Advances in Biology Laboratory Education
Publication of the Association for Biology Laboratory Education
Volume 41, Article 7, 2020

FlyBuilder: A Multimodal Dry Lab Curriculum Teaches Mendelian Genetics through the Lens of *Drosophila* Balancer Chromosomes

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Mendelian genetics is often taught through student lab work with live *Drosophila melanogaster* (fruit flies). This approach can be limited by institutional resources and time requirements imposed by the *Drosophila* lifecycle. FlyBuilder overcomes these practical limitations by using a reductionist paper doll *Drosophila* toolkit. Here, we offer FlyBuilder as a free multimodal genetics curriculum that can be integrated into existing curricula. FlyBuilder uses *Drosophila* balancer chromosomes and visible 'marker' mutations to illustrate and apply examples of recessive lethality, genotype-phenotype pairing, phenotypic dominance, and *Drosophila* transgenesis. We have used FlyBuilder in introductory, intermediate and advanced topic biology and neuroscience courses at two universities where it generated positive student feedback and understanding.

Keywords: genetics, *Drosophila*, inquiry-based learning

Introduction

When teaching Mendelian genetics, the fruit fly *Drosophila melanogaster* is commonly used in complementary experimental labwork. However, the two-week lifecycle of *Drosophila* can slow the experimental process significantly, delaying a student's application of lecture material. Additionally, microscopes and consumables required for *Drosophila* labwork are costly. We wanted to accelerate the teaching timeline and remove the burden of resource restrictions for *Drosophila* labwork. To do so, we invented a paper-doll *Drosophila* 'FlyBuilder' kit that allows for fast exchange of phenotypic traits. The FlyBuilder kit is used in our novel FlyBuilder curriculum to effectively apply the principles of Mendelian genetics.

Collectively, FlyBuilder is a three-part progressive drylab curriculum that teaches students the basics of Mendelian genetics through the lens of the *Drosophila* genetic system. This curriculum includes a background lecture (Module 1), a prelab reading packet (Module 2), and a hands-on lab activity using the FlyBuilder Kit (Module 3). FlyBuilder is appropriate for intermediate to advanced courses and can be adapted to introductory levels. The learning outcomes for FlyBuilder as presented here include:

- Understanding the practical use of mutant model organisms in a research setting.
- Identifying common *Drosophila* genetic tools used in crossing schema (balancers and markers).
- Applying knowledge of Mendelian genetics to predict progeny from given crosses.
- Integrating knowledge of balancers, markers and Mendelian principles to critically design practical mating schema.

The appeal of this curriculum is that it (1) can be tailored to meet a variety of learning levels, (2) can be easily integrated into existing curricula piecemeal or as a whole, (3) allows for an accelerated application of Mendelian genetics, and (4) is easily implemented, due to its use of inexpensive widely available materials and minimal set up time.

Time Requirements

Preparation and Setup

Modules 1 and 2 are deliverable to students electronically, individually, or in lecture form. Module 3 requires (a) a worksheet and balancer table, printed for each lab and (b)

the one-time ~15-20 minutes printing and assembly of the FlyBuilder kit (Appendix A). The FlyBuilder kits are reusable and one kit is required for each pair of students. Module 3 setup involves distribution of FlyBuilder kits and printed worksheets and balancer tables to your students.

Student Performance

Taught together, the entire curriculum can be completed in one 3-hour laboratory session. However due to the volume of information presented in FlyBuilder, we recommended that instructors temporally space out the three modules to allow students to optimally integrate the information provided. Module 1 requires ~20 minutes of in-class lecture or at-home viewing; Module 2 is self-paced assigned reading; Module 3 can be completed in a single 90-minute laboratory class. The instructor has a large amount of freedom to alter the required time by omitting specific concepts or activities from the laboratory or by splitting the lecture and activity components over multiple class sessions.

Module Descriptions

Module 1

Module 1 is a brief lecture that assumes students have been introduced to Mendelian genetics and using model organism for basic research. Module 1 reviews the basics of Mendelian cross predictions by highlighting and explaining key practical genetic tools one uses when working with *Drosophila*. Specifically, this module introduces the requirement for and concept of *Drosophila* balancer chromosomes and how they enable the maintenance of deleterious mutations in mutant fly lines. Upon completion of this module students will be able to:

- Explain research uses for mutant model organisms.
- Understand why some mutations are hard to maintain in populations, and how they are removed.
- Explain what *Drosophila* balancer chromosomes are, and how they allow maintenance of mutations in *Drosophila* populations.
- List two essential features of balancer chromosomes, and how those features are used in a lab.

Module 2

Module 2 (Appendix B) is a short self-paced reading assignment. It explains *Drosophila* genotypic nomenclature, anatomy, common balancers and marker mutations, and provides a reference to review Punnett squares. Upon completion of this module students will be able to:

- Recognize *Drosophila* genotypic nomenclature in proper format.
- Understand the concept of marker mutations, including the *white* gene and how these mutations are used in laboratory applications.
- Identify basic *Drosophila* anatomical features relevant to Module 3 and relate them to common balancers and marker mutations.
- Use a Punnett Square to design and execute mating schemas.

Module 3

Module 3 is a drylab worksheet activity that engages student pairs in tasks of progressive difficulty as they work towards designing a multi-step *Drosophila* cross schema to produce a specific genotype. Throughout, students use a hands-on novel FlyBuilder kit (Figure 4; Appendix A) to 'build' flies and reinforce the pairing of genotype and phenotype. Module 3 requires students to deduce genotype from phenotype and vice-versa while re-familiarizing them with the common balancers and marker mutations introduced in Module 2. It reviews Punnett squares and asks students to generate Punnett squares for flies of given genotypes, and to identify progeny phenotypes and 'build' progeny flies. Students then advance in their comprehension by generating multiple cross schema; this allows students to synthesize their knowledge of Mendelian genetics with the practical tools of balancers and markers. Upon completion of this module students will be able to:

- Use basic genotypic nomenclature.
- Use common *Drosophila* balancers to determine genotype using phenotypic markers.
- Use common *Drosophila* balancers and Punnett squares to predict the viable genotypic and phenotypic outcomes of genetic crosses.
- Design multi-generational genetic crosses to produce a final specific genotype.

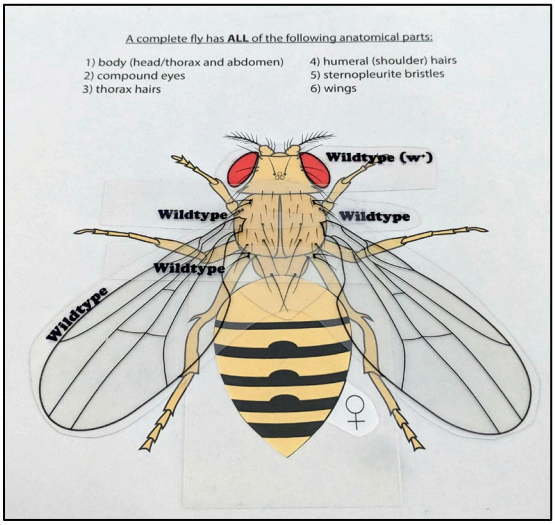


Figure 1. A fly fully assembled on the FlyBuilder mat using FlyBuilder kit interchangeable anatomy pieces. The mat instructions (top) request placement of categorical body components: body, compound eyes, hair/bristle types, and wings.

Student Outline

Introduction

Your instructor will provide all relevant materials for FlyBuilder, which is a three-module curriculum designed to teach you to the principles of Mendelian genetics using important practical features of the *Drosophila melanogaster* (fruit fly) model organism. Below is an introductory overview to orient you to FlyBuilder module content and expectations of having completed the FlyBuilder curriculum.

Module 1

Module 1 is a brief ~20min lecture where you will learn about powerful genetic tools that allow scientists to use *Drosophila* in basic research. PowerPoint slides will be presented in class or provided to you by your instructor for independent viewing. Before beginning Module 1, you should be familiar with the use of mutant organisms for basic research. By the end of Module 1, you should be able to:

- Explain what kinds of questions one can ask using mutant organisms. Can you provide an example of using mutant *Drosophila* in basic research?
- Understand why some mutations are hard to maintain in mutant organisms, and how they are removed from *Drosophila* populations.
- Explain what balancer chromosomes are, and how they allow maintenance of mutations in a population.
- List two essential features of balancer chromosomes, and how those features are used in a lab.

Module 2

Module 2 is a self-paced reading assignment that builds on concepts introduced in Module 1. In Module 2, you will learn information essential to working practically with *Drosophila.* Module 2 materials will be provided to you by your instructor. Understanding the contents of Module 2 is essential for attempting Module 3. By the end of Module 2, you should be able to:

- Write *Drosophila* genotypic nomenclature in proper format.
- Explain the role of the *white* gene, and how it is used to track mutations.
- Understand the concept of marker mutations. Can you think of how they would be useful, in combination with their host balancers, for identifying progeny in single and multiple-cross schemas?
- Identify basic *Drosophila* anatomical features relevant to Module 3.
- List common balancers and their phenotypic marker mutations.
- Design and execute a basic Punnett square.

Module 3

Module 3 is a laboratory activity that you will complete with a partner. Using your knowledge of Mendelian inheritance, balancer chromosomes, and marker mutations you will work towards designing multiple-cross schema to produce a specific genotype of fly. To complete Module 3, your instructor will provide a worksheet, a balancer table, and a FlyBuilder kit. At the end of Module 3, you will have learned to:

- Use basic genotypic nomenclature.
- Use common *Drosophila* balancers and markers to determine genotype from phenotype.
- Use common *Drosophila* balancers, markers and Punnett squares to predict the viable genotypic and phenotypic outcomes of genetic crosses.
- Integrate knowledge of Mendelian genetics and *Drosphila* balancer and marker mutations to design multi-generational genetic crosses that produce a final specific genotype.

Note: The FlyBuilder kit contains an inventory sheet to ensure retention of all its various components. Upon completion of the Module 3 worksheet, please return your FlyBuilder kit to your instructor with all its contents included.

Materials

Instructors can download the following free materials at go.brandeis.edu/FlyBuilder or through direct contact with the authors:

- Module 1 Lecture Slides (.pdf) (.ppt)
- Module 2 Reading (.pdf) (.docx)
- Module 3 Balancer Table (.pdf) (.docx)
- Module 3 Worksheet: Student Copy (.pdf) (.docx)
- Module 3 Worksheet: Instructor Copy (.pdf) (.docx)
- Module 3 FlyBuilder Kit (.pdf) (.ai)

Module 1

Materials Needed:

- Lecture slides
- Projector system (or digital distribution)

Module 2

Materials Needed:

- Reading
- Color printer (or digital distribution)

Module 3

Materials Needed:

- Worksheet: Student Copy (1 per student)
- Balancer Table (1 per student)
- Worksheet: Instructor Copy (1 per instructor)
- FlyBuilder Kit* (1 per student pair, ~$2.50 US each)
- A camera (1 per student pair, cellphone camera works well) - optional
- Color printer (LaserJet preferred)

*Please see Appendix A for comprehensive materials and one-time assembly instructions.

Notes for the Instructor

Initially, FlyBuilder Modules 1 and 3 were given as a pilot during a 3hr introductory neuroscience lab course at Salve Regina University. Students struggled to integrate the material. In response, we generated Module 2 background content (Appendix B) and split the Modules temporally to allow for content absorption. FlyBuilder in its current form was taught in a second-year level lab course at Brandeis University and ran very effectively. While the initial lecture was given to ~350 students simultaneously, it worked best to have approximately 1 instructor per 30 students in each Module 3 lab. Students doubled their ability to infer phenotype from genotype (and vice versa) as well as their ability to predict progeny from parent crosses, and most students expressed enjoyment of the lab activity. Students worked best as pairs; it is not recommended to make Module 3 an independent activity.

To promote information retention and highlight key concepts, it may be helpful to give short assessments at the conclusion of each Module. A brief in-class quiz or short written homework would suffice, aimed at evaluating student understanding of the Module's learning objectives (provided in Introduction and Student Outline).

We received requests for more instruction on course level adaptability. We suggest specific modifications to Module content to suit introductory and intermediate course levels (Appendix D). In response to requests to increase supporting content in Module 3, we have added problem examples in the student copy, and have generated an instructor's copy containing those modifications as well as instructor notes and an answer key (Appendix C).

Acknowledgments

Thank you to Dr. Melissa Kosinski-Collins at Brandeis University, who very kindly helped us to pilot FlyBuilder in BIOL18A; and to the BIO-220 and BIO-425 students at Salve Regina University that provided feedback on the FlyBuilder workshop.

About the Authors

Joey Flyer-Adams is a doctoral student in neuroscience at Brandeis University where she studies learning and memory in the Griffith lab using *Drosophila*. Joey mentors undergraduates in a research setting and works as a Fellow in the Brandeis SciComm Lab, where she coaches members of the Brandeis science community to communicate their work effectively. She invented FlyBuilder as a gift for her good friend Dr. Barbagallo in celebration of her professorship.

Dr. Belinda Barbagallo has been an Assistant Professor at Salve Regina University since 2018, where she teaches introductory Cell Biology courses and advanced topic Neuroscience courses in addition to running a research program using *Drosophila* with undergraduate students. Dr. Barbagallo is a R.I.-INBRE investigator.

Dr. Leslie Griffith is a full professor at Brandeis University, where her lab has studied molecular mechanisms and neural circuits of memory and sleep since 1992.

Appendix A: FlyBuilder Kit Assembly Instructions

Each FlyBuilder model kit only requires assembly once and is indefinitely reusable.

Materials

- Downloaded 'Module3_FlyBuilderKit_ai.zip' OR 'Module3_FlyBuilderKit_pdf.zip'
- LaserJet color printer
- Standard white 8.5x11 printer paper
- Cold lamination pouches (2 per kit): we suggest Scotch Self-Sealing Laminating Pouches, 9.0x11.5in, Gloss Finish (Amazon)
- Overhead transparencies (1 per kit): we suggest Apollo Transparency Film for Laser Printers, Black on Clear (Amazon)
- Scissors
- 1qt zip-top baggies (1 per kit)
- Large manila envelopes (1 per kit; *optional*)

Preparation

1. Uncompress the downloaded FlyBuilderKit file to find 'FlyBuilder_body' and 'FlyBuilder_transparency'.
2. Print 'FlyBuilder_body' to standard white printer paper.
3. Print 'FlyBuilder_transparency' to transparency. For best results, print on rough side, using a LaserJet printer.

Assembly

1. Cut pieces from the transparency sheet (Figure 2A). Do not separate grouped text/anatomy.
   a. Before cutting the pieces, we strongly recommend cold laminating the transparency for weight and durability. For best results apply the adhesive side to the rough printed-on side of the transparency.
2. Cut square building mat and male and female abdomens from printed body sheet (Figure 2B). Cut abdomens as close to their edges as possible.
3. Using a single cold laminate pouch, laminate male and female abdomens and building mat. Cut pieces apart again according to dotted lines in Figure 2B. (Make sure to leave ¼" laminated edges around each piece.)
4. Place all pieces cut from Figure 2A and male and female abdomens from Figure 2B in a small baggie (24 pieces).
5. Store laminated building mat and baggie of pieces in a manila envelope to form one kit (optional).

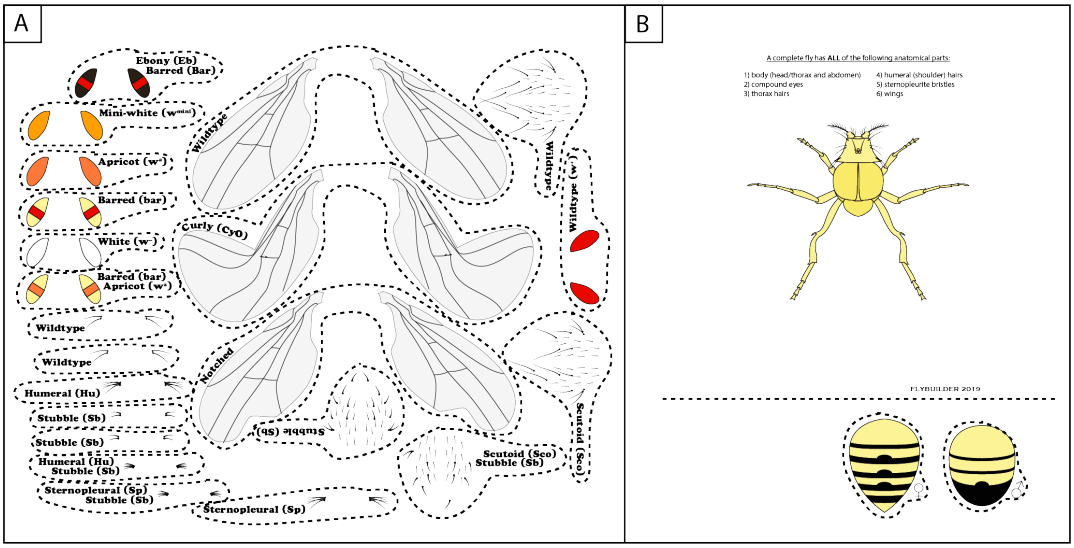


Figure 2. Pieces to be cut from printed materials. Cut along dotted lines shown for (A) transparency, yielding 22 pieces and (B) for paper building mat and bodies, yielding 3 pieces.

Note: (1) To prevent loss of contents, we include a list of all pieces in each kit, printed on standard printer paper (2) There are pieces supplied in the transparency that are not used in the current version of the FlyBuilder curriculum.

Appendix B
Module 2: FlyBuilder Pre-Lab Background Material

Note: Please ensure comprehension of Module 1 materials before reading.

Drosophila Genotypic Nomenclature

Drosophila have four chromosomes: Ch. #1- 4. *Drosophila* genotypes are written in numerical chromosomal order and read left to right. Chromosomes are separated by a semicolon (1 ; 2 ; 3 ; 4), while paired chromatids are separated by a divider (SisterA/SisterB). Genes are usually written with the allele in superscript (example: $gene^{allele}$). Wildtype is reported either as '*wt*,' '+,' or an absence of text (inferred). In this way the allele of each chromatid is reported either specifically or by inference.

For example, the full genotype of a wildtype Canton-S fly (originating from Canton, Ohio) could be written as

$$+^{CS}/+^{CS}\ ;\ +^{CS}/+^{CS}\ ;\ +^{CS}/+^{CS}\ ;\ +^{CS}/+^{CS}$$

OR

$$+^{CS}\ ;\ +^{CS}\ ;\ +^{CS}\ ;\ +^{CS}$$

OR

$$wt^{cs}$$

Additionally, handwritten genotypes often show the / as a horizontal division bar, and multiple mutations on a chromosome are separated by a comma. Thus, a mutation Z in gene *abc* and mutation Y in gene *def,* both heterozygous on Ch. 2, can be written as

; +/abc^{Z}*, def*^{Y}*; ;* OR *;* $\frac{\quad +\quad}{}$ *; ;*

$$abc^{Z},\ def^{Y}$$

(Remember, you can infer that this fly is wildtype on Ch. 1, 3 and 4 due to lack of text.)

If an allele is homozygous, the divider is commonly left out to avoid repetition. In this way the same mutation Z, homozygous instead of heterozygous, is written as

; abc^{Z} *;;*

Lastly, the fourth chromosome is extremely small and often not included in everyday notation. So the above Ch.2 homozygous mutation (again, wildtype on all other chromosomes) would usually be written as below (note the lack of the last semicolon)

; abc^{Z} *;*

Remember, always include as much information as possible in a genotype to avoid confusion! (Your colleagues and future self will thank you).

The *White* Gene and its Role in Tracking Transgenic Mutations

If you recall from Module 1, transgenic mutations are pieces of DNA (or transgenes) added to the genome that aren't normally there. This is done by injecting the transgenic DNA into a *Drosophila* embryo, where the transgene is inserted into the genome of *some* of the embryo's germline cells. Since only *some* of the germline cells take up the transgene, when the embryo grows to an adult fly and mates, only a few of the progeny will carry your transgene. To easily select just the few flies (out of many) which carry your transgene, it is extremely helpful to have a visible phenotypic marker for your mutation - something you can see on the outside of the fly, to tell you your transgene is present in that fly's DNA. This is where the *white* gene comes into the story.

Found on Ch.1, the *white gene (w)* encodes a transporter protein that shuttles many types of molecules in a cell, including ones responsible for the red colored eyes of wildtype flies (Fig. 3). *White* was discovered when it was first mutated, which produced flies with white eyes. Now, many *white* mutants have been generated with a variety of colors (on the white → red spectrum). The several *white* mutants which all have white eyes are broadly referred to as *w*- mutants, though specific mutant alleles obviously exist.

But how does the *white* gene play a role in tracking transgenic insertions into the genome? Interestingly, inserting a small piece of the *white* gene (commonly known as a 'mini-w') anywhere in the genome partially restores the red eye pigmentation. This becomes helpful if a mini-w is attached to a transgene that is then injected into a *w*-[2] embryo; any *w*- transgenic progeny will have colored eyes, in contrast to the white eyes of their *w*- non-transgenic siblings. Presence of a mini-w at a transgenic insertion creates flies with eye color shades anywhere from pale yellow to nearly red, depending on the insertion location. In this way, we say that the transgene is 'marked' by the mini-w.

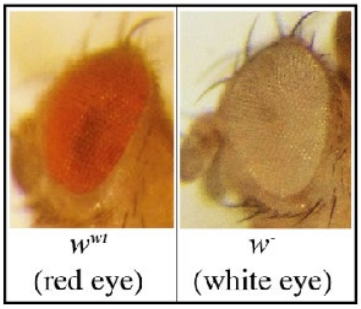


Figure 3. The w^{wt} and *w*- eye color phenotypes

How Does a Balancer Get 'Marked'?

Used to preserve mutations in a fly population, balancer chromosomes are chromosomes that contain multiply-inverted regions of DNA. The multiple inversions ultimately prevent recombination with a sister chromatid along the length of the balancer (for review, see Module 1). These multiple inversion events are not benign; they generate mutations in genes within the endogenous chromosome. When a mutation results in a visible phenotype, we say that mutation and its phenotype is a 'core' marker of the balancer.

Balancers can, and usually do, have more than one core marker – but often the most obvious one is used to determine the presence of the balancer and in everyday communication[1], as shown in Table 1: A Brief List of Common Balancers and Markers. In fact, it's so common to associate a balancer with its most easily visible marker phenotype that sometimes scientists, new to the field, are unaware of additional core markers. For example, the Curly of Oster (CyO) balancer on Ch.2 actually contains four different core marker mutations. The most obvious one is the Cy^1 mutation that causes wings to curl upwards, rather than lay flat and straight as wildtype wings do (Fig.4). Because the CyO phenotype is so pronounced, scientists rarely utilize the three additional markers, which give more subtle or confusing phenotypes.

In addition to their core markers, balancers can have markers added by recombination. As the balancer inherently prevents recombination within its chromosomal boundaries, these added markers are difficult to achieve. However, once incorporated these markers similarly resist loss by recombination, precisely due to their close chromosomal distance to the balancer.

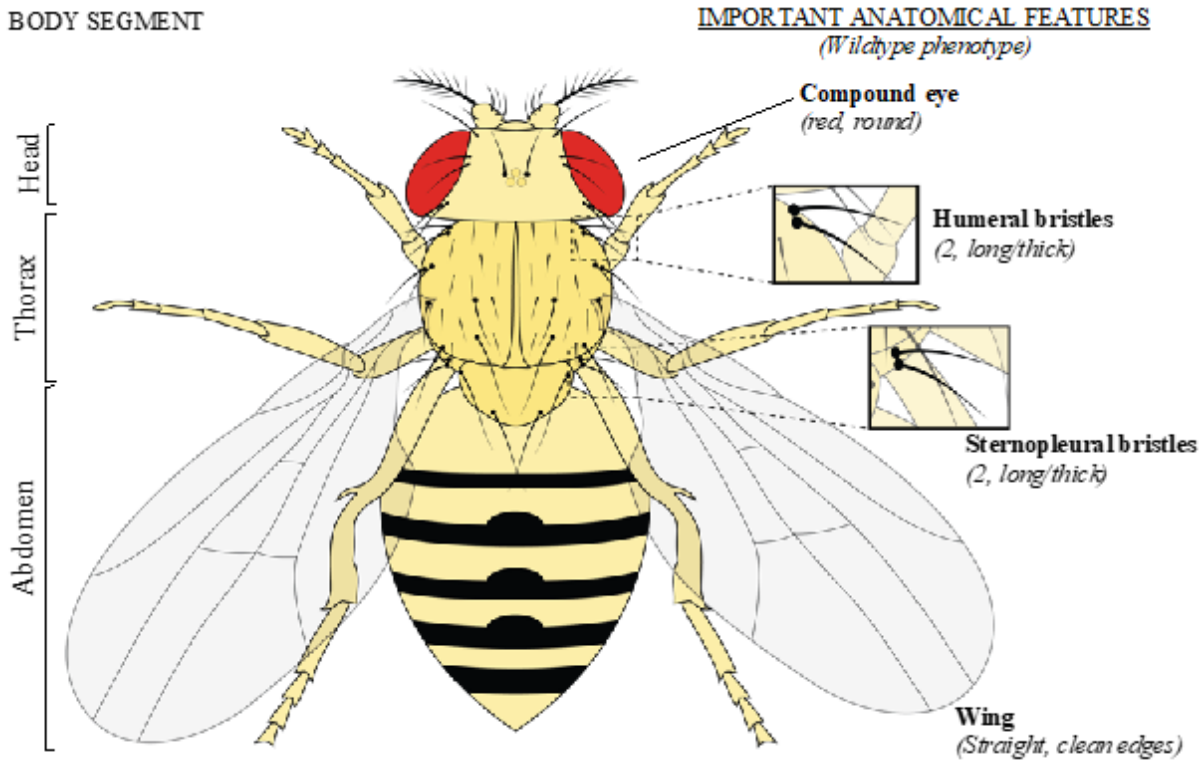
[1] To view common balancers and the entirety of their core markers, see Bloomington Drosophila Stock Center: Core Balancer Definitions https://bdsc.indiana.edu/stocks/balancers/balancerdefs.html

2 While it is technically correct to use a superscript(-) to note a white white eye mutant, we have chosen to remove the superscript for ease of reading

Common Anatomy Affected by Marker Mutations

Module 3 works with commonly used balancers containing markers that affect (1) eye color and shape, (2) wing shape, and (3) hair number, length and count. Please study in detail the wildtype fly anatomy as shown in Fig. 4A; for instance, note the many symmetrical short and long hairs on the thorax, such as the humeral bristles on the 'shoulder.' Try to identify in this real fly the features in the photograph of a real fly (Fig. 4B).

A



B

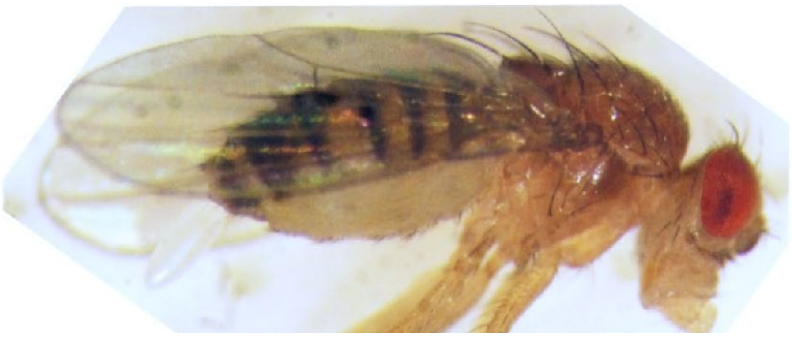


Figure 4. A) Highlights of anatomy on a wildtype female *D. melanogaster* fruit fly (dorsal view); B) Photographed image of a wildtype female *D. melanogaster* fruit fly lying on its side (image cuts off leg tips).

Common Balancers and Markers

In this section you'll find a table containing balancers and markers for chromosomes 1(X), 2 and 3 that will be used in Module 3. There are many more existing balancers[3] but these were selected for familiarization, being often used as they prevent recombination along the most length of their indicated chromosome. Some are included to highlight the exceptions that can occur with balancer-marker pairings, which can be highly problematic if not observed and are discussed below. Importantly, in everyday genotypic nomenclature the balancer name is written without the marker mutation (exception: markers without balancer association).

Table 1: A Brief List of Common Balancers and Markers

Ch #	*Balancer Name*	*Most Visible Phenotypic Marker Mutation (**Abbr.**)*	*Phenotype*	*Homozygous Lethal (IrnV)*
1 (X)	—	White (**w-**)	White eye	N
1 (X)	FM7a	Barred (**Bar**), apricot (w^a)	Rectangular ('barred') apricot-colored eye	N
2	CyO (Curly of Oster)	Curly (**CyO**, or Cy')	Curly wings (cannot fly, only jump)	Y
2	—	Sternopleural (**Sp**)	Extra bristles on the stemopleurite	Y
2	—	Scutoid (**Sco**, or sna^{Sco})	Loss of hairs on thorax in varying numbers	N
3	MKRS	Stubble ($\mathbf{Sb^1}$)	Length reduction of thick hairs on thorax	Mostly
3	TM1	Stubbloid lethal ($\mathbf{Sb^{sbd\text{-}l}}$)	Length reduction of thick hairs on thorax	Y
3	TM6B	Humeral (**Hu**, or $Antp^{Hu}$)	Extra hairs on shoulder	Y

Exceptions Discussed

In the Module 1 lecture slides, we noted that a key useful feature of balancer chromosomes is that they are homozygous lethal. Two exceptions to this are the Ch. 1(X) balancer FM7a, and Ch. 3 MKRS. In fact, MKRS has the same phenotype as another Ch.3 balancer, TM1, but the difference in lethality can confound progeny selection.

On another note, the Sp marker doesn't actually mark a balancer, although it is a homozygous lethal mutation. Though both Sp and Sco markers are often used over a Ch.2 balancer (like CyO) to mark progeny in multi-generational cross schemas, they shouldn't be used to actually balance a mutation.

[3] For some balancer lines which are commercially available, you can again visit 'Bloomington Drosophila Stock Center: Core Balancer Definitions' https://bdsc.indiana.edu/stocks/balancers/balancer_defs.html

Important Concepts for Practical Applications of Cross Design

Assumption: Male progeny receive Y from their father and X from their mother

Just like mammals, *Drosophila* have both X and Y chromosomes, which are located at Ch.1—as such, Ch.1 is often referred to as the 'X' chromosome. While not always true[4], you can usually assume females to be XX and males to be XY. When designing crosses, one assumes that fertile male progeny received their Y chromosome from the male parent and their X chromosome from the female parent. (This is important when dealing with mutations that are on the X chromosome, or `X-linked'). Thus when writing Ch.1 genotypes, a *w*-;; female is implied homozygous *w-/w-,* but a *w*-;; male is implied as *w-/Y*.

If a female is not virgin, you cannot accurately predict her progeny

Importantly, after mating, sperm from a male can remain in the female and comingle with sperm from subsequent partners. To ensure predictability of progeny genotypes, it is essential to always set up *Drosophila* crosses with virgin females, i.e., those that have never mated before.

Punnett Squares

It is assumed that FlyBuilder students have completed an introductory genetics course in which they received exposure to and some practice using Punnett squares used for progeny prediction. These skills are heavily utilized in Module 3, and thus students are strongly encouraged to (re)-familiarize themselves with the practice of setting up and completing Punnett squares. A good resource for review is Griffiths AJF, Miller RI, Suzuki DT, Lewontin RC, Gelbart WM. 2000. Patterns of Inheritance. In: An Introduction to Genetic Analysis, 7th edition. New York: W. H. Freeman, available free through NCBI Books at https://www.ncbi.nlm.nih.gov/books/NBK22098/

[4] Unlike mammals, *Drosophila* sex is not determined solely by the presence of a Y chromosome_ *Drosophila* sex is determined by the ratio of the number of X chromosomes to the number of autosomes (or the X:A value), and the Y chromosome confers sperm viability to males_ Females have an X: A ratio of 2:2=1, and males 1:2=0.5

Appendix C
Module 3: FlyBuilder Lab Instructor Guide and Answer Key

Instructor Notes

(1) For completion of Module 3, students are provided a review Balancer Table containing the appropriate genotypic nomenclature and the recessive lethality of each balancer and marker mutation (content sourced from Module 2). However, given a varied level of Module 2 content comprehension, students may find it helpful to additionally reference Module 2 Reading materials during the Module 3 laboratory assignment, and should be permitted to do so.

(2) A check for understanding between student and instructor is recommended after students complete the first problem in each Part. Otherwise, students compound their errors throughout the assignment.

(3) Providing a reference wild type fly while using the FlyBuilder kit allows students to reference the placement of the various parts of the fly, especially sternopleural and humeral bristle placement. A reference fly can be found in Module 2.

(4) Students may turn in their completed Worksheet at the end of Module 3, or instructors can request a formal lab writeup of Worksheet contents, which can then include the pictures of 'built' flies students take in Parts II-III of the lab.

(5) Additional Instructor Notes can be found in *red italics* throughout the body of this lab.

(6) Answers can be found in red beneath each question throughout the body of this lab.

Module 3: FlyBuilder Lab

Materials Needed:

a) FlyBuilder Kit
b) Balancer Table
c) Worksheet

Assumptions:

1. All unaltered chromosomes are wildtype and should be written as '+'.
2. All genotypes should be written out in full, including balancers, mutations or wildtype **for both homologous chromosomes** (when heterozygous). Leaving off Ch.4 is acceptable.
3. Balancers should be written as their name, not their phenotypic marker mutation.
4. **'mut#' is a mutation that is marked with a mini-w**. Numbers indicate different mutations.
5. Each fly that you build must have all important anatomical features (see body template card for list) with phenotype as indicated by genotype.
6. For this lab: Eye color due to presence of a mini-w is always orange, regardless of how many mini-w transgenes are present in the genotype.*

**Instructor Note: In reality, dose dependence (ie: color variation) due to copy number or chromosomal location of a mini-w does exist. Thus, more than one mini-w in the genotype can sometimes be used as a selection marker when choosing progeny, but should not be relied upon, and is thus not introduced in this lab.*

Part I. Write the genotype of each fly with the given sex and phenotype:

---[Example: FEMALE, stubble hairs could be written as ♀ *+ ; + ; +/MKRS*]---

a. MALE, extra shoulder hairs, red eyes

♂ *+ ; + ; +/TM6B* OR *+/y ; +/+ ; +/TM6B* OR *+/y; + ; +/TM6B*

b. FEMALE, stubble hairs, barred apricot eyes, extra sternopleurite hairs (also stubble)

♀ *FM7a ; +/Sp ; +/MKRS* OR *FM7a/FM7a ; +/Sp ; +/MKRS*

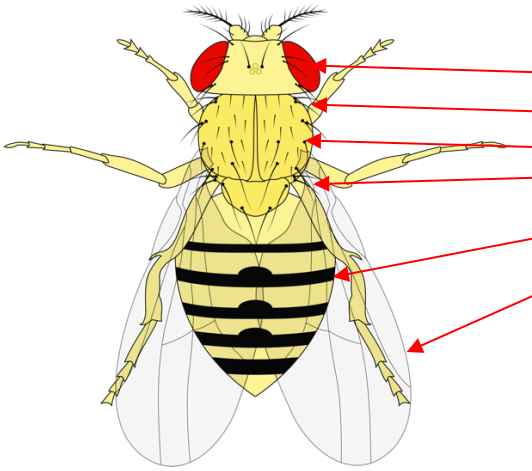
c. MALE and FEMALE (write separately): white eyes, curly wings, reduced number of thick thorax hairs, extra shoulder hairs

♂ *w- ; Sco/CyO ; +/TM6B* OR *w-/y ; Sco/CyO ; +/TM6B*

♀ *w- ; Sco/CyO ; +/TM6B* OR *w-/w- ; Sco/CyO ; +/TM6B*

Part II. Using your FlyBuilder kit, build flies of the given genotypes on your provided building mat. Take a picture of each for your lab writeup.

Example: A wildtype FEMALE (♀) + ; + ; +

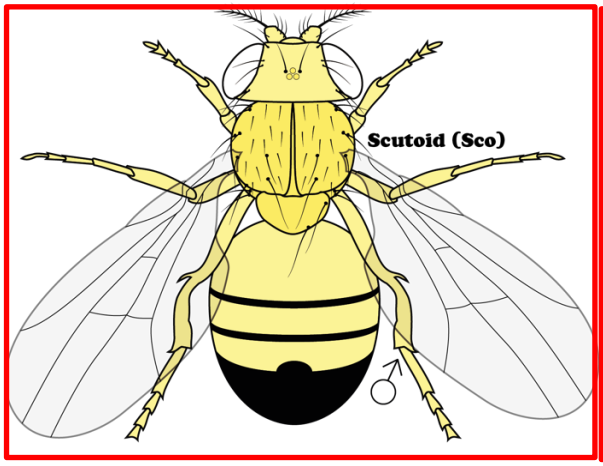


Instructor Note: Every fly built should have

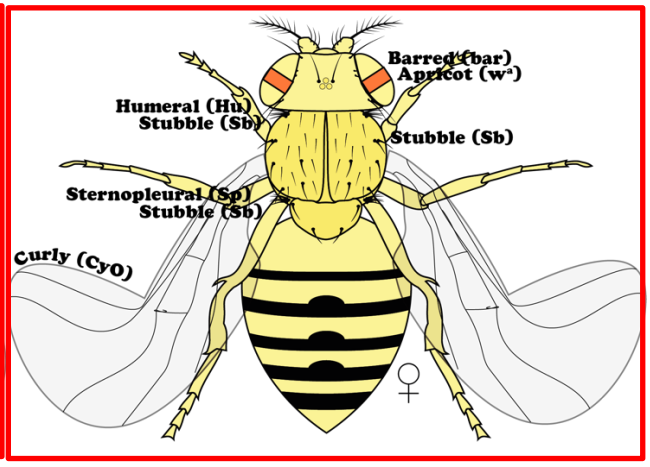
- *Compound eye*
- *Humeral bristles*
- *Thorax hairs*
- *Sternopleural bristles*
- *Abdomen*
- *Wings*

Students will place these six components atop their FlyBuilder body mat to build a complete fly.

a. MALE (♂) *w- ; Sco/+ ; +*

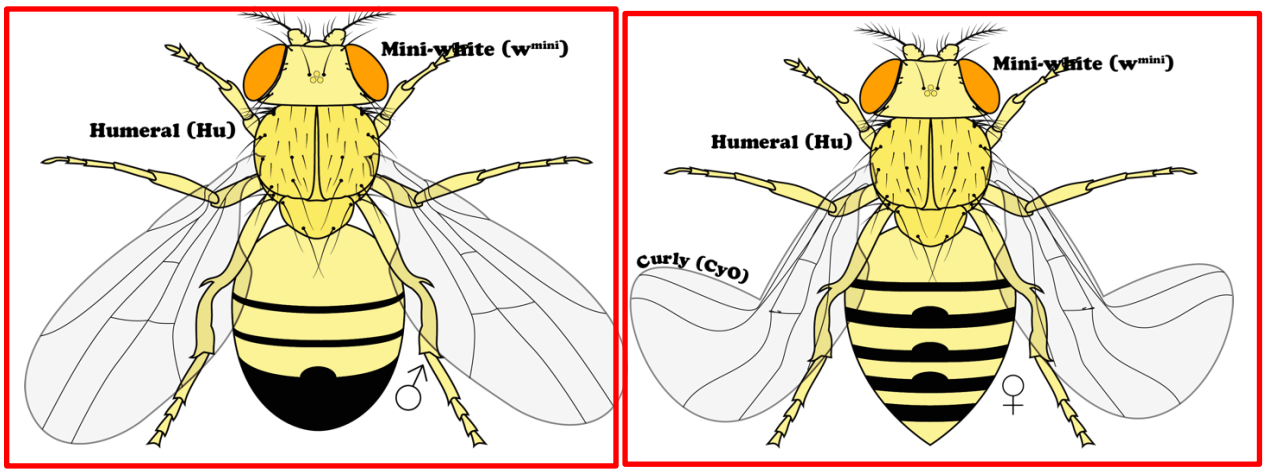


b. FEMALE (♀) *FM7a ; Sp/CyO ; MKRS/TM6B*



c. MALE (♂) *w- ; + ; mut3/TM6B*

d. FEMALE (♀) *w- ; mut2/CyO ; +/TM6B*

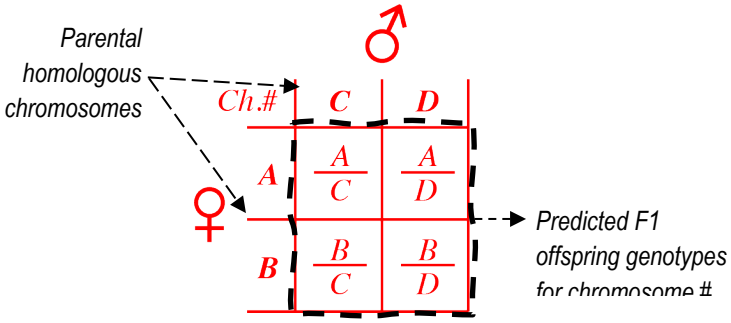


Part IIIa. Write out the Punnett squares for the following fly crosses. After crossing out any non-viable progeny, use your FlyBuilder kit to build an example of each type of viable progeny. Include an image of each progeny in your writeup. (Where males and females have the same phenotype, provide one image with written indication of male AND female.)

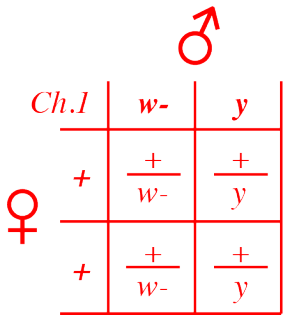
Instructor Note: For simplicity, let students write individual 2x2 squares for each chromosome #1-3. Students should demonstrate understanding of balancer homozygous lethality by crossing out any non-viable progeny squares after indicating the Mendelian cross output.

It may be helpful to remind students of Punnett squares by demonstrating the following mock and example crosses:

On any particular chromosome, for parents of genotype female A/B and male C/D,



Example: for parents of genotypes MALE (♂) *w- ; + ; +* x FEMALE (♀) *+ ; + ; +*
(only need to set up Ch.1 square, since all other chromosomes are wildtype)



F1 progeny will be predicted to be 50% female *+/w- ; + ; +* , and 50% male *+/y ; + ; +* (or, ♂*+ ; + ; +*). Males will appear wildtype with red eyes; females will have reduced red eye pigmentation due to the heterozygous *white* mutation. Students may build both male and female flies of the same phenotype or build one and indicate in writing that the cross would also produce a male/female fly of the same phenotype.

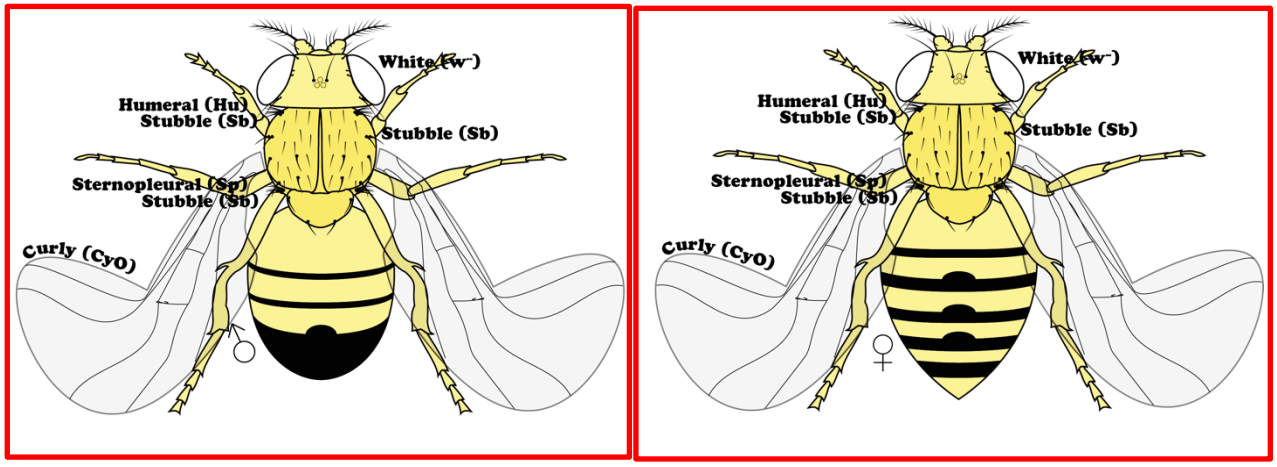
a. MALE (♂) *w- ; Sp/CyO ; MKRS/TM6B* x FEMALE (♀) *w- ; Sp/CyO ; MKRS/TM6B*

♀ \ ♂	*w-*	*y*
w-	*w-* / *w-*	*w-* / *y*
w-	*w-* / *w-*	*w-* / *y*

♀ \ ♂	***Sp***	***CyO***
Sp	*Sp* / *Sp*	*Sp* / *CyO*
CyO	*CyO* / *Sp*	*CyO* / *CyO*

♀ \ ♂	*MKRS*	*TM6B*
MKRS	*MKRS* / *MKRS*	*MKRS* / *TM6B*
TM6B	*TM6B* / *MKRS*	*TM6B* / *TM6B*

Flies will be male *(w-/y)* or female *w- ; Sp/CyO ; MKRS/TM6B.* Students should build a single fly of *w-; Sp/Cyo ; MKRS/TM6B* and indicate they will have male and female progeny of this phenotype in their writeup (both shown below for reference).



b. MALE *w- ; mut2/ CyO ; +* x FEMALE *w- ; + ; mut3/TM6B*

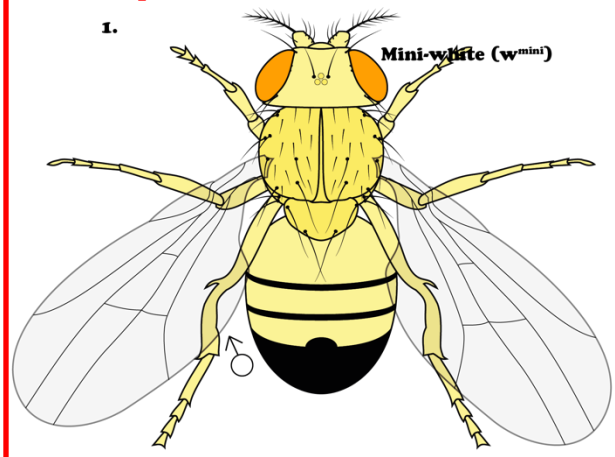
♀ \ ♂	*w-*	*y*
w-	*w-* / *w-*	*w-* / *y*
w-	*w-* / *w-*	*w-* / *y*

♀ \ ♂	***mut2***	***CyO***
+	*+* / *mut2*	*+* / *CyO*
+	*+* / *mut2*	*+* / *CyO*

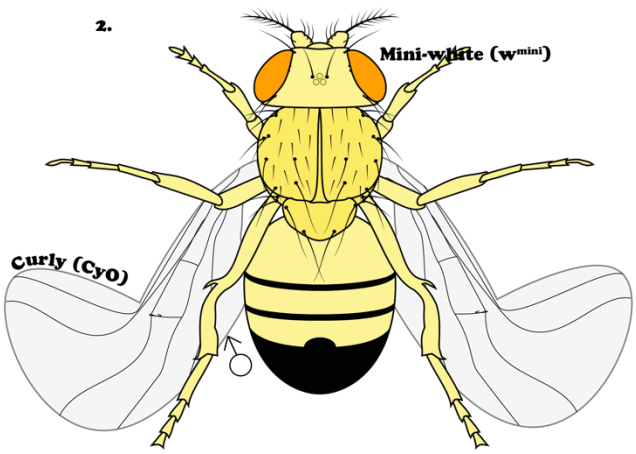
♀ \ ♂	*+*	*+*
mut3	*mut3* / *+*	*mut3* / *+*
TM6B	*TM6B* / *+*	*TM6B* / *+*

Viable progeny will be male (*w-/y*) or female (*w-*) of the following genotypes/phenotypes (shown as male):

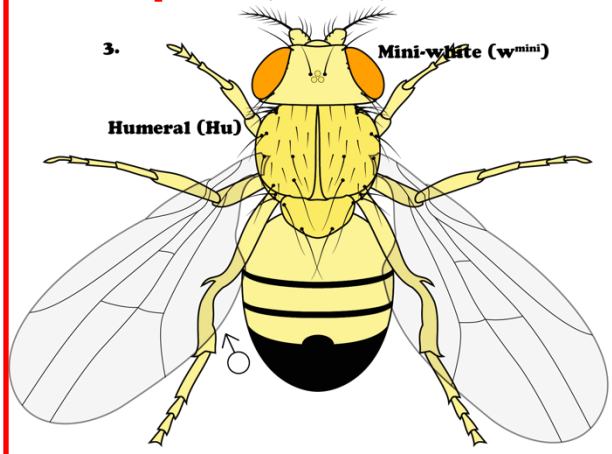
1. ♀or ♂*w- ; +/mut2 ; mut3/+*



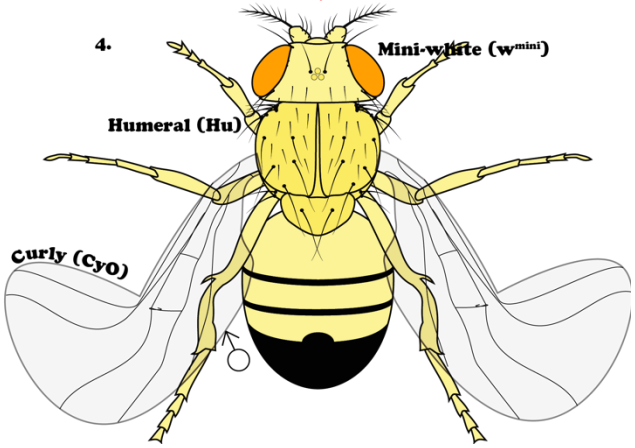
2. ♀or ♂*w- ; +/CyO ; mut3/+*



3. ♀or ♂*w- ; +/mut2 ; TM6B/+*



4. ♀or ♂*w- ; +/CyO ; TM6B/+*



c. MALE *w- ; mut2/CyO ; +* x v.FEMALE *w-, mut1; + ; mut3/MKRS*

Instructor Note: This cross calls attention to X-linked mutations. If the F1 progeny of this cross are used in a subsequent cross, only the F1 males will predictably pass on mut1 to 100% of F2 females. This is a useful tactic when designing cross schema in Part IVa(b) and PartIVb(b).

♂

♀	Ch.1	w-	y
	w-, mut1	w-, mut1 / w-	w-, mut1 / y
	w-, mut1	w-, mut1 / w-	w-, mut1 / y

♂

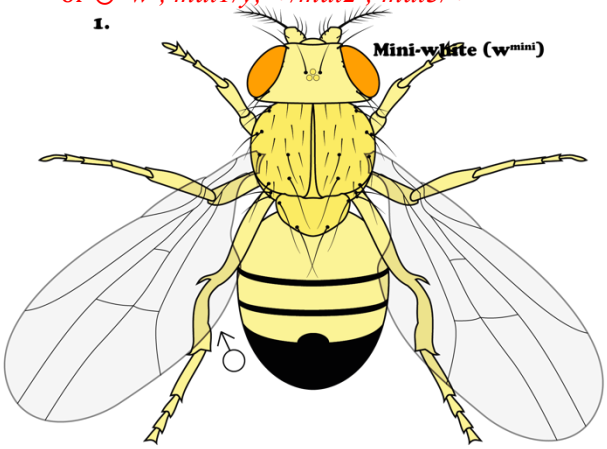
♀	Ch.2	mut2	CyO
	+	+ / mut2	+ / CyO
	+	+ / mut2	+ / CyO

♂

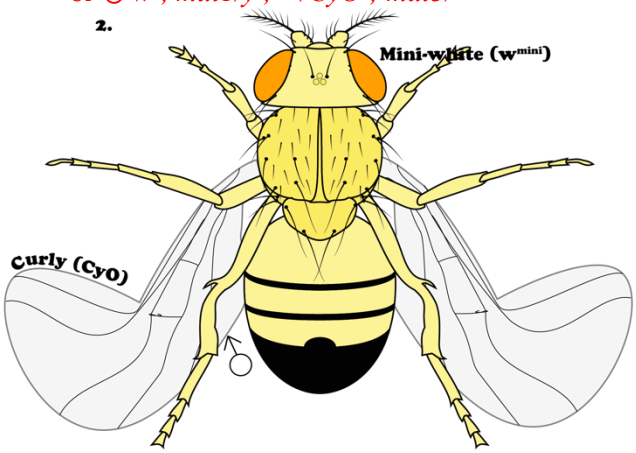
♀	Ch.3	+	+
	mut3	mut3 / +	mut3 / +
	MKRS	MKRS / +	MKRS / +

Flies will have the following genotypes/phenotypes (shown as male):

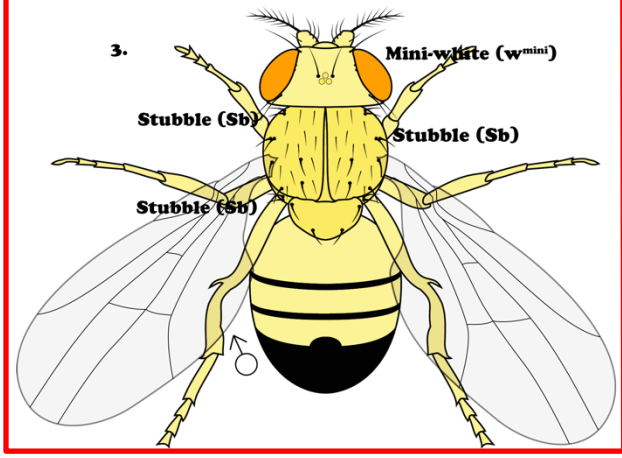
1. ♀ *w-, mut1/w-; +/mut2 ; mut3/+*
or ♂ *w-, mut1/y; +/mut2 ; mut3/+*



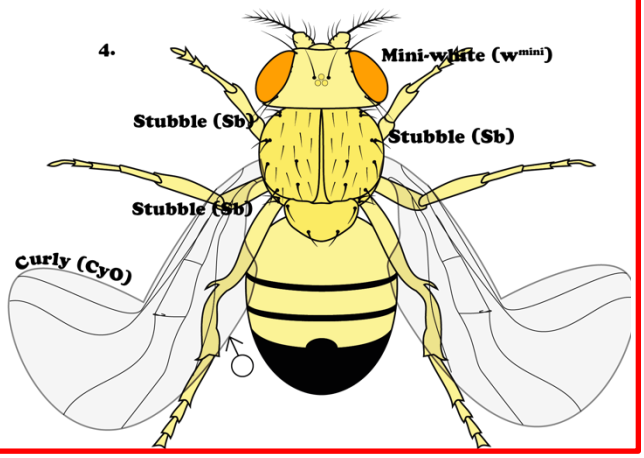
2. ♀*w-, mut1/w-; +/CyO ; mut3/+*
or ♂*w-, mut1/y ; +/CyO ; mut3/+*



3. ♀*w-, mut1/w- ; +/mut2 ; MKRS/+*
or ♂*w-, mut1/y ; +/mut2 ; MKRS/+*



4. ♀*w-, mut1/w- ; +/CyO ; MKRS/+*
or ♂*w-, mut1/y ; +/CyO ; MKRS/+*



Part IIIb. A stable line is a population of flies where mating among any of them, throughout multiple generations, will only produce a single predicted genotype (or a single balanced genotype, which may or may not homozygose depending on the mutation it balances. Regardless, you will always know the genotype(s) in that population.) *Which cross above generates a stable line?* Part III A cross

Part IVa. A critical skill of any Drosophila researcher is the ability to generate a fly of a specific genotype from existing stable 'parent' fly lines in the lab. This often involves setting up multiple generations of crosses using progeny from a prior cross. A plan of these crosses, designed beforehand, is known as a 'cross schema.'

Here, you must design a cross schema to generate a fly with the indicated target genotype, from the stable parent line genotypes provided (in boxes below). *For each cross in your schema, make sure to indicate:* (1) which parent will be male and which will be virgin female, and (2) what balancer and corresponding phenotype you will select for (or against) to ensure correct genotypic selection of progeny.

EXAMPLE (single cross)

Stable parent lines:

FM7A ; mut2; +	*+ ; Sp/CyO ; +*

P ♂+ ; Sp/CyO ; + ✖ ♀FM7a ; mut2 ; +

Select ONLY ♂ F1

***For:* CyO, FM7a *Against*: w-, Sp**

Selected male flies will have curly wings AND barred, apricot colored eyes. They will NOT have extra sternopleural hairs or white eyes.

♂ FM7a ; mut2/CyO ; +

a) Target: ♀ ***w- ; mut2/CyO ; mut3/TM6B***

Stable parent lines:	*w- ; mut2; +*	*w- ; +; mut3/TM6B*	*w- ; Sp/CyO ; MKRS/TM6B*

Instructor Note/Hint: You need to set up multiple parental (P) crosses simultaneously and mate their respective F1 progeny.

P ♂w- ; mut2 ; + ✖ ♀w- ; Sp/CyO ; MKRS/TM6B

Select ♀ F1

***For:* Sp, TM6B *Against*: CyO, MKRS**

Selected female flies will have extra humeral AND sternopleural hairs. They will NOT have curly wings or stubble hairs.

♂w- ; + ; mut3 ✖ ♀w- ; Sp/CyO ; MKRS/TM6B

Select ♂ F1

***For:* CyO, MKRS *Against*: Sp,TM6B**

Selected male flies will have curly wings AND stubble hairs. They will NOT have extra humeral or sternopleural hairs.

Cross #2 ♀ w- ; mut2/Sp ; +/TM6B ✖ ♂w- ; +/CyO ; mut3/MKRS

Select only ♀F2

***For:* CyO, TM6B *Against*: Sp, MKRS**

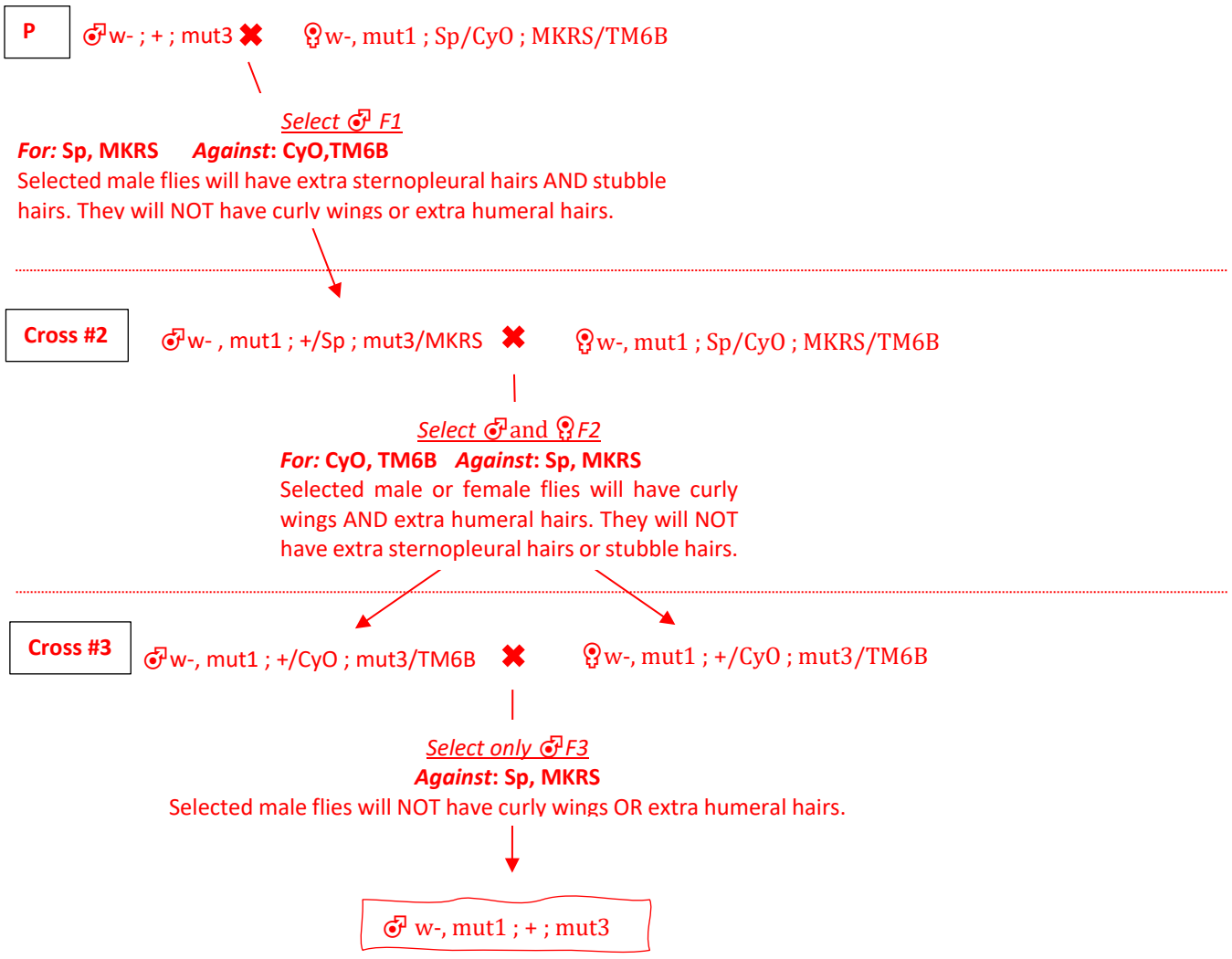
Selected female flies will have curly wings AND extra humeral hairs. They will NOT have extra sternopleural hairs or stubble hairs.

♀ w- ; mut2/CyO ;

b) Target: ♂ ***w-, mut1 ; + ; mut3***

Stable parent lines:	*w-, mut1 ; Sp/CyO ; MKRS/TM6B*	*w- ; + ; mut3*

Instructor Note/Hint: Students will need to understand that having stable parent lines means they can mate future progeny back to flies of the original parent line genotype.



Part IVb. Now, design the cross schema that will generate stable lines (as explained in **Part IIIb**) of the 'target' lines indicated in parts *a* and *b* in the space below. For each cross, make sure to label which parent will be male and which will be virgin female. At each progeny selection, indicate what phenotype/balancer you will select for (or against) to ensure correct genotypic selection.

a) Target: STABLE LINE ***w- ; mut2/CyO ; mut3/TM6B***

Stable parent lines:	*w- ; mut2; +*	*w- ; +; mut3/TM6B*	*w- ; Sp/CyO ; MKRS/TM6B*

All crosses and selection criteria will be the same as Part IVa, EXCEPT students should select both male and female F2 siblings of the specified phenotype and cross them to each other. The resulting progeny will be a stable line of *w-; mut2/CyO ; mut3/TM6B*

b) Target: STABLE LINE ***w-, mut1 ; + ; mut3***

Stable parent lines:	*w-, mut1 ; Sp/CyO ; MKRS/TM6B*	*w-;+ ; mut3*

All crosses and selection criteria will be the same as Part IVb, EXCEPT students should select both male and female F3 siblings of the specified phenotype and cross them to each other. The resulting progeny will be a stable line of *w-, mut1; ; mut3.*

Appendix D: Adaptations For Different Instruction Levels

In the provided form, the FlyBuilder curriculum is best suited for intermediate and advanced level students that have already taken introductory coursework in biology and genetics. However as mentioned, this curriculum can also complement less advanced coursework. By including or excluding content within Modules 2 and 3, one can adjust the depth of learning from evaluating and creating tasks in an upper level course to basic understanding and remembering tasks in an introductory course. The FlyBuilder kit in particular can be used independently at a range of levels: it can provide students hands-on visualization of Mendelian trait inheritance (introductory) or it can enable exposure to the practicalities of working with Drosophila genetic tools before beginning multi-week crossing schemas (advanced). Below are some suggestions for adaptation to introductory and intermediate level coursework.

Please Note: At all levels, Module 2 contains some information for students working with live Drosophila, which can be removed according to the instructor's preference.

Introductory Level

An instructor may use FlyBuilder during a single laboratory or classroom session as introduction to the topic of Mendelian genetics. At an introductory level, the instructor should spend additional Module 1 lecture time discussing genetic nomenclature and the relationship between genotype and phenotype. Students then reinforce these concepts by completing all of Module 2 and Module 3 Parts I and II.

Suggested content removal: Module 2 Section VI, Module 3 Parts III & IV.

Intermediate Level

At this level, the instructor can use the FlyBuilder Kit to teach students how to apply basic concepts in Mendelian Genetics to practical laboratory applications. We suggest that the instructor provides a lecture on genetic nomenclature, *Drosophila* balancer chromosomes and practical applications for these genetic tools in the laboratory prior to completion of the lab. Students will then use this information to predict progeny from given mating schemas, create crossing schemas of their own to produce given progeny and critically evaluate their proposed mating schemas to determine their usefulness in the laboratory.

Suggested content removal: Module 3, Part IV.

Advanced Level

At the advanced level, the instructor should cover all topics discussed in the intermediate level with the addition of critical evaluation of proposed mating schemas to determine their usefulness in the laboratory. At this level, students will reinforce concepts by completing the entirety of the FlyBuilder workshop. At the advanced level we suggest pairing the FlyBuilder workshop with wet lab experiments involving live Drosophila. Some suggestions are: (1) Having students sort a mixed population of animals by phenotype (2) Design and execute a mating scheme, sorting and counting progeny to evaluate genetic ratios.

Suggested content removal: None.

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Citing This Article

Flyer-Adams JG, Barbagallo B, Griffith LC. 2020. FlyBuilder: A multimodal dry lab curriculum teaches Mendelian genetics through the lens of *Drosophila* balancer chromosomes. Article 7 In: McMahon K, editor. Advances in biology laboratory education. Volume 41. Publication of the 41st Conference of the Association for Biology Laboratory Education (ABLE). **https://doi.org/10.37590/able.v41.art7**