Performing a fly cross

1 Overview

Next month, we will perform one of the classic experiments in developmental biology, making measurements of morphological features of *Drosophila* embryos. In preparation for that experiment, we need to mate flies to produce the desired mutant we want to study. This mutant contains a single functional copy of the gene *bicoid*, abbreviated as *bcd*. The idea of our experiment is to change the "dosage" of the *bcd* gene and measure how it changes patterning of the developing embryo.

As you mate the flies, you will learn some basic skills of fly handling and genetics. This is a useful skill because, like *E. coli* (which you have already encountered in this course), *Drosophila melanogaster* is an important **model organism**. Historically, many major findings in biology have come from this organism, including the discovery that genes are carried on chromosomes. *Drosophila* continues to be an important model for studying of genetics, pathogenesis, neuroscience, behavior, and, as we will study in Bi1x, development.

2 Basic fly genetics

2.1 Notation

Drosophila have four pairs of homologous chromosomes. The chromosome pairs are written as "fractions," with the numerator describing what mutations are on one of the homologous chromosomes and the denominator describing the mutations on the other. Wild type (no mutations) is denoted with a "+." The chromosome "fractions" are separated by semicolons. So, if a fly has mutation m on one copy of chromosome 3 and the other copy is wild type, the chromosomal composition would be written as $;\,;m/+$. (Note that if both homologous chromosomes are wild type, their "fraction" is typically omitted.) If it had the mutation m on both chromosomes, it would be written as $;\,;m/m$. A fly with such chromosomal makeup is said to be **homozygous**. Conversely, a fly with $;\,;m/+$, or $;\,;m_1/m_2$ (where m_1 and m_2 are two different mutations) is **heterozygous**. Finally, multiple mutations on the same copy of a chromosome are separated by commas, e.g. $;\,;m_1, m_2/+$.

2.2 Heredity

When two flies are mated, the offspring inherit one copy of each chromosome from each parent. As an example, consider mating a female (♀) fly with genotype $;\,;m/b,s$ with a wild type male (♂) fly. Since chromosomes 1, 2, and 4 are wild type in both parents, all offspring will have wild type chromosomes

female chromosome 3 urn   male chromosome 3 urn

Figure 1: The chromosomes that an offspring inherits can be thought of as draws from urns, one for the mother, and one for the father. Note that each "ball" in the urn contains a whole chromosome, inclusive of all mutants. Therefore, the mutations b and s come together as a package.

1, 2, and 4. For chromosome 3, the mother may donate a chromosome with genotype m or b, s. The male will necessarily donate a chromosome with + genotype.

You can think of the genotype of the offspring as being derived from two draws from urns (Figure 1). The urn corresponding to female chromosome 3 has two types of balls, m and b, s. The male urn has only one type of ball, +. The offspring chromosome pair gets one ball from the female urn and one from the male urn. The offspring have a 50/50 chance of being male or female, so all possible offspring from this example cross are as follows.

Cross: ♀ $\frac{m}{b}$ × ♂ $\frac{+}{+}$
♀ $\frac{b,s}{+}$
♀ $\frac{m}{+}$
♂ $\frac{b,s}{+}$
♂ $\frac{m}{+}$

3 Making a *bcd*/+ fly

In this experiment, we will cross the following flies.

$$♂\ ;\,;\ \frac{bcd}{bcd} \times ♀\ ;\,;\ \frac{+}{+}.$$

All offspring will therefore be $;\,;\, bcd/+$ because the offspring will get one chromosome 3 with *bcd* from the father and one wild type from the mother. This is our desired mutant, since the *bcd* mutant is not functional, but the wild type (+) is. Note that we cannot see any effects of the *bcd* mutation in adult flies. (Next month, we will measure the effects of this mutation on embryos.)

The male flies come from a vial that contains a mixture of bcd/bcd and $bcd/TM3, Sb^1$. The vial is mixed because a) *bcd* is homozygous sterile, meaning that female flies with $;\,;\, bcd/bcd$ cannot produce offspring, and b) TM3 is homozygous lethal, meaning that we will never see offspring with genotype

Figure 2: Male and female flies, from FlyMove. Males are typically smaller than females. The rear abdomen of males is also darker. The best way to distinguish males from females is to look at their genitalia; see Figure 3.

$;;TM3,Sb^1/TM3,Sb^1$.* The heterozygous flies have the Sb^1 mutation, which we can see visually in the adult fly (as described below). Therefore, for our cross, we need to find which males are *bcd*/*bcd* in our mutant vials, and put them in a vial with virgin wild type flies. The females have to be virgins so we can be sure they did not mate with any other males besides the ones we want them to mate with.

4 Mechanics of a cross

4.1 Determining the sex and virginity of a fly

We need to identify male and female flies, as well as female virgins (☿, though the virgin identification will be done by your TAs). Figures 2 and 3 show visual cues. Your TAs will have virgin wild type flies ready for you. You need to pick out the males from the mutant stock.

4.2 Identifying phenotypes

To pick out which flies are homozygous in the mutant stock, we need to determine which flies have the Sb^1 mutation and which do not. We want to pick out the ones that do **not**. Figure 4 describes how to identify this phenotype.

*To check your mastery of the rules of heredity, can you write out all mating pairs that occur in this vial and their offspring to verify that the vial will contain the mixture of genotypes we say it does?

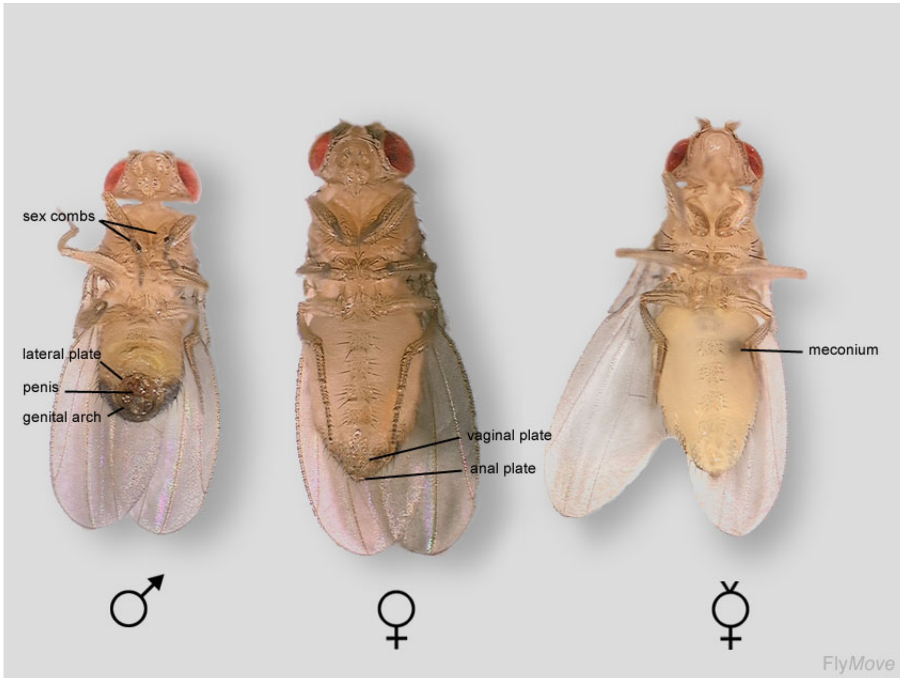

Figure 3: The underside of male, female, and virgin flies, from FlyMove. The genitalia of males and females is clearly different. Virgin females are often slightly larger and lighter in color. Importantly, the meconium, a dark spot remaining from the fly's last meal as a larva, is visible.

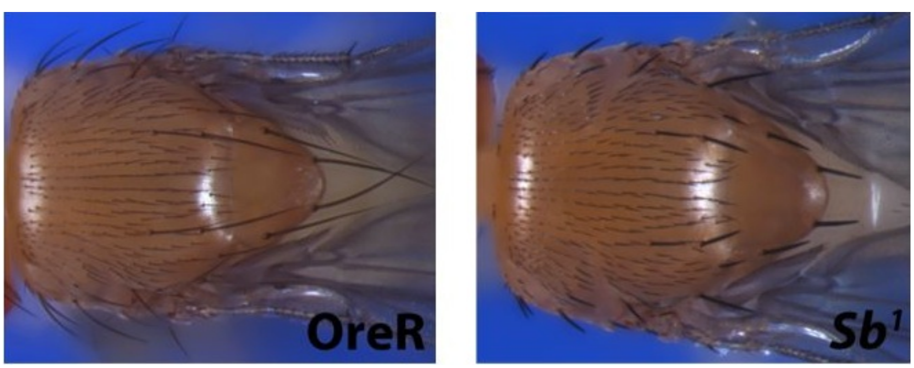

Figure 4: Images of the thorax of adult fruit flies from FlyBase. Oregon R is wild type. The Sb^1 phenotype features shorter bristles that lack the taper and point of wild type. The easiest way to identify whether a fly is Sb^1 or wild type is to look at the back two bristles, which cross each other in wild type.

4.3 Handling flies

Flies are kept in plastic vials with porous cotton plugs on them. The bottom of the vials contains food. If the plug is removed, the flies will fly away. Therefore, they must be anesthetized. We use carbon dioxide for this purpose. To put the flies to sleep, insert a needle connected to a CO_2 source into the vial through the cotton plug. The vial should be inverted (cotton plug down) while you do this to ensure that the flies do not get stuck in the food. When the flies stop moving in the vial, remove the plug and let the flies fall onto a flypad. The flypad is connected to a CO_2 source, so the flies continue to sleep on the pad. Push the flies around with a paint brush while looking at them using a dissection microscope. You can brush the flies into fresh vials as needed for crosses. Flies you wish to discard go to the "morgue." For this purpose, we use a beaker of soapy water in an ice bucket.

5 Protocol for making the *bcd*/+ fly

Following the fly handling guidelines of section 4, do the following:

1. Your TAs will provide you with vials of wild type virgins.
2. Collect males that have wild type bristles from the vial containing the mutant flies.
3. Place two or three of these males in each vial of wild type virgins.
4. After about 8 or 9 days, adult flies from the cross will begin to eclode (emerge after completion of metamorphosis). Collect the flies.[†]

[†]You can keep the males, but it is the females that are important, since we are studying the effect of *bcd*. *Bcd* is a **maternal effect gene**, which means that the genotype of the mother alone determines what happens in the development of the offspring. I.e., the genotype of the offspring is unimportant, and therefore so is the genotype of the father. We will cover this in more depth when we look at the effects of *bcd* on development next month.