruct it where and when to start transcription. There are hundreds of transcription factors: each one binds specific DNA sequences in the genome, thereby recruiting RNA polymerase to start transcription at specific genes.
Moving the yeast Gal4-UAS system into flies

Gal4 is a transcription factor that binds to an "upstream activating sequence" or UAS site that matches the consensus sequence 5’-CGG-N11-CCG-3’ (Traven et al., 2006). Although Gal4 is widely used in Drosophila research, it is not an endogenous fly gene. Gal4 is a gene from the yeast, S. cerevisiae. In yeast, Gal4 helps RNA polymerase initiate the transcription of galactose metabolism genes, such as Gal1 and Gal10 that have UAS sites in their promoter regions. See Figure 8.1.

The discovery that Gal4 could work in Drosophila was made in 1988 by Mark Ptashne and his colleagues, pioneers in the transcription field (Fischer et al., 1988). They showed that Gal4, when expressed in Drosophila, can do what it does in yeast: it can direct transcription of UAS-linked genes.

Drosophila does not naturally have UAS-linked genes, so Ptashne and his colleagues engineered a fly strain with a synthetic UAS-linked transgene integrated into the fly genome. In their trans-species experiment (also called a "heterologous" experiment), they created two strains of flies, one that expressed the yeast Gal4 gene in larval tissues (under the control of the Adh promoter) and one that contained a UAS-linked "reporter gene" called LacZ. LacZ is a commonly used reporter gene because its expression is easy to detect in cells: it encodes an enzyme called β-galactosidase that converts a colorless substrate called X-gal into a blue precipitate. Ptashne and colleagues found that flies that carried the UAS-LacZ reporter gene alone did not produce any blue precipitate when X-gal was added, indicating that the UAS-LacZ gene was not expressed. However, when flies carried both the Gal4 transgene and UAS-LacZ, the progeny produced blue precipitate specifically in all the cells where Gal4 was known to be expressed. This proved that the yeast Gal4 transcription factor could activate expression of the UAS-LacZ gene in Drosophila!
Using Gal4-UAS to generate phenotypes

The demonstration that yeast Gal4 could work in Drosophila shed light on the conserved nature of transcription from yeast to flies. However, the utility of using the yeast Gal4 transcription factor to manipulate the genetics of Drosophila was not evident until five years later, with the groundbreaking work and insights of Andrea Brand and Norbert Perrimon (Brand and Perrimon, 1993). Brand and Perrimon reasoned that Gal4 could be used to activate not only reporter genes like LacZ, but also normal and mutant Drosophila genes, thereby providing a new way to study gene function. As a proof-of-principle, they demonstrated that Gal4 could be used to change the developmental fate of cells. Figure 8.2 shows a modern adaptation of Brand and Perrimon’s results, in which the Gal4 transcription factors are used to express a mutant version of a gene called Notch, resulting in a dominant wing-notching phenotype. In this example, the gene encoding Gal4 is integrated at the genomic location (locus) of a gene that is normally expressed in the developing fly wing. At this position in the genome, the Gal4 gene also gets expressed in the developing fly wing. It is called a “wing driver” because it drives the expression of UAS-transgenes in the developing wing. In this example, the UAS-transgene encoded a dominant-negative allele of Notch, which when expressed in the wing results in a dominant notched-wing phenotype.

Brand and Perrimon’s paper and the accompanying reagents that they created revolutionized how we do genetics in Drosophila. Even before their paper was published, they started to share their newly created Gal4 driver lines and DNA cloning vectors. In fact, the first paper using the Brand and Perrimon Gal4-UAS system was reported in a letter to Nature (Greig and Akam, 1993) before the now classic Brand and Perrimon paper was in print. The Brand and Perrimon paper is the most highly cited paper in the Drosophila field, with over 8,000 citations and counting.
This cross results in a fly with both the engrailed-Gal4 segmentation driver and the Gal4 responsive UAS-Green Fluorescent Protein (GFP) transgene. UAS-GFP is an excellent reporter to visualize where Gal4 drivers are expressed.

Figure 8.3

Brand and Perrimon didn’t stop with their discovery that Gal4 can be used to create mutant phenotypes. They also created several new Gal4 “enhancer trap” lines by randomly inserting the gene encoding Gal4 in different positions of the fly genome. By crossing flies containing randomly inserted Gal4 transgenes to flies carrying the UAS-LacZ reporter, Brand and Perrimon could deduce where each inserted Gal4 transcription factor was expressed. This and related work has led to the creation of thousands of Gal4 “driver” lines that are each expressed in specific tissues such as muscle, brain, ovary, testis, and gut. For example, Figure 8.3 shows the expression pattern of the “engrailed-Gal4” driver in an L3 larva, which as you can see is expressed in a segmental pattern.

More recently, several large-scale projects have been funded to refine Gal4 expression to subsets of cells within organs. For example, the FlyLight project at HHMI’s Janelia Farm recently produced thousands of Gal4 lines that are each expressed in a subset of cells in the fly brain. Efforts are now underway to create Gal4 lines using CRISPR-Cas9 genome editing to precisely express Gal4 downstream of hand-selected genes (reviewed in Kondo, 2014). Conversely, Gal4 is also being used to direct tissue specific expression of genetically engineered versions of CRISPR-Cas9 to ectopically upregulate and downregulate gene expression (Ewen-Campen et al, 2015).

UAS-Responder Lines

You may wonder what good it is to express the yeast Gal4 transcription factor in specific cells of the fly brain or in other tissues. As shown in Figures 8.2 and 8.3 you can cross Gal4 expressing flies, also called Gal4 drivers, to flies carrying UAS-linked responder genes, encoding mutant genes, like NotchΔN and the reporter gene GFP. In addition, fly researchers have access to thousands of other UAS-responder lines including UAS-microRNAs, UAS-
cDNAs, and reporter flies such as UAS-red fluorescent protein and UAS-luciferase.

The most popular UAS responder lines in addition to UAS-GFP reporters are the UAS-RNAi (RNA interference) fly lines. Each UAS-RNAi fly line is designed with a specific RNAi “hairpin” construct that knocks down the expression of a specific gene. In fact, for each of the 14,000 genes in the fly genome, there is a corresponding UAS-RNAi line that you can order from a Drosophila stock center (see Resources Chapter) to knockdown expression of each gene. In theory, you could use UAS-RNAi lines to test the role of every gene in any cell that interests you, as long as there is a Gal4 driver that is specifically expressed that cell. However, it is often more practical to screen a subset of genes in the genome, to learn the role of a specific subclass of genes in the system you are studying. For example, to identify which transcription factors are playing an important role in the system you are studying, you could order UAS-RNAi lines against each of the 700 genes encoding transcription factors.

The Power of Gal4-UAS
The power of the Gal4-UAS system lies precisely in the fact that it is not endogenous to flies. This means that fly researchers can “mix-and-match” Gal4 lines with UAS responder lines to elicit precise responses determined by the researcher, not the fly. For example, Gal4 can be used to map neuronal circuits in the brain, manipulate hormone gradients, force cells to divide, force cells to die, and change fly behavior.

In total, there are over 10,000 Gal4 lines and over 20,000 UAS-lines. In theory, they can be crossed in every pairwise combination, resulting in over 200 million experiments. While I do not recommend that you embark on such an expedition, take some time to think about how you can take advantage of the Gal4-UAS system to answer questions of interest to you.

To get started, check out the Gal4 Driver and UAS-responder lines available at the Bloomington Indiana Stock Center

Try this application of Gal4-UAS: marking cells with GFP
To get a feel for the Gal4 system, set up a cross with a Gal4 driver line and a UAS-GFP responder line. A good driver to start with is with the ubiquitous Gal4 line: Actin-Gal4/CyO. Actin is a gene that is essential to life and is expressed ubiquitously (in every cell) in the fly. When an Actin-Gal4/CyO driver fly is crossed to a homozygous UAS-GFP/UAS-GFP responder fly, all of the non-CyO progeny will have one copy of the Actin-Gal4 transgene and one copy of the UAS-GFP responder. What will the progeny look like? See the cartoon at the beginning of this chapter for a hint. To see for yourself, you’ll need a way to visualize GFP, either a high intensity blue LED flashlight and a yellow filter, or a fluorescence dissecting microscope.
Drosophila researchers have been awarded five Nobel Prizes in Physiology or Medicine! You can read about every Nobel prize ever awarded and the recipients of each prize, called “Nobel laureates” at the Nobel Prize website: Nobelprize.org.

Fly Nobel Prize #1 — 1933
The first Nobel Prize for Drosophila research was awarded in 1933 to Thomas Hunt Morgan “for his discoveries concerning the role played by the chromosome in heredity.” Remarkably, the discoveries celebrated by this Nobel prize were mostly made by two students who joined Morgan’s lab at Columbia University as undergraduates and continued as graduate students: Alfred Sturtevant and Calvin Bridges. Their work established that genes reside on chromosomes in a linear order and that genes can be exchanged between chromosomes by a process called “crossing-over.”
Fly Nobel Prize #2 — 1946
The second Nobel prize for Drosophila research was awarded in 1946 to Hermann Joseph Muller “for the discovery of the production of mutations by means of X-ray irradiation.” Muller also began in Morgan’s lab as a graduate student, but performed his groundbreaking work with X-rays later in his own lab at UT Austin. His demonstration that X-rays cause heritable mutations paved the way for experimentalists to purposefully create mutations in DNA. This is important because “breaking genes” is one of the most powerful ways to figure out the functions of genes. Muller’s results were first brought to the public’s attention in a 1927 New York Times article, “Altered Heredity of Flies by X-ray.” The timing of his Nobel Prize, two decades later, immediately after the United States detonated two atomic bombs in highly populated Japanese cities, was likely to call attention to the possible dangers of nuclear weapons on generations to come.

Fly Nobel Prize #3 — 1995
The third Nobel prize for Drosophila research was awarded in 1995 jointly to Edward Butts Lewis, Christiane Nüsslein-Volhard, and Eric Francis Wieschaus “for their discoveries concerning the genetic control of early embryonic development.” Nüsslein-Volhard and Wieschaus used chemicals (rather than Muller’s X-rays) to cause mutations in DNA and then performed a screen to find all the genes that when mutated interfered with fly embryonic development in interesting ways. For example, they found mutations that resulted in embryos with two heads and others with two tails. In total, they discovered 131 new genes that are required to pattern the embryo so that it develops with the head, tail, belly, back, and other parts in the right places at the right times. Interestingly, most of these genes have evolutionary counterparts (homologs) in humans and play similar roles in development as well as disease. Lewis independently worked on genes called Hox genes, that pattern discrete regions of the embryo. Thousands of researchers across the globe, from fly geneticists to human geneticists, study the genes discovered by these researchers.
Fly Nobel Prize #4 — 2011
The fourth Nobel prize for Drosophila research was awarded in 2011 jointly to Bruce A. Beutler and Jules A. Hoffmann “for their discoveries concerning the activation of innate immunity” and Ralph M. Steinman “for his discovery of the dendritic cell and its role in adaptive immunity.”

Hoffman pioneered the study of innate immunity with his work in Drosophila on the Toll receptor, a gene that was initially discovered for its role in embryonic patterning by Nüsslein-Volhard and Wieschaus.

Fly Nobel Prize #5 — 2017
The fifth Nobel prize for Drosophila research was awarded in 2017 jointly to Jeffrey C. Hall, Michael Rosbash, and Michael W. Young “for their discoveries of molecular mechanisms controlling circadian rhythm.”

These researchers identified genes whose protein products, including Period and Timeless, function as oscillating machines that rise and fall in activity on an approximate 24-hour cycle. These genes are highly conserved in all animals and function to synchronize all the cells in your body to function in accord with the light/dark cycle of the Earth. The timing of this award in 2017, for research that was performed in the 1980’s, is speculated to highlight the importance of basic research in a political climate that puts funding of scientific research at risk at all levels.
10. Drosophila Researchers: Then & Now

Thomas Hunt Morgan’s Lab at Columbia University is the place where the model organism Drosophila melanogaster and its researchers first became famous. Drosophila had been studied in a handful of labs before Morgan took them into his own, including the lab of his former Ph.D. student Nettie Stevens, who had a faculty position at Bryn Mawr (Kohler, 1994). However, it was in Morgan’s lab that the power of Drosophila genetics was first realized, attracting dozens of students, postdocs, visiting scientists and technicians to train in his lab. Some of the scientists, in fact the most famous of them, such as Alfred Sturtevant, Calvin Bridges, and Lilian Vaughn Morgan, remained in Morgan’s lab for years after earning their graduate degrees. Most others, including Herman Muller, Jack Shultz, and Helen Redfield, had briefer stays and continued their Drosophila research in independent positions.

We are indebted to Morgan, not just for the discoveries that came out of his lab, but for creating an open environment where fly researchers freely discussed ideas and shared reagents, thereby leading to the success of Drosophila and its researchers (Kohler, 1994).
In fact, most Drosophila researchers today can track their academic lineage or lineages to Morgan himself! For example, the author of this book has three independent lineages to Morgan: through her two Ph.D. advisors, Michael Levine and Edwin ("Chip") Ferguson and through her postdoctoral advisor, Norbert Perrimon, as shown in Figure 10.1. To see how you or your advisor(s) connect to Morgan, shown in Figure 10.2, check out FlyTree. If you are not in the database, you can add your name simply by registering.
Hidden Figures

If you Google images of Morgan’s fly room or read the major historical account on early Drosophila research from 1910-1940, Lords of the Fly (Kohler, 1994), you might get the impression that women did not contribute to the science of early Drosophila research. Indeed, women were not included in the official photographs of Morgan’s lab, nor were they encouraged to attain faculty positions after earning their Ph.D. degrees. However, as highlighted by Michael Dietrich and Brandi Tambasco’s study of the Drosophila Information Services archives (1934-1970), women made up about 1/3 of the technical workforce of the early Drosophilists in the 1930s, and an even greater share after World War II (Dietrich and Tambasco, 2007). While many of the women and men with technical positions held Bachelor’s and Masters degrees, some had Ph.D. degrees. In fact, 4 of the 24 Ph.D. graduate students trained by Morgan in the field of Drosophila genetics from 1910-1930 were women (Kohler, 1994).

Tracking the contributions of female Ph.D. scientists from Morgan’s lab and others has been difficult in part because these scientists were often referred to by their marital status “Miss” and “Mrs.” rather than by their academic title, “Dr.,” giving the false impression that they were in the lab as “helpers” and “wives” rather than as the fully trained Ph.D. scientists that they were. Unfortunately, this confusing naming convention was continued by historical accounts of early Drosophila scientists, rendering invisible these scientists and their contributions. For example, Dr. Helen Redfield was faculty at Stanford University when she took a position as a Fellow in the Morgan laboratory to study chromosome dynamics and crossing over (Smithsonian Archives). However, the major historical account on Drosophila research, Lords of the Fly, refers to Dr. Redfield only as “Miss Redfield,” obscuring the fact that she was a Ph.D. scientist.

Perhaps even worse than Redfield’s treatment, many women have been ignored altogether in the telling of early Drosophila research. For example, Dr. Katherine Suydam Brehme Warren, who co-authored the first major go-to classic for Drosophila geneticists, “The Mutants of Drosophila melanogaster,” also known as “Brehme and Bridges,” (Ashburner and Drysdale, 1994), is entirely absent from Lords of the Fly, which claims that early Drosophila genetics was essentially a man’s game. Luckily, other historians like Marsha Richmond (Richmond, 2007), Michael Dietrich, and Brandi Tambasco, have brought to light the contributions of previously overlooked hidden figures in genetics research in the US and abroad.

As scientists we can begin to identify the contributions of hidden figures that were previously missed by historical accounts, by going directly to the primary literature of published research. In fact, a look at early papers in the field shows that many of the tools that we take for granted in Drosophila research today were either discovered or invented by female scientists. These include the mutants Curly, Tubby and attached-X (discovered between 1919-1943), as well as the Drosophila S2 cell line and Schneider’s Drosophila culture medium (created in the 1970s). In addition, significant biological insights, such as the discovery that the sex of an individual is determined by their chromosomes (1905), and the idea that flies can be used as a model for cancer research (1919), were pioneered by female Ph.D. scientists. The women behind these contributions are highlighted in the remainder of this chapter.

Footnote¹: I am indebted to Margot Lee Shetterly for the term “hidden figures,” which is the title of her inspirational book, Hidden Figures: The Story of the African-American Women Who Helped Win the Space Race (2016) and the film adapted from the book. As she writes on her website, “We all know what a scientist looks like: a wild-eyed person in a white lab coat and utilitarian eyeglasses, wearing a pocket protector and holding a test tube. Mostly male. Usually white. Even Google, our hive mind, confirms the prevailing view. Just do an image search for the word “scientist.”
Hidden Figures Trained by Morgan

Morgan is known for training Alfred Sturtevant, Calvin Bridges, Herman Muller, and Jack Shultz, as well as several additional men. However, he began his career training female scientists, having started his first academic position at Bryn Mawr, a women’s college (Sturtevant, 1959). The most famous female scientist trained by Morgan was Nettie Stevens (Figure 10.3) who completed her Ph.D. with him in 1903 at Bryn Mawr, where she then attained a faculty position (Brush, 1978). Two years after earning her Ph.D., Stevens made the brilliant discovery in her own laboratory at Bryn Mawr that sex is determined by chromosomes. This may seem obvious to you now, but at the time, there was no proof that genes were on chromosomes, nor was it clear whether the oddly mis-matched chromosomes (or “elements”), that you know of today as the X and Y chromosomes, were even really chromosomes. Stevens was able to make sense of the confounding data of the time by examining the chromosomal content of the germ cells and somatic cells of both sexes of several insect species. She was the first scientist to deduce that sperm carrying a small chromosome (now called Y) result in male offspring, whereas those carrying the normal sized counterpart, which she called “element X,” (and now is simply called “X”) result in female offspring (Stevens, 1905; Brush, 1978; Richardson, 2013). Interestingly, Stevens brought Drosophila into her laboratory even before Morgan did (Kohler, 1994). Her chromosomal insights paved the way to Morgan’s deduction in 1910 that the white gene must be X-linked, an integral discovery leading to the Nobel prize he was awarded in 1933.
Another hidden figure who began in Morgan’s laboratory at Bryn Mawr and who made significant contributions to Drosophila research was Lilian Vaughan Morgan, nee Sampson, Figure 10.4. Sampson started at Bryn Mawr as an undergraduate and graduated as the top student of her year, with concentrations in math and biology, having taken 11 courses in each (Keenan, 1983). This put her on the fast track to becoming a professional scientist. For example, she was awarded the European Fellowship to support her study of chitons at the University of Zurich, after which she returned to Bryn Mawr and earned a Master’s Degree with Morgan in 1894. Sampson then went to Woods Hole to work independently on regeneration and embryology. Woods Hole is also where Thomas Hunt Morgan spent every summer (Sturtevant, 1959). Ten years after earning her Master’s degree, Sampson married Morgan, took a leave of absence from research to raise their four children, and then recommenced with research, in Morgan’s lab starting in 1918. She published 18 papers in her lifetime, including the discovery and characterization of the first attached-X chromosome, in which two X chromosomes are attached to the same centromere (LV Morgan, 1922), Figure 10.5. This discovery led to important experiments in Morgan’s lab and the Drosophila field in general, including the ability to maintain recessive X-linked lethals, studies of crossing over, and proof that sex in Drosophila is due to the X:autosome ratio (Keenan, 1983). LV Morgan’s most cited work was on her discovery of the first ring chromosome in Drosophila (LV Morgan, 1933), which opened the door to creating and studying mosaics.
Another influential hidden figure who trained with Morgan was Mary B. Stark. Stark earned her Ph.D. with Morgan at Columbia University, where she published the first-ever report of a heritable tumor-causing mutation in Drosophila, under the title, "A benign tumor that is hereditary in Drosophila" (Stark, 1919). Stark not only mapped the tumor-inducing mutation, but also showed that tumors could be transplanted and retain their tumorigenic properties (Figure 10.6). Stark’s interest in translational science was decades ahead of her time. It appears that Stark found a way to continue her Drosophila tumorigenesis work at New York Medical College and Flowers Hospital. In total, she published 6 papers, including a traditional mapping paper with Calvin Bridges. This history, however, is missing from historical accounts of the early Drosophilists.

Hidden Figures Who Produced Tools You Likely Use

Virtually all fly researchers today, whether working with Drosophila strains or Drosophila cells in tissue culture, work with biological materials discovered or created by women scientists, hidden from historical accounts. For example, the Curly mutant that distinguishes the CyO balancer was discovered, mapped, and characterized as a recessive lethal by Lenore Ward, who in 1923 published a single-author paper in Genetics on the first Curly balancer (Ward, 1923), Figure 10.7. In this paper, she lists a residential address in Sioux City, Iowa, and acknowledges support from her research mentor, A.F. Shull at the University of Michigan, as well as Muller and Bridges for their “invaluable” input on her project. It is likely that Ward characterized Curly as part of a Ph.D. thesis. However, since women were typically discouraged from climbing the ranks of academic science, it appears that Ward did not continue in science and instead became a “silent scientist” — a female scientist who contributed to mainstream science of the day in her dissertation but disappeared due to a host of factors (Richmond, 2007). Although Ward did not appear to continue in science, her contribution of Curly lives on as major resource for the Drosophila community today.

Figure 10.6
Illustration of fly tumors by Mary Stark with “aid of a camera lucida” from her 1919 Genetics paper, "A Benign Tumor That is Hereditary in Drosophila.”

Figure 10.7
Illustration of Curly Wing by Lenore Ward from her 1923 paper, "The Genetics of Curly Wing in Drosophila. Another Case of Balanced Lethal Factors.”
Tubby is another invaluable marker (Auerbach, 1943) that was discovered by a female scientist Charlotte “Lotte” Auerbach. Figure 10.8. Auerbach earned her Ph.D. in 1935 in the laboratory of Francis Crew at the University of Edinburgh, where she wrote her thesis, “Development of the legs, wings, and halteres in wild type and certain mutant strains of D. melanogaster,” (Beale, 1993). Auerbach continued in Crew’s lab as a researcher and became interested in the power of mutagenesis while overlapping with Herman Muller (who later received the 1946 Nobel Prize in Physiology or Medicine for showing that X-rays cause heritable mutations). It was through talks with Muller that Auerbach became hooked on the idea of finding chemicals to dissect gene function. Muller and others had tried for decades to find a chemical method to cause mutations, but had failed.

Auerbach reasoned that since X-rays and mustard gas cause similar burns on skin, mustard gas, like X-rays, may have mutagenic properties (Auerbach, 1967). She went to great lengths to test her hypothesis, conducting experiments on the rooftop of Crew’s lab and burning her hands several times in the process (Beale, 1993). However, her work paid off. Auerbach along with her collaborator J.M. Robson, were the first to demonstrate that chemicals can cause heritable mutations (Auerbach and Robson, 1946), opening the door to chemical mutagenesis screens such as the Nobel Prize winning work by Christiane Nusslein-Volhard and Eric Weischause, using EMS, not mustard gas, in the 1980s.

Auerbach devoted her career to studying mutagenesis, with the hope of finding a way to make “directed mutations,” of the type only recently made possible with the development of Cas9 mediated “genome editing” by Jennifer Doudna and Emmanuelle Charpentier (Doudna and Charpentier, 2014). While Auerbach is not widely recognized today, she was far from being a hidden figure in her day. She published 91 papers and was awarded the Royal Society’s Darwin Medal in 1976 “in recognition of her discovery of and continuing work on chemical mutagenesis.”
The hidden figures of Drosophila research are not restricted to early classical geneticists. For example, few Drosophila researchers today recognize that today’s commonly used Drosophila tissue culture tools (Figure 10.9) were developed in the 1970s by Imogene Schneider, a Ph.D. scientist employed at the Walter Reed Army Institute of Research. She developed the Drosophila S2 line, as well as the famous “Schneider’s medium” that remain integral to virtually all Drosophila cell culture experiments today (Schneider, 1971; Schneider, 1972).

Unfortunately, the biographical information and photographs of many hidden figures, including Imogene Schneider, Mary Stark, and Lenore Ward, are not readily available online (Figure 10.10). I welcome information and photos from the community to more fully honor these and other, hidden figures of Drosophila research.
11. Resources for Students and Teachers

1. Recommended iPhone App: Fly Genetics
A handy iPhone app helps you identify females vs. males and shows you how to spot dominant markers such as Scutoid, Sternopleural, Stubble, Serrate, Humeral, and Tubby.

2. modENCODE’s Primer on Drosophila
http://modencode.sciencemag.org/drosophila/introduction

3. Manchester Fly Facility: How and why carry out research with flies
http://www.flyfacility.manchester.ac.uk/

4. FlyBook — Great for Students and Teachers
An open access compendium of review articles on Drosophila research published by The Genetics Society of America (GSA).

5. Genetics on the Fly: A primer on the Drosophila Model System

6. First in Fly by Stephanie Mohr
This book highlights major discoveries in science that originated with Drosophila research. Named one of the top 10 science books in 2018 by Smithsonian Magazine!

Essential Websites

Flybase
http://flybase.org/
This is the go-to site to find all the information about any fly gene, including available mutant stocks. For example, you can find more information about balancers from this site.

The Interactive Fly
The Interactive Fly
In-depth information on virtually every paper that discusses fly genes involved in cell-cell signaling and development.
12. Major Drosophila Stock Centers

1. The Bloomington Stock Center
   https://bdsc.indiana.edu/
   This is where most researchers in the United States get their fly stocks. See special pages for Gal4 drivers, UAS-responders, fluorescent reporters, RNAi lines, etc.

2. The RNAi Project (TRiP) at Harvard
   https://fgr.hms.harvard.edu/
   The Harvard TRiP hosts several freely available online tools and creates transgenic flies by scientist-driven nominations.

3. Vienna Drosophila Stock Center
   https://stockcenter.vdrc.at/control/main
   A great resource in Europe specializing in RNAi stocks.

4. The National Institute of Genetics Stock Center
   https://shigen.nig.ac.jp/fly/nigfly/
   This stock center in Japan pioneered the creation Cas9 genome editing stocks, and contains about 20,000 UAS-RNAi stocks.

5. The Kyoto Stock Center
   https://kyotofly.kit.jp/cgi-bin/stocks/index.cgi
   A major stock center in Japan with about 27,000 different fly stocks including Gal4 drivers and UAS-RNAi stocks.
References

Chapter 1

Chapter 6

Chapter 7

Chapter 8

Chapter 10


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