

## Lecture 7

Last time we discussed how to measure the distance between two genes on the X chromosome. To do this we used the trick of looking only at male progeny so the genotype of the X chromosome could be scored directly since these flies only carry one copy of the X. For autosomes, we can have the same ability to score all recombinant classes by crossing to a homozygous recessive individual. This is known as a test-cross.

Consider the recessive traits vestigial wings and short bristles that are specified by two different genes on the same autosome (non-sex chromosome).

$$\begin{array}{c} \text{♀} \quad \frac{\mathbf{vg}}{\mathbf{vg}} \quad \frac{\mathbf{sh}}{\mathbf{sh}} \quad \times \quad \text{♂} \quad \frac{+}{+} \quad \frac{+}{+} \\ \downarrow \\ \text{All F}_1: \quad \frac{\mathbf{vg}}{+} \quad \frac{\mathbf{sh}}{+} \end{array}$$

The F<sub>1</sub> flies are heterozygous for both genes so we are in position to see how often crossovers between these chromosomes occur in meiosis by doing a test-cross.

$$\begin{array}{c} \text{♀} \quad \frac{\mathbf{vg}}{+} \quad \frac{\mathbf{sh}}{+} \quad \times \quad \text{♂} \quad \frac{\mathbf{vg}}{\mathbf{vg}} \quad \frac{\mathbf{sh}}{\mathbf{sh}} \\ \downarrow \end{array}$$

(Note that the progeny have distinct phenotypes)

$$\frac{\mathbf{vg}}{\mathbf{vg}} \quad \frac{\mathbf{sh}}{\mathbf{sh}} \quad 458$$

$$\frac{\mathbf{vg}}{+} \quad \frac{\mathbf{sh}}{+} \quad 442$$

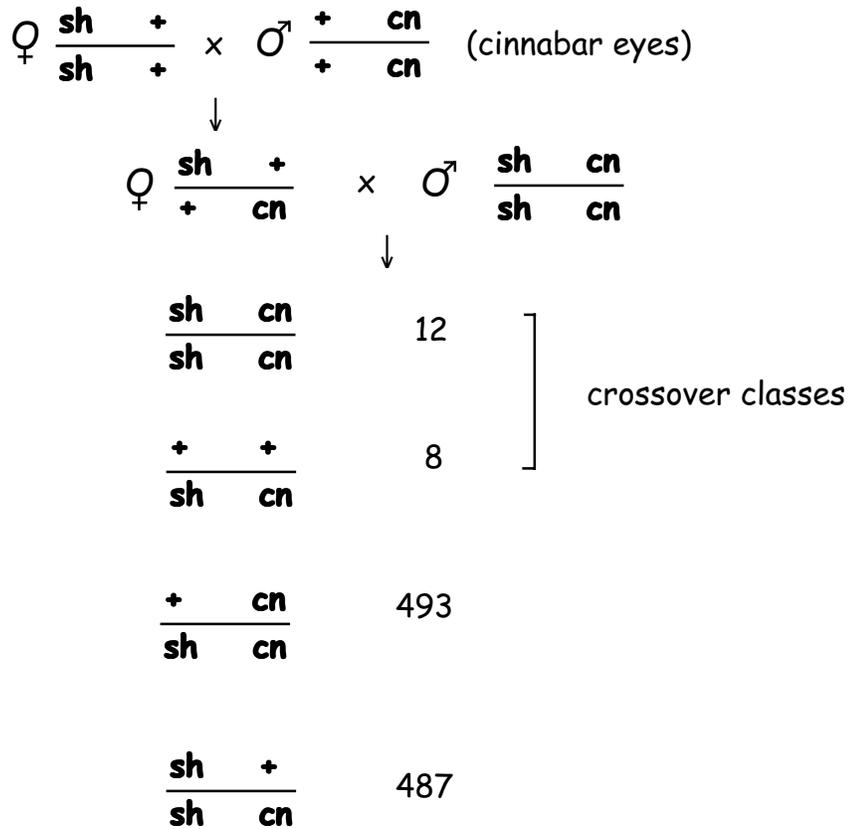
$$\frac{\mathbf{vg}}{\mathbf{vg}} \quad \frac{+}{\mathbf{sh}} \quad 47$$

$$\frac{+}{\mathbf{vg}} \quad \frac{\mathbf{sh}}{\mathbf{sh}} \quad 53$$

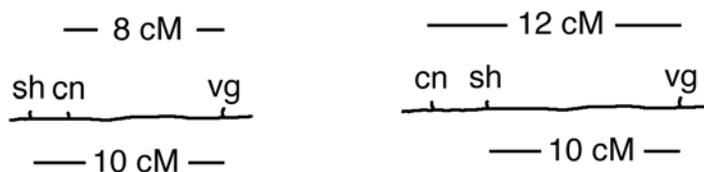
(crossover classes)

The distance between **vg** and **sh** =  $100 \times \frac{100}{1000} = 10 \text{ cM}$

Now we'll do a second cross. Note that the key is to set up a parent that is heterozygous at two loci.



Distance between **sh** and **cn** = 2 cM



There are two possible orders. We could resolve them by measuring the **cn** to **vg** distance, which should be either 8 cM or 12 cM depending on the order. However, it's difficult in practice to get a statistically significant measurement that would cleanly distinguish between these possibilities.

A better way to find the order is to set up all three heterozygous markers at the same time and to look at the frequencies of the eight different gamete genotypes.

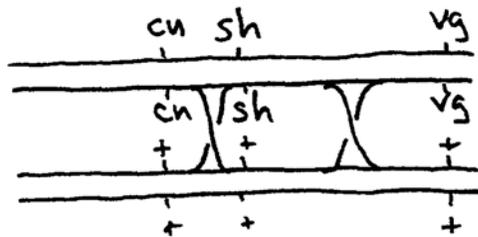
This is known as a **3 factor cross**

$$\text{♀} \begin{array}{ccc} \text{cn} & \text{sh} & \text{vg} \\ \hline + & + & + \end{array} \times \text{♂} \begin{array}{ccc} \text{cn} & \text{sh} & \text{vg} \\ \hline \text{cn} & \text{sh} & \text{vg} \end{array}$$

<b>cn</b>	<b>sh</b>	<b>vg</b>	900
+	+	+	912
<b>cn</b>	+	+	2
+	<b>sh</b>	<b>vg</b>	1
<b>cn</b>	<b>sh</b>	+	75
+	+	<b>vg</b>	70
<b>cn</b>	+	<b>vg</b>	18
+	<b>sh</b>	+	22

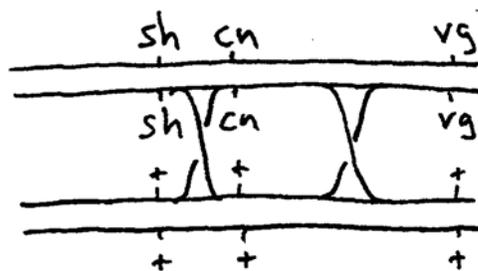
These are all of the possible combinations. One pair of these gamete classes must be the result of double crossovers. This class will be very rare ( $0.1 \times 0.02 = 2 \times 10^{-3}$ ). By finding the rare class we have a qualitative test to determine gene order.

The double crossover classes for the two possible orders are:



$$\begin{array}{ccc} \text{cn} & + & \text{vg} \\ \text{or} & & \\ + & \text{sh} & + \end{array}$$

(If this were the order, these would be the rare classes)



$$\begin{array}{ccc} \text{sh} & + & \text{vg} \\ \text{or} & & \\ + & \text{cn} & + \end{array}$$

(Since these are the rare classes we know this to be the order)

There is a simple system for evaluating 3-factor crosses:

- 1) Group recombinant classes into reciprocal pairs.
- 2) The most frequent pair is the parental classes.
- 3) Derive the gene order from the least frequent pair, which are the double crossover classes.
- 4) The single crossover frequency for the two intervals can be obtained by adding the frequency of each of the single crossover class pairs to the frequency of the double crossover class pair. (In the present example the double crossovers are so rare that their inclusion doesn't matter).

