

BIO440 Genetics Laboratory *Drosophila* crosses - gene mapping

Objectives:

- to review and extend your understanding of transmission genetics--how traits are passed from parent to offspring
- to give you practice confronting difficult data, and using the scientific method to make sense of complex phenomena
- to review how mapping calculations are performed

In the Genetics Lecture course, you probably spent the first several weeks of class talking about Mendelian Genetics. Fundamental concepts such as the significance of chromosomes, sorting of chromosomes in meiosis, dominance/recessiveness, and sexual determination, to mention only a few, came from direct observations of transmission genetics. Many insights concerning the molecular basis of genetics came from observing the patterns of transmission of easily-observable morphological traits in the common fruitfly. *Drosophila* continues to be an important organism in contemporary genetics, particularly developmental genetics and genomics, and was one of the first eukaryotes to have the nucleotide sequence of its entire genome determined.

In the introductory lecture, we will review basic Mendelian genetics and statistical analysis related to the crosses that we will be carrying out. We will then go over *Drosophila* culture, anesthetizing *Drosophila*, and determining sex in *Drosophila*.

An introduction to *Drosophila melanogaster*

What is it and why bother about it?

Drosophila melanogaster is a fruit fly, a little insect about 3mm long, of the kind that accumulates around spoiled fruit. It is also one of the most valuable of organisms in biological research, particularly in genetics and developmental biology. *Drosophila* has been used as a model organism for research for almost a century, and today, several thousand scientists are working on many different aspects of the fruit fly. Its importance for human health was recognized by the award of the Nobel prize in medicine/physiology to Ed Lewis, Christiane Nusslein-Volhard and Eric Wieschaus in 1995.

Drosophila melanogaster was first recorded in New York City in 1875, and in New Haven in 1879, introduced again and again in fruit shipments from the south. In the wild, adults and larvae feed on yeast and bacteria growing on rotting fruit. In the Lab they can be fed on the yeast cells growing on a high carbohydrate prepared diet.

Why work with *Drosophila*?

Part of the reason people work on it is historical - so much is already known about it that a) it is easy to ask specific, answerable questions, and b) it is easier to interpret results, Part of the reason people still work with *Drosophila* has to do with why people worked with it in the first place; it's a small animal, with a short life cycle of just two weeks, and it's cheap and easy to keep large numbers of the flies. Mutant flies, with defects in any of several thousand genes are available, and the entire genome has been sequenced. Work using *Drosophila* is on the increase -- detailed gene expression studies are now being

carried out using microarray analysis. *Drosophila* is also widely used in developmental biology, looking to see how a complex organism arises from a relatively simple fertilized egg. Embryonic development is where most of the attention is concentrated, but there is also a great deal of interest in how various adult structures develop in the pupa, mostly focused on the development of the compound eye, but also on the wings, legs and other organs. Scientists study simple model systems in hopes of understanding principles that can apply to complex systems. Some *Drosophila* genes have homologs (corresponding genes with similar structure and functions), in other animals, including vertebrates. Some vertebrate genes when introduced into flies do the job of the homologous fly gene. It seems that there are more similarities of design among organisms than there are differences. This universality helps biologists understand the complexities of living things.

Life cycle of *Drosophila*

The *Drosophila* egg is about half a millimeter long. It takes about one day after fertilization for the embryo to develop and hatch into a worm-like larva. The larva eats and grows continuously, molting one day, two days, and four days after hatching (first, second and third instars). After two days as a third instar larva, it molts one more time to form an immobile pupa. Over the next four days, the body is completely remodeled to give the adult winged form, which then hatches from the pupal case and is fertile after another day. (timing is for 25°C; at 18°, development takes twice as long.)

The *Drosophila* genome

Drosophila has four pairs of chromosomes: the X/Y sex chromosomes and the autosomes 2,3, and 4. The fourth chromosome is quite tiny and rarely heard from (not many genes there). The size of the genome is about 165 million bases and contains an estimated 14,000 genes (by comparison, the human genome has 3,300 million bases and may have about 70,000 genes; yeast has about 5800 genes in 13.5 million bases). The genome was (almost) completely sequenced in 2000, and analysis of the data is now underway. Females normally have two X chromosomes; males have one X and one tiny Y chromosome. But sex is determined by the X to autosome ratio, not merely the presence or absence of the Y chromosome. (One of chromosome #2, #3, #4 = one set.) 2 X chromosomes to 2 sets of autosomes = 1:1 ratio = female. 1 X chromosome to 2 sets of autosomes = 1:2 ratio = male. For this reason, a fly with only one X and no Y chromosome (XO) is a male. This male is sterile because it lacks a Y chromosome, however externally it appears normal. A XXY fly is a normal female.

Red-eye/ white eye cross

The work with *Drosophila*, which Thomas Hunt Morgan began at his Lab at Columbia University in 1909, set the stage for his discovery of sex linkage in fruit flies. Our genetic cross is based on these experiments. He found that there is a mutation which affects eye color, changing it from the normal “wild type” red found in nature, to seemingly unpigmented and therefore white eyes. When a white-eyed male was crossed with a wild type female, all the offspring were red-eyed. This shows red-eyes to be dominant over white. When these red-eyed offspring were mated, the results followed a predictable 3:1 Mendelian ratio of red to white. But the interesting thing about these results were that all

the white-eyed flies were males. Half of the males were red-eyed, and all of the females were red-eyed. Morgan realized that these results could be explained if the gene for red vs white eye color were located on the X chromosome. In this experiment the parent (P) females have two X chromosomes each with a dominant red-eyed gene (homozygous for this trait) Males have only one X, in this case carrying the white-eyed mutation, and no gene for eye color on the Y. (This is termed hemizygous because the male can only have one possible copy of a gene, since the Y carries none.) The first generation (F1) offspring would be females all be carrying a red-eyed gene, and would therefore be red-eyed. In the F2 generation, all females would be red-eyed because male gametes could only have the red-eyed gene. Males would have a 50/50 chance of being red or white-eyed because half of the female gametes carry a white-eyed gene.

Making a Chromosome Map:

During meiosis, each homologous pair of chromosomes becomes doubled, forming 2 sets of sister chromatids. A synapsed (joined) bundle of 4 chromatids is called a tetrad. During Prophase I, the first phase of meiosis, the strands of the tetrad sometimes twist around each other. Non-sister chromatids may exchange segments at this point. This is crossing-over or recombination. In *Drosophila* it is only the females in which crossing over may occur. (The reason for this is not yet known.)

Maps of relative positions of linked genes on a chromosome can be constructed by noting the frequencies of crossing-over between genes. The closer two genes are together, the less likely they will show crossing-over. Conversely, the greater the distance between two genes on a chromosome, the greater the chance that a cross-over will happen between them. The probability of crossing over can be expressed as a distance or value (the % of crossovers that occur between 2 points on the chromosome). One map unit, (m.u.) is the distance between linked genes in the space where 1% of crossing-over occurs, or is the distance between genes for which one result of meiosis out of 100 is recombinant. (A map unit is sometimes called a centimorgan [cM] in honor of Thomas Hunt Morgan.) The position on the map where a gene is located is called the gene locus. On *Drosophila* chromosome 1 (X), for instance, the locus of the cross-veinless wings (cv) is 13.7. The locus of cut wings (ct) is 20.0, so the distance is 6.3 m.u. The relationship could be shown like this cv_____ (6.3 m.u.) _____ ct.

If the recombination frequency between cv and ct is 6.3, and ct and vermilion eyes (v) is 13, the order on the chromosome could either be cv-ct-v, or ct-cv-v. We can determine which of these is correct by measuring the recombination frequency between cv and v. If cv and v are found to recombine with a frequency of 19.3 %, then we deduce that ct is located between them.

Chromosome mapping is still very important in these days of genomics and proteomics. Many genes that have been sequenced do not have a known function, and the genes that are responsible for most genetic diseases have yet to be identified. Most of the disease genes that *have* been identified, were located by a process which relies on the mapping of the chromosomal location of the disease gene. This process of identifying a disease gene from knowledge of its chromosome location is known as positional cloning. This process has a number of steps that are performed sequentially:

Positional Cloning -

Collect families with disease of interest->
Genotype markers across genome->
analyze linkage to localize gene->
identify gene in linked interval->
identify mutations

Mapping Experiments in *Drosophila melanogaster*

We are going to analyze the inheritance of 3 genes located on the X chromosome in *Drosophila*. The first thing to work on is making sure that you can identify and distinguish male and female flies, as well as each of the traits that we are examining - bristles (long, **straight** vs small, *forked*), wing shape (**long** vs *short*) and eye color (**red** vs *white*). Each of the bolded traits is dominant over the italicized recessive traits.

The cross will be examining the inheritance of these three traits in an attempt to determine:

- gene order on the X chromosome
- distance between the genes

We will also analyze whether or not the short wing phenotype is inherited in a sex-linked fashion. To free up laboratory time for other experiments, I carried out a cross of wildtype males and mutant females. Now that two weeks have passed, we will examine the F1 progeny and carry out F1 crosses. Then, after an additional two weeks have passed, we will examine the F2's.

Timing Details

Day 1. Establishing (first cross) Parental cross from true-breeding flies (that yields F1 generation) Cross virgin mutant females with wildtype males

(why this cross - and not virgin wildtype females with mutant males?)

In the very early morning on the day of this experiment, your beloved instructor cleared all the adult flies from the various culture vials. That afternoon, all of the flies in the vials were known to have emerged within the last 8 hours. Because *D. melanogaster* females don't mate until 10 hours after emergence, all of the flies in your vials should be virgins. It is important to use virgin females, because female *Drosophila* stores sperm from previous copulations.

The first thing to work on is making sure that you can identify and distinguish male and female flies (red vs white eyes is somewhat unmistakable). You will not be able to tell sex based on abdomen color as young males and females look the same (light butts). The only way to tell is by looking for sex combs on legs of males as we'll discuss in lab.

Day 7. Clearing Adults

The day we added males to virgin females was day 1. By day 8, there were larvae on the side of vial. The larvae on the sides of the vials will hatch into your F1 generation. The adult flies were discarded.

Days. 14, 19. **Counting F1 offspring** – F1 offspring emerge on days 14-20, determine wing shape and sex for ~ 50 offspring per cross (save some to start your next cross – see next step, and discard the others....do not put them back into the vials). Tally numbers for each phenotypic class in table below.

Day 14-19. Establishing second cross (cross of F1 progeny that yields F2 generation)

All your F1 males from a given cross should have been identical; all your F1 females from a given cross should be identical. If not – speak with your lab instructor.

You do not need virgin females for these second crosses.

As your F1s emerge – collect (3-5) males and (3-5) females (siblings), and add them to a new vial with fresh media. The day you mix the males and females = day 1b.

Day 26 or 28. Clearing Adults

The day you added your males to your females was day 1b. Wait 7 days, until Day 7b. Look for larvae on side of vial – if there are none, come back next day and check again. If still no larvae – talk with your lab instructor. On Day 7b (7 days after you started your second crosses) – you need to clear vials of adult flies (these are the F1 offspring you made your 2nd cross with). The larvae that should be visible on the sides of the vials will hatch into your F2 generation (or progeny). You need to re-verify that these adults have the expected phenotypes

6. Counting F2 offspring – F2 offspring emerge on days 10b-19b, determine eye color and sex for ~ 100 offspring per cross discard them as you count/tally - do not put them back into the vials). Tally numbers for each phenotypic class in table below.

Due 10/25/07 Observations and Analyses Name: _____

Results: F1 phenotypic classes - Wing shape

_____ short female _____ long female _____ short male _____ long male

F2 phenotypic classes

| wing, eye, bristle | Males | Females | Total |
|---------------------------|--------------|----------------|--------------|
| Long, red, wild | | | |
| Long, red, forked | | | |
| Long, white, wild | | | |
| short, red, wild | | | |
| Long, white, forked | | | |
| short, Red, forked | | | |
| short, white, wild | | | |
| short, white, forked | | | |

From the table above, extract the following data
F2 phenotypic classes - Wing shape

_____ short female _____ long female _____ short male _____ long male

Analysis of Inheritance of Wing Shape:

1. What were the genotypes and phenotypes of the parental flies?

2. What was the expected genotypic and phenotypic distribution of the F1 flies?

3. What was the expected genotypic and phenotypic distribution of the F2 flies?

4. For your group and for the entire class, what were the observed phenotypic distributions of F1 and F2 flies for each of the crosses that you performed?

5. Using the results of the whole class (or a subset, as will be discussed in class) carry out a chi-squared analysis to determine if the results deviate significantly from those expected for a sex-linked trait. Attached is a chi-squared table of p values for help in evaluating your results.

Chi-squared Table of p values

Frequency of Chance Occurrence

| ° of Freedom | 70% | 50% | 30% | 20% | 10% | 5% (Signif) | 1% |
|---------------------|------------|------------|------------|------------|------------|--------------------|-----------|
| 1 | 0.148 | 0.455 | 1.074 | 1.642 | 2.706 | 3.841 | 6.635 |
| 2 | 0.713 | 1.386 | 2.408 | 3.219 | 4.605 | 5.991 | 9.210 |
| 3 | 1.424 | 2.366 | 3.665 | 4.642 | 6.251 | 7.815 | 11.341 |

Mapping Analysis: Combined Class data - F2 phenotypic classes

| Phenotype wing, eye, bristle | Total |
|---|--------------|
| Long, red, wild | |
| Long, red, forked | |
| Long, white, wild | |
| short, red, wild | |
| Long, white, forked | |
| short, Red, forked | |
| short, white, wild | |
| short, white, forked | |

1. The parental allele combinations are expected to be the most prevalent. What are the parental combinations? Were they the most prevalent?

2. The Double crossovers (DCO) are the least prevalent, and reveal which gene is in the middle of the other two (the middle gene is out of the parental combination). Which group are the DCO? Which gene is in the middle?

3. Using the class data, calculate the distance between the middle gene and the outer genes to construct a map. Show your work.

Discussion questions:

1. What do you think is the physiological basis of the white-eyed phenotype? Are the white-eyed flies blind? How could you experimentally test this question?

2. Were equal ratios of males and females produced? Assume that more females were produced than males -- devise a hypothesis to explain this phenomenon. Describe an experiment designed to test this hypothesis.

3. Briefly discuss the following statement: "Because the long-winged trait is dominant to the short-wing trait, we expect that more wild fruit flies will have long wings than will have short wings".