Lecture 7

What we are going to do is to use crossing over during meiosis to map genes relative to one another. To begin, we need two genes on the same chromosome. Consider two mutations on the X chromosome of Drosophila; minature wing and white eye.

Genotype	Phenotype
$X^{w+m+}\ y$	wild-type
$X^{w+m-}\ y$	minature wings
$X^{w-m+}\ y$	white eyes

$X^{w-m+}\ y$ x $X^{w+m-}\ X^{w+m-}$ (true breeding)

All of the daughters from this cross will have two different X-chromosomes, which differ at two loci: $X^{w+m-}\ X^{w-m+}$

We want to follow these X chromosomes into the next generation so after a cross we look only at the male flies.

parental classes: $X^{w+m-}\ y$ and $X^{w-m+}\ y$

crossover classes: $X^{w-m-}\ y$ and $X^{w+m+}\ y$
(minature, white) (wild type)

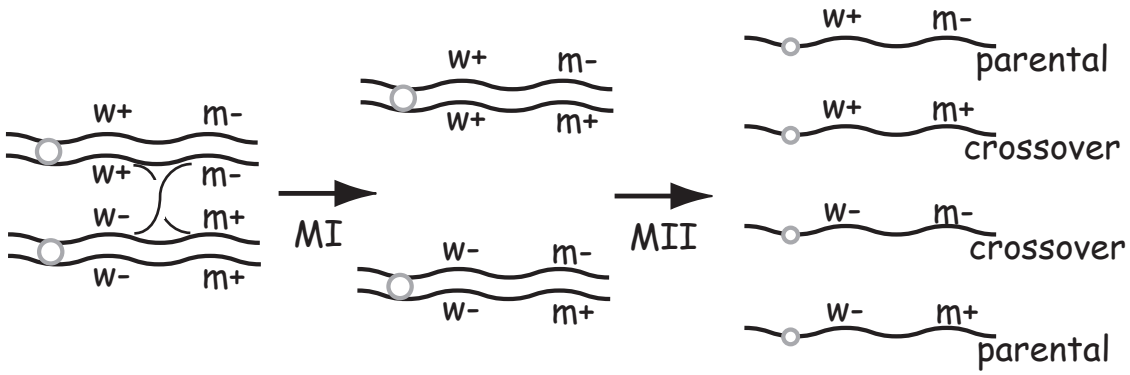
In the crossover classes the alleles appear to have separated and moved from one X to the other. Genes on the same chromosome often do not assort independently. Such behavior is known as **Linkage**. When we talk about gene position the term **locus** is often used to designate the chromosomal location of a gene.

unlinked — crossover classes are at approximately the same frequency as parental classes. (This will be the case for genes on different chromosomes and that are far apart on the same chromosome)

weakly linked — crossover classes appear statistically significantly more often than parental classes.

tightly linked — crossover classes appear rarely or never. (Tightly linked alleles may be in the same gene)

The diagram below shows the X chromosome in the female in which a crossover occurs between two chromatids in an interval between the w and m genes. This will lead to gametes of either the parental or crossover types.



Crossovers between homologous chromosomes occur more or less at random during meiosis. To give you a rough idea of how frequent these crossovers are, in several different well studied organisms (Yeast, Drosophila, and humans) there is about one crossover per chromosome arm per meiosis. The geneticist uses these random crossovers as a tool to measure distance. Distance can be obtained because crossovers between two points that are close together will rarely occur whereas crossovers between points that are far apart will occur frequently.

Definition of genetic distance:

$$\text{map distance (m.u. or cM)} = 100 \times \frac{\text{crossover gametes}}{\text{total gametes}}$$

♀ $\frac{w+\ m-}{w-\ m+}$ x ♂ $\frac{w+\ m+}{y}$ (Note new notation to indicate linked genes)

In order to detect both dominant and recessive alleles coming from the gametes in the mother we can use the trick of looking at male offspring (who receive only one copy of the X chromosome from the mother only)

phenotype	males	females
$w^+\ m^-$	23	0
$w^-\ m^+$	33	0
$w^+\ m^+$	6	56
$w^-\ m^-$	7	0

Number of crossover gametes = 13 Total gametes = 69 (total males)

$$\text{Distance} = 100 \times \frac{13}{69} = 19 \text{ cM}$$

For measurements of map distance it is important to keep track of the accuracy of the measurement. A useful way to estimate the statistical error in herent in counts of the number of recombinants is to use the Poisson Distribution, which provides an estimate of the number of occurrences of an event that happens rarely but has many opportunities to happen.

$$p(x=k) = \frac{n^k e^{-n}}{k!}$$

For the Poisson distribution the mean = n and standard deviation = $\sqrt{n}$; we usually represent the error associated with a count of the number of recombinants as: $n \pm \sqrt{n}$.

Thus in our example the count of recombinants would be 13 ± 3.6 and the distance would be 19 ± 5.2 cM

We will now use recombinational mapping to determine whether two mutations are in the same or in different genes. Consider an easily scored trait aristaless. Imagine that you have isolated two different autosomal dominant aristaless mutations (designated $A1^D$ and $A2^D$). Since the mutations are dominant, we can't use a complementation test to distinguish them. However, we can measure the distance between the mutations by a cross and use this information to ascertain whether the mutations are likely to be in the same gene. We can use the following cross between true breeding strains to produce a double heterozygous female:

♀ $\frac{A1^D\ A2^+}{A1^D\ A2^+}$ × ♂ $\frac{A1^+\ A2^D}{A1^+\ A2^D}$

↓

All F_1: $\frac{A1^D\ A2^+}{A1^+\ A2^D}$

We are now in position to see how often crossovers between these chromosomes occur in meiosis by doing a test-cross to a double homozygous recessive (wild type) male.

♀ $\frac{A1^D\ A2^+}{A1^+\ A2^D}$ × ♂ $\frac{A1^+\ A2^+}{A1^+\ A2^+}$

↓

	genotype	phenotype	number
parental classes	$\frac{A1^D\ A2^+}{A1^+\ A2^+}$	aristaless	96
	$\frac{A1^+\ A2^D}{A1^+\ A2^+}$	aristaless	
crossover classes	$\frac{A1^D\ A2^D}{A1^+\ A2^+}$	aristaless	4
	$\frac{A1^+\ A2^+}{A1^+\ A2^+}$	normal aristae	

The total number of crossover gametes is estimated to be $(4 \pm 2) \times 2 = 8 \pm 4$

Thus the measured distance between A1 and A2 = $100 \times \frac{8 \pm 4}{100} = 8 \pm 4$ cM

Now the question is whether two mutations in the same gene can be 3 cM apart. To answer this question in Drosophila or other organisms we need to know the sizes of the largest genes in terms of cM. It is important to remember that genetic distances are measured using a property of meiosis (genetic recombination) that varies from one organism to another. The relationship between genetic distance and actual physical distance can be summarized in this way:

Genetic distance = physical distance x recombination rate

The actual relationship between genetic distance in cM and physical distance in base pairs (bp) depends on the recombination rate and is different for different organisms.

For example: Drosophila; 3.3 cM/Mbp Human; 1.3 cM/Mbp Yeast; 360 cM/Mbp

[Sometimes recombination rates in the male and female of a species are different. In Drosophila there is no recombination in the male so the genetic distance between markers on the same chromosome are always zero when examined by meiosis in the male. In humans the recombination rate (and therefore map distances) in the female are twice that of the male.]

The largest genes in these organisms are approximately:
Drosophila; 0.1 Mbp Human; 2.3 Mbp Yeast; 0.005 Mbp

Thus the maximum distances in cM between mutations in the same genes are:
Drosophila; 0.3 cM Human; 3 cM Yeast; 1.8 cM

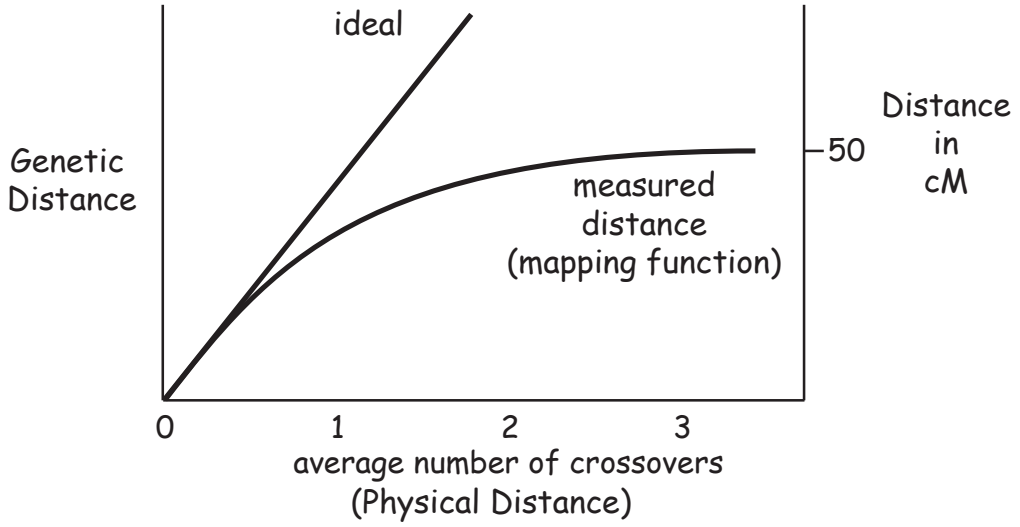
So the conclusion is that the two aristaless mutations are in different genes.

The physical length of a genetic interval is proportional to the frequency of crossovers that occur in that interval during meiosis, but in a cross we actually count the number of recombinant progeny that are produced – not the number of crossovers.
The number of recombinants provide a good approximation of distance for short intervals but as the interval length increases, multiple crossovers are possible making the relationship between frequency of recombinants and crossovers not linear.

Crossovers between the same pair ofchromatids in the same genetic interval	Recombinant progeny
1	yes
2	no
3	yes
4	no

This produces a relationship between physical distance and measured distance that is known as a **Mapping Function.**

Here the mapping function for two markers based on recombination compared to an ideal linerar function of physical distance.



If the number of recombinants is not statistically different from 50% then we say that the genes are unlinked. However, distances greater than 50 cM can be obtained by adding shorter intervals. For example, if all the intervals between linked genes in the human genome are added together the total length of the genome (in males) is 2,500 cM.

Once a map distance between two genetic markers has been established this distance can be used to calculate the expected numbers of each type of progeny. For example, if you know that two mutations are 20 cM apart then you should expect that 10% of the progeny from a cross will be of each recombinant class.