Lecture 8

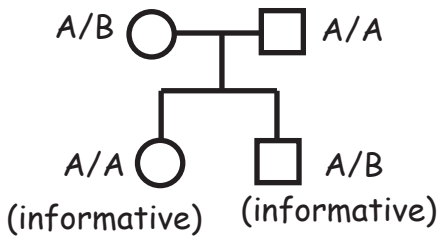
In this lecture we will see how the basic test for linkage between markers can be used to find the position of a mutation of interest on the genetic map of Drosophila. Conceptually, mapping a mutation simply involves doing a series of two-factor crosses to markers of known position to find the chromosome and then the region of that chromosome to which a marker is linked. There are not enough standard phenotypic markers in Drosophila to do this efficiently so we will make use of a new kind of genetic marker known as a DNA-based Marker. DNA markers: 1) are present in different allelic forms that are easy to discern, 2) have a position that can easily be placed on the genome sequence, and 3) are highly abundant allowing high resolution mapping.

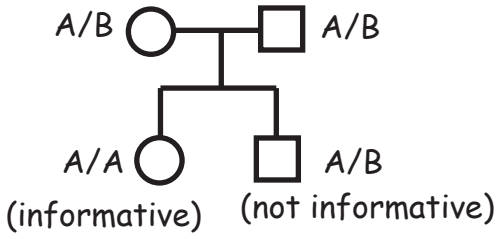
The most common DNA-based markers are SNPs (Single Nucleotide Polymorphisms). The different SNP alleles amount to single base pair differences at a given location in the DNA base sequence. The vast majority of SNPs are not in genes so the different alleles have no phenotypic consequences. SNP alleles can be discerned by direct sequencing of the DNA region of interest. Alternatively, if a SNP lies in a restriction enzyme cleavage site the different SNP alleles can lead to DNA that either can or cannot be cleaved by a particular enzyme and thus leads to obvious differences in the size of a particular restriction fragment of DNA.

A second type of DNA-based Marker is a SSR (Simple Sequence Repeat). SSR markers consist of a stretch of DNA composed of a series of repeated sequences usually of 2, 3, or 4 nucleotides. Different alleles of a given SSR have different numbers of repeats and can also be distinguished as DNA bands of different sizes.

First, let's see how the genotype of a DNA-based marker would be scored. Imaging a marker that has two different alleles called A and B. The A allele gives a particular DNA band of higher molecular weight than the B allele. Thus the A/A, B/B and A/B genotypes can each be identified:

For mapping experiments we will need to score the gamete genotypes that come from an individual that is heterozygous for a given DNA marker. The following pedigrees show that a cross of a heterozygous female to a homozygous male will always be informative whreas a cross to a heterozygous male is not always informative.





Now let's see how we would map a recessive autosomal trait such as wingless (wg^-) with respect to a DNA based marker with alleles A and B. We would first construct a female that is heterozygous for both wingless and the DNA marker by crossing two true breeding strains.

$$\text{♀}\ \frac{wg^-\ A}{wg^-\ A} \times \text{♂}\ \frac{wg^+\ B}{wg^+\ B}$$

$$\downarrow$$

$$\text{♀}\ \frac{wg^-\ A}{wg^+\ B}$$

Then we would do a test-cross to an appropriate male that is homozygous for both markers (eg a male from the true breeding wingless strain).

$$\text{♀}\ \frac{wg^-\ A}{wg^+\ B} \times \text{♂}\ \frac{wg^-\ A}{wg^-\ A}$$

The gamete genotypes from the heterozygous female can be scored from the progeny by the presense or absence of the wingless trait and marker genotype A/A or A/B.

Gamete Genotype	Number
wg^- A	45
wg^+ B	40
wg^+ A	7
wg^- B	8

$$\text{Distance} = 100 \cdot \frac{15 \pm 4}{100} = 15 \pm 4\ \text{cM}$$

Consider a case in which the wingless mutation is thought to map in the vicinity of two DNA markers (M1 and M2) that are about 30 cM apart.

M1 — 30 cM — M2

Instead of crossing wg^- to each marker individually, it is more efficient to consider segregation of all three markers at once. This is known as a **3-factor cross**. The strategy to set up a 3-factor cross is the same as for a 2-factor cross.

♀ $\frac{wg^-\ \text{M1-A}\ \text{M2-A}}{wg^-\ \text{M1-A}\ \text{M2-A}}$ X ♂ $\frac{wg^+\ \text{M1-B}\ \text{M2-B}}{}$

↓

♀ $\frac{wg^-\ \text{M1-A}\ \text{M2-A}}{wg^+\ \text{M1-B}\ \text{M2-B}}$ X ♂ $\frac{wg^-\ \text{M1-A}\ \text{M2-A}}{wg^-\ \text{M1-A}\ \text{M2-A}}$

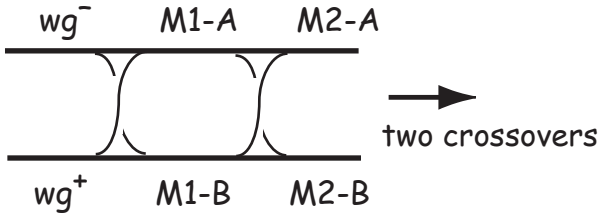
Note that for three markers there are 8 possible gamete genotypes.

	Gamete Genotype	Number	
1	wg^- M1-A M2-A	60	**128**
	wg^+ M1-B M2-B	68	
2	wg^- M1-A M2-B	20	**42**
	wg^+ M1-B M2-A	22	
3	wg^- M1-B M2-A	12	**22**
	wg^+ M1-A M2-B	10	
4	wg^- M1-B M2-B	3	**8**
	wg^+ M1-A M2-A	5	

The first step in analyzing a 3-factor cross is to group the 8 genotypes into 4 reciprocal pairs which should occur at equal frequency. We can also add together the total for each pair. Next, note the genotype of the rarest reciprocal pair. In this case the rare genotypes are: wg^- M1-B M2-B and wg^+ M1-A M2-A. We can now determine the order of wg with respect to M1 and M2.

The key to determining the order of markers from a 3-factor cross is to realize that the rare class of gametes (in this example it is reciprocal class #4) will be the product of a double crossover whereas the more abundant classes can be produced by a single crossover. Consider the three possible orders of M1 M2 and wg.

Order 1:



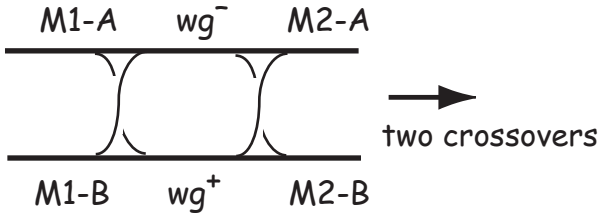
Gamete genotypes produced

wg^- M1-B M2-A

wg^+ M1-A M2-B

#2

Order 2:

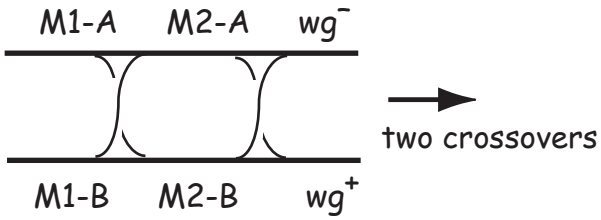


wg^- M1-B M2-B

wg^+ M1-A M2-A

#4

Order 3:



wg^- M1-A M2-B

wg^+ M1-B M2-A

#3

Through this analysis we can see that Order 2 will produce the correct rare class #4 as the product of double crossovers. The map can be completed by calculating the two relevant 2-factor distances.

$$\text{M1 — wg distance} = 100 \cdot \frac{30 \pm 5.5}{200} = 15 \pm 2.7 \text{ cM}$$

$$\text{M2 — wg distance} = 100 \cdot \frac{50 \pm 7.1}{200} = 25 \pm 3.5 \text{ cM}$$

The final map would be represented as follows: