

5 GENETIC LINKAGE AND MAPPING

5.1 Genetic Linkage

So far, we have considered traits that are affected by one or two genes, and if there are two genes, we have assumed that they assort independently. However, It should be obvious that there are many more genes than there are chromosomes in all organisms. In this segment of the course, you will learn how to determine if genes are linked on the same chromosome, and how to determine how far apart the genes are. This is the basis of **genetic mapping**. First, let's revisit the independent assortment of genes that are located on different chromosomes.

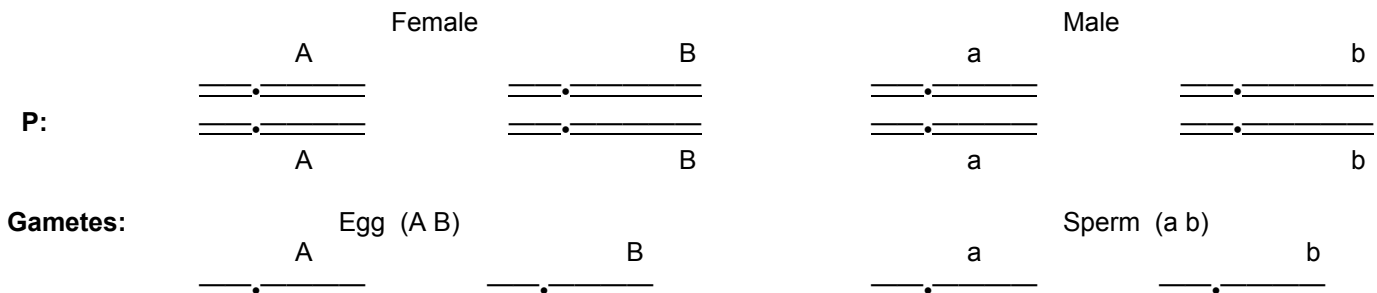
Mendel's Law of Independent Assortment (during gamete formation, segregation of one gene pair is independent of other gene pairs) derived because the traits he studied were determined by genes on different chromosomes.

First, consider two genes, each with two alleles A a and B b on separate chromosomes

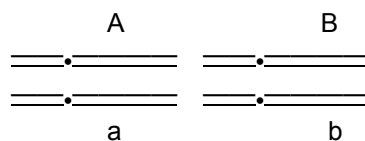
NEW NOTATION: $\begin{array}{c} A \\ \text{---}\cdot\text{---} \end{array}$ represents the two chromatids of a metaphase chromosome, bearing allele A of the

a locus. $\text{---}\cdot\text{---}$ represents the same chromosome in the single-chromatid stage.

So the cross AA BB * aa bb could be represented as:



F1 (after the S phase):



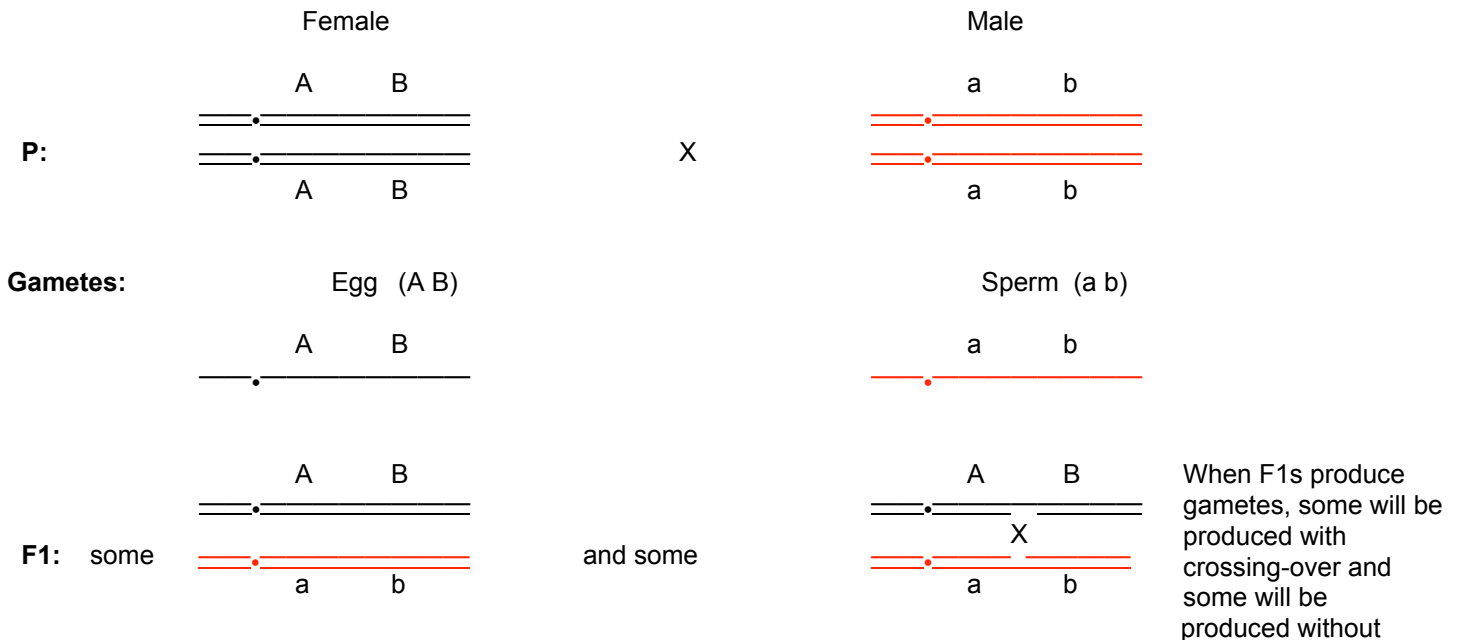
Gametes (non-homologous chromosomes assort independently at anaphase):

			<u>Gamete Genotype</u>	<u>Expected Proportion</u>
$\text{---}\cdot\text{---}$	$\text{---}\cdot\text{---}$	Parental	A B	1/4
$\text{---}\cdot\text{---}$	$\text{---}\cdot\text{---}$	Parental	a b	1/4
$\text{---}\cdot\text{---}$	$\text{---}\cdot\text{---}$	Recombinant	A b	1/4
$\text{---}\cdot\text{---}$	$\text{---}\cdot\text{---}$	Recombinant	a B	1/4

So, when genes are on different chromosomes, **50%** of the gametes produced by a doubly-heterozygous individual are **recombinant**, when compared to the gametes produced by its parents. The other 50% are **parental**.

If two genes occur on the same chromosome, they may not assort independently at anaphase of meiosis. These genes are said to be **linked** and demonstrate **linkage** in genetic crosses. Linkage is present when *fewer than 50% of the gametes produced by a double heterozygote are recombinant*.

Now consider a case where the two genes are on the same chromosome: (A and B are **linked**)



Gametes resulting from no crossover:

	Expected Prob.
Parental	1/4
Parental	1/4
Parental	1/4
Parental	1/4

Gametes resulting from crossover:

	Expected Prob.
Parental	1/4
Parental	1/4
Recombinant	1/4
Recombinant	1/4

If there were no **crossing over**, all the alleles on a single chromosome would segregate together and would end up in the same gamete (depicted on the left, above). But, with crossing over, we get **recombination** of alleles on the same chromosomes (depicted on the right, above). Since crossover occurs in the 4-strand stage of meiosis, and involves only two of the four chromatids, each crossover event results in **50% recombinant gametes, and 50% parental gametes**.

So,

Genes on different chromosomes → 50% recombinant gametes after meiosis

Genes on the same chromosome ≤ 50% recombinant gametes after meiosis

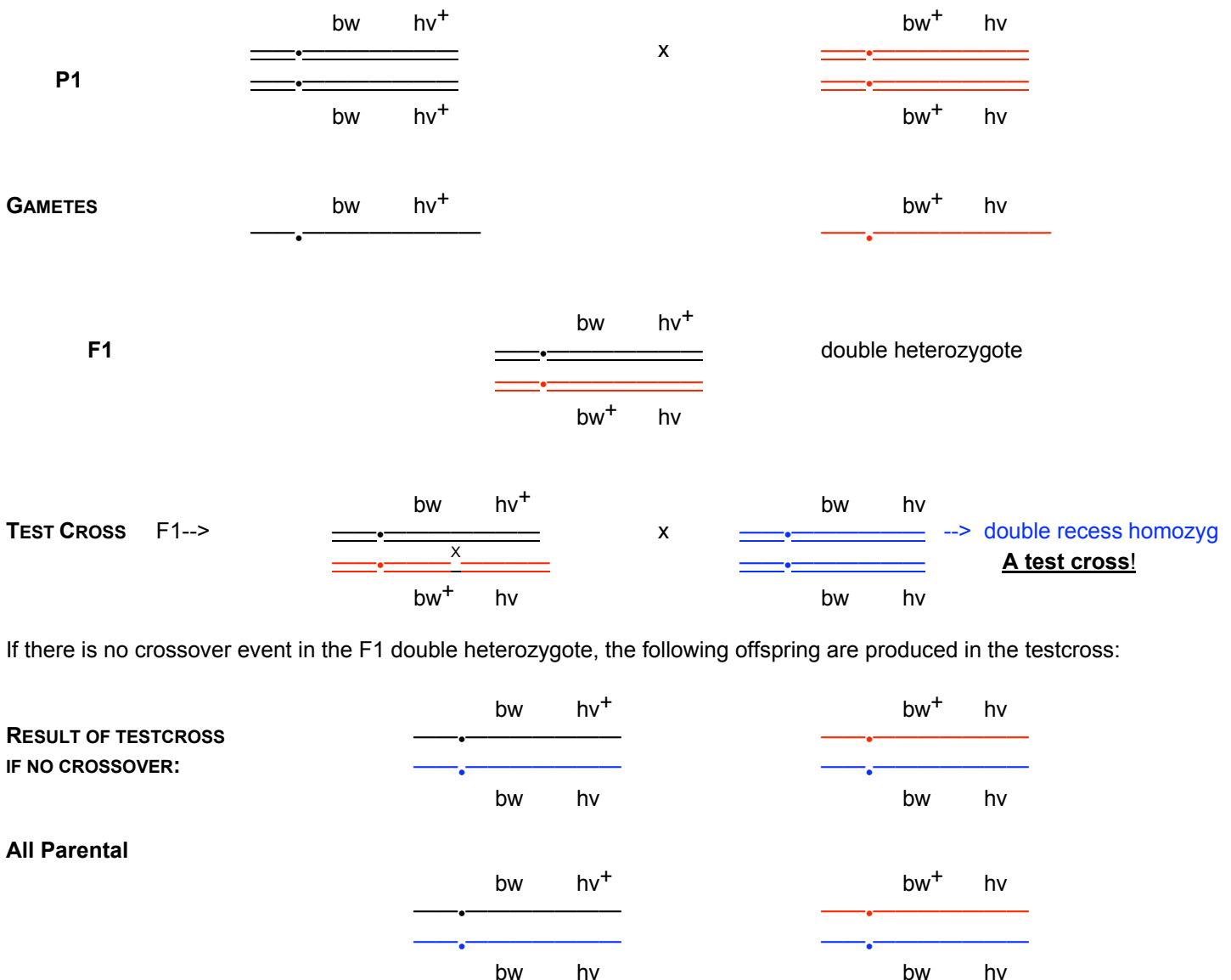
5.2 Genetic Mapping

Genes with recombination frequencies less than 50% are present in the same chromosome (linked). Two genes that undergo independent assortment, indicated by a recombination frequency of 50 percent, are either on non-homologous chromosomes or are located far apart in a single chromosome.

However crossing over does not occur between linked genes in every meiotic event, especially when the positions of the genes on the chromosome are very near one another. The frequency with which crossing over occurs between any two linked genes is proportional to the distance between the loci along the chromosome.

1. At very small distances, crossover is very rare, and most gametes are parental.
2. As the distance between two genes increases, crossover frequency increases. More recombinant gametes, fewer parental gametes.
3. When genetic loci are very far apart on the same chromosome, crossing over nearly always occurs, and the frequency of recombinant gametes approaches 50 percent.

How do we determine how much crossing-over occurs between linked genes (and therefore get an idea of how far apart two loci are)? The answer is critical, because this is the first step in constructing a **genetic map**. The following shows the use of a testcross to determine how much crossing-over occurs between the genes for *brown eyes* (*bw*) and *heavy-veined* (*hv*) in the fruit fly *Drosophila melanogaster*.



If there is a crossover event in the double heterozygote, half the offspring produced by the gametes resulting from crossing over will be like those above,



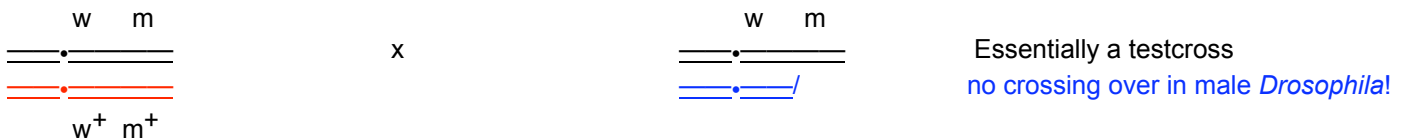
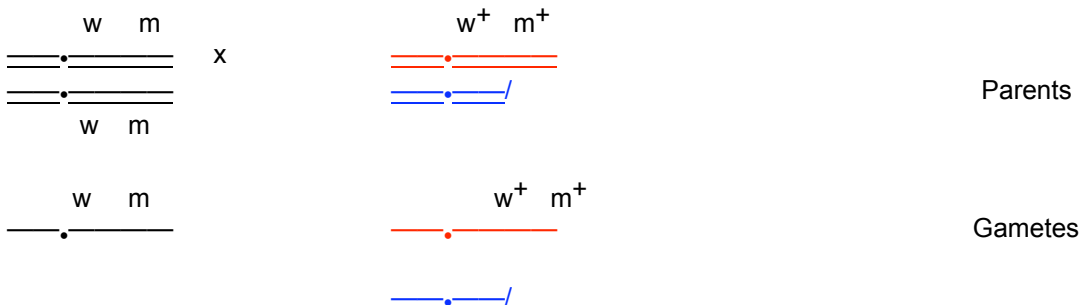
and the other half will be:

**RESULT OF TESTCROSS
WITH CROSSING OVER
IN 4-STRAND STAGE**



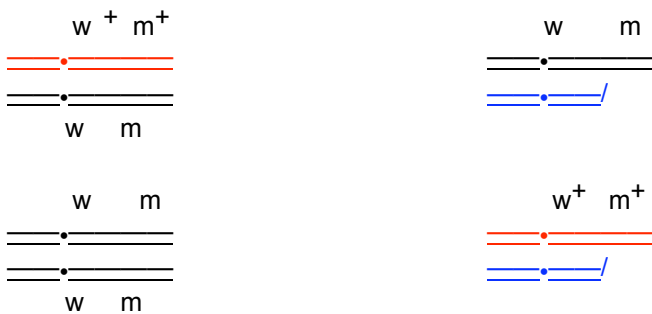
So half the offspring are **recombinant** with respect to the parental combinations of alleles at the two loci!

Another example, this time using a **hemizygous** male to detect recombination on the X chromosome:
white eyes, miniature wings (w w ; m m) on *D. melanogaster* X chromosome.



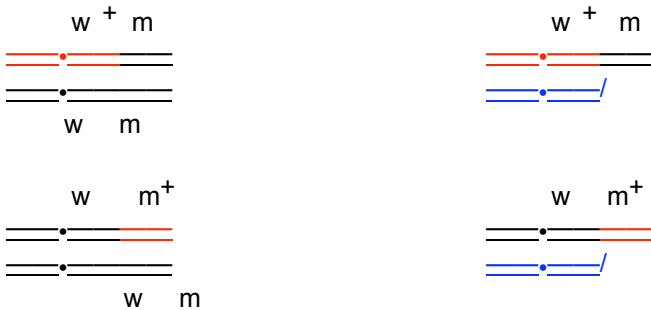
Two kinds of offspring

No crossing over → all offspring are parental.



With crossing over, 1/2 of offspring will be parental

1/2 will be recombinant:



Lets' say we do this cross and find **62.8% non-recombinant offspring**, and **37.2% recombinant offspring**.

37.2 % is the frequency of recombination.

5.3 Map units

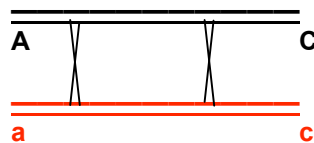
When large numbers of mutations are available for a species, genes on the same chromosome will show evidence of linkage to one another (<50% recombination frequency). Genes will fall into **LINKAGE GROUPS**. The number of linkage groups will equal the haploid number of chromosomes. The linkage of genes on a chromosomes can be represented on a genetic map (linkage map/chromosome map). A genetic map shows the linear order of the genes along a chrom. with distance **proportional to the frequency of recombination**. Unit of distance in linkage map is a map unit--**1 map unit is equal to 1 percent recombination**. A map unit is also equivalent to the physical distance along a chromosome which will experience 1 crossover event in every 50 meiotic divisions (1 crossover in 50 meiotic divisions = 2 recombinant gametes in every 200 = 1 % recombination). So two genes that recombine with a frequency of 1% are said to be 1 map unit apart.

See *Drosophila* linkage map (Text Figure 5.7).

5.4 Multiple Crossover Events

Unless genes are very close together, more than one crossover event can occur in a single meiotic division. **Multiple crossovers can make mapping inaccurate if genes are far apart on the same chromosome.**

**Heterozygote
(cis configuration)**



On the other hand, double crossovers can be very useful if three or more mutations are available on the same chromosome. If there are 3 mutations available on the same chromosome, their ordering wrt one another, and their map distances can be determined by following the procedure.

5.5 Three-Locus Mapping

If a multiply heterozygous individual is crossed to an individual homozygous at all loci, the relative positions of 3 loci can be mapped. (Cross to homozygote so gametic genotypes can be recognized):

Maize: l l—lazy or prostrate growth
 g g—glossy leaves
 s s--sugary endosperm

Order Unknown, so L G S IS ARBITRARY ORDER

Triple Heterozygote: L G S / l g s X Recessive homozygote: l g s / l g s

Progeny Phenotype	Genotypes of offspring	Number
wildtype	L G S / l g s	286
lazy	l G S / l g s	33
glossy	L g S / l g s	59
sugary	L G s / l g s	4
lazy,glossy	l g S / l g s	2
lazy,sugary	l G s / l g s	44
glossy,sugary	L g s / l g s	40
lazy,glossy,sugary	l g s / l g s	272
Total		740

Rule 1: The two most-frequent types of gametes are **nonrecombinant**. These provide the linkage phase (cis vs. trans) of the alleles in the heterozygous parent. This is because recombinant gametes can **never** account for more than 50% of the gametes, even if genes are unlinked.

Therefore: L G S and l g s are the **nonrecombinant** (parental) classes

Rule 2: The **double-recombinant** gametes will be the least frequent type.

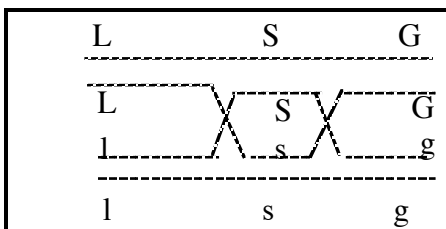
Therefore: L G s and l g S are the **double crossover** classes

Rule 3: The effect of double crossovers is to **interchange the members of the middle pair of alleles** between the chromosomes. (look at figure above)

Therefore: S occurs between L and G, and the arrangement of the genes on the chromosome is:

L S G

Why? Because this is what double-crossovers do:



The gene in the middle gets shuffled

Single crossover between L and S loci====> L s g and l S G

Single crossover between S and G loci====> L S g and l s G

Can now label the genotypes with the processes that produced them

In parents, L goes with S and l goes with s. So any time these combinations get broken up --> recombination between L and S loci.

In parents, S goes with G and s goes with g. So any time these combinations get broken up --> recombination between S and G loci.

Progeny Phenotype	Genotypes of offspring	Number	Crossover or Non-X over?
wildtype	L G S / l g s	286	Parental
lazy	l G S / l g s	33	single Xover between L and S
glossy	L g S / l g s	59	single Xover between S and G
sugary	L G s / l g s	4	double Xover
lazy,glossy	l g S / l g s	2	double Xover
lazy,sugary	l G s / l g s	44	single Xover between S and G
glossy,sugary	L g s / l g s	40	single Xover between L and S
lazy,glossy,sugary	l g s / l g s	272	Parental
Total		740	

Rule 4: Reciprocal products expected to occur in approximately equal numbers, so $LGS \approx lgs$, ($286 \approx 272$) $LgS \approx lGs$ ($59 \approx 44$), $Lgs \approx lGS$ ($40 \approx 33$), and $LGs \approx lgs$ ($4 \approx 2$).

Q: Calculate the frequency of recombination between L and S, given that the parental classes are L G S and l g s:

l G S	33
L g s	40
L G s	4
l g S	<u>2</u>
	79

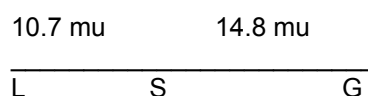
79/740 or 10.7% of gametes recombinant between L & S. So, map distance between L & S = **10.7 map units**.

Q: Calculate the frequency of recombination between S and G, given that the parental classes are L G S and l g s:

L g S	59
l G s	44
L G s	4
l g S	<u>2</u>
	109

109/740 or 14.8 % of gametes recombinant between S & G. So, map distance between S & G = **14.8 map units**

So, the data are give the following map:



Rule 5: Don't forget to include the double recombinants when calculating recombination frequency!

5.6 V. Interference

Using information from 3-point mapping, we can determine if crossing over in two different regions of the same chromosome occur **independently**. If they are independent, crossing over in one region has no effect on the probability of crossing over in another region of the same chromosome. **Assuming independence, the percentage of expected double crossovers is calculated by multiplying the probability of exchange in one region by probability of exchange in other (Product Rule).**

MAIZE EXAMPLE:

Prob of recombination between L and S is 10.7% = 0.107, and that between S and G is 14.8% = 0.148.

Using the **product rule** (see Lecture 2): The probability of a detectable double crossover should then be

$$0.107 * 0.148 = 0.0158$$

So, in 740 events, the double crossover classes of gametes should occur

$$0.0158 * 740 = 12 \text{ times}$$

So the expected expected double crossovers between L and G should be about 12, but we observed only 6. Such deficiencies are common, and indicate that **crossing over in one region of a chromosome reduces the probability of crossing over in closely adjacent regions**. This phenomenon is called **INTERFERENCE**.

The **coefficient of coincidence** is the observed number of double recombinants **divided by** the expected number. It provides measure of the degree of interference.

$$\begin{aligned} \text{Coefficient of coincidence} &= 6/12. & \text{Interference} &= 1 - (\text{Coeff. of coincidence}) = 1 - 0.5 = 0.5 \text{ in this case.} \\ & & &= 1 - (6/12) = 1/2 \end{aligned}$$

Observed number of double crossovers is just 1/2 of that expected. At distances less than about 10 map units (in *Drosophila*) no double crossovers are observed (interference is complete, $I=1$). Conversely, at distances greater than about 45 map units, Interference disappears, and the observed number of double crossovers is usually nearly equal to the expected (interference is absent, $I=0$).