EXERCISE 7 - LINKAGE, CROSSING-OVER, & GENE MAPPING IN *DROSOPHILA*

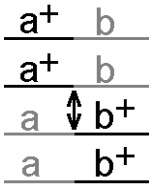
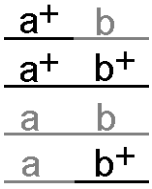
LINKAGE AND CROSSING-OVER

According to Mendel's principle of independent assortment, a dihybrid cross with unlinked markers ought to produce a 1:1:1:1 ratio. If a significant deviation from this ratio occurs, it may be evidence that for **linkage**, that is, that the loci are located close to each other on the same chromosome pair.

During meiosis, a pair of synapsed chromosomes is made up of four chromatids, called a **tetrad**. The phenomenon of a **cross - over** occurs when homologous chromatids in the tetrad (one from each of the two parents) exchange segments of varying length during prophase. The point of crossover is known as a **chiasma** (pl. **chiasmata**). A tetrad typically has at least one chiasma along its length. Generally, the longer the chromosome, the greater the number of chiasmata. There are two theories on the physical nature of the process. The **classical theory** proposes that cross-over and formation of the chiasma occur first, followed by breakage and reunion with the reciprocal homologues. According to this theory, chiasma formation need not be accompanied by chromosome breakage. Alternatively, according to the **chiasmatype theory**, breakage occurs first, and the broken strands then reunite. Chiasmata are thus evidence, but not the causes, of a cross-overs. Recent molecular evidence favours the latter theory, although neither is a completely satisfactory explanation of all of the evidence.

Gametes produced by a dihybrid heterozygous individual with linked loci

Condition	Meiotic tetrad	Gametes	Combination
No crossover	$a^+ \ b^+$ $a^+ \ b^+$ a b a b	$a^+ \ b^+$ $a^+ \ b^+$ a b a b	**Parental** **P** **P** **P**
Single crossover: markers in *cis* **a^+b^+//ab**	$a^+ \ b^+$ $a^+ \ b^+$ a ↕ b a b	$a^+ \ b^+$ a^+ b a b^+ a b	**P** **Recombinant** **Recombinant** **P**

Single crossover: markers in *trans* **$a^+b//ab^+$**			**P** **Recombinant** **Recombinant** **P**

In dihybrid crosses, an arrangement in which the wild-type alleles of both loci are contributed by one parent is referred to as a ***cis* configuration**; the alternative arrangement is called a ***trans* configuration**. A gamete that shows the same configuration as the parent is referred to as a **parental** type; where the configuration is altered, the gamete is referred to as a **recombinant** type. *Cis* and *trans* configurations are altered by recombination.

Linkage between loci is indicated when the recombinant phenotypes occur less frequently than the parental types. The frequency of crossing over (% recombination) between two loci is directly related to the physical distance between those two loci. Percent recombination in a test cross equals **map distance** (1 map unit = 1 % recombination).

eg. P_1 **$a^+b//a^+b$** x **$ab^+//ab^+$**

F_1 **$a^+b//ab^+$** x **ab//ab** (test cross)

$F_{2:}$ **$a^+b//ab$** , **$ab^+//ab$** 90% - parental combinations

$a^+b^+//ab$, **ab//ab** 10% - recombinant

10% recombinant indicates that loci **a** and **b** are 10 map units apart.

Gene Map **a** ——— **b**
10 m. u.

Conversely, if it is known that loci **b** and **c** are 16 map units apart, then the expected proportions of parental and recombinant phenotypes in a test cross can be predicted:

eg. P_1 **$b^+c//b^+c$** x **$bc^+//bc^+$**

F_1 **$b^+c//bc^+$** ♀ x **bc//bc** ♂ (test cross)

In a test cross

♀ / ♂	8 % **b^+c^+**	8% **bc**	42% **b^+c**	42% **bc^+**
100 % **bc**				

In a F_1 x F_1 cross

♀ / ♂	8% **b^+c^+**	8% **bc**	42% **b^+c**	42% **bc^+**
50% **b^+c**				
50% **bc^+**				

Multiple Crossovers

Analysis of the genetic behaviour of three or more linked loci may show evidence of multiple cross-overs. When three loci are involved, there will be two parental types, four recombinant classes with single cross-overs and two recombinant types showing cross-overs between all three loci (= double cross-over). The two parental types will be most abundant, the four single cross-over (SCO) recombinants will be next while the two double cross-over (DCO) recombinants will be least abundant.

Each cross-over situation results in two parental gametes and two recombinant gametes. The maximum recombination between any two loci is 50% (since ½ of the gametes are parental type).

Gametes produced by trihybrid heterozygous individual. **a^+ b^+ c^+ / a b c**

	Meiotic tetrad	Gametes	Combination
Single Crossover (**a-b**)	$a^+\ b^+\ c^+$ $a^+\ b^+\ c^+$ a ↕ b c a b c	$a^+\ b^+\ c^+$ a^+ b c a $b^+\ c^+$ a b c	**P** **R** **R** **P**
Single Crossover (**b-c**)	$a^+\ b^+\ c^+$ $a^+\ b^+\ c^+$ a b ↕ c a b c	$a^+\ b^+\ c^+$ $a^+\ b^+$ c a b c^+ a b c	**P** **R** **R** **P**
Double Crossover	$a^+\ b^+\ c^+$ $a^+\ b^+\ c^+$ a ↕ b ↕ c a b c	$a^+\ b^+\ c^+$ a^+ b c^+ a b^+ c a b c	**P** **R** **R** **P**

MAKING A GENE MAP

The recombination frequency is constant for any pair of linked loci and represents the "genetic" distance between them. Each 1 m.u. is the distance that will generate 1% recombination.

It is possible to develop a gene map, showing the order of the loci and the distance between them by observing the number of offspring showing recombinant phenotypes.

Example 1: A standard problem in genetics is to determine the order of three loci known to be linked on one pair of the autosomes. Solution of the problem requires (1) a determination of the relative order of loci, and (2) the map distances between loci.

A cross is made between homozygous wild-type female *Drosophila* (**$a^+a^+b^+b^+$ c^+c^+**) and triple-mutant males (**aa bb cc**) (the order here is arbitrary). The F_1 (**a^+a b^+b c^+c**) females are test crossed back to the triple-mutant males and the F_2 phenotypic ratios are as follows:

"a^+ b c"	18
"a b^+ c"	112
"a b c"	308
"a^+ b^+ c"	66
"a b c^+"	59
"a^+ b^+ c^+"	321
"a^+ b c^+"	102
"a b^+ c^+"	15
	1000

1. The gene order can be determined by examination of the relative frequencies of the F_2 phenotypes.

   a. Because linked loci tend to stay together, the non-crossover (NCO) or parental phenotypes should be most frequent (and equal in number). In this case
   $a^+b^+c^+$ (321) and **a b c** (308)

   b. Because simultaneous crossovers between the outside and middle loci are unlikely, the double-crossover (DCO) genotypes should be the least frequent. We observe
   a^+ b c (18) and **a b^+c^+** (15)

   c. Then, to determined the physical order of loci, compare the parental and double-crossover phenotypes. ***The marker that appears to "switch places" is in the middle*** [technically, this marker is said to be "out of phase"]. Here, the **$a^+b^+c^+$** NCO and **a b^+c^+** DCO phenotypes indicate that the **a** locus falls between the **b** and

c loci. The correct order of the loci is **b a c** . [Note that this order is equivalent to **c a b** , and that the order of the outside markers is arbitrary].

d. The **coupling phase** of the trihybrid F_1 is $\mathbf{b^+a^+c^+ / b\ a\ c}$.

2. The remaining two pairs of phenotypes correspond to single-crossovers (SCO) events in the region between either **b** and **a**, or between **a** and **c**.
   a. $\mathbf{b^+a\ c}$ (112) and $\mathbf{b\ a^+c^+}$ (102) phenotypes indicate crossovers between **b** & **a.**
   b. $\mathbf{b^+a^+c}$ (66) and $\mathbf{b\ a\ c^+}$ (59) phenotypes indicate crossovers between **c** & **a**.
3. The percent recombination between two markers indicates the map distance between them: 1% recombination = 1 map unit (m.u.). To determine the map distance between a pair of loci, ***count the number of SCO and DCO events***, and use the following formula [the most common error is to neglect the DCO classes].

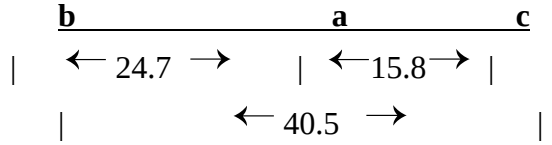
$$\text{Map distance} = \%\ \text{recombination} = \frac{(\#\ \text{in SCO phenotypes} + \#\ \text{in DCO phenotypes} \times 100)}{(\text{total}\ \#\ \text{progeny})}$$

(**b**$\leftrightarrow$**a**) Map distance = $\frac{112 + 102 + 18 + 15}{1000} \times 100 = 24.7\% = 24.7$ m.u.

(**a**$\leftrightarrow$**c**) Map distance = $\frac{66 + 59 + 18 + 15}{1000} \times 100 = 15.8\% = 15.8$ m.u.

(**b**$\leftrightarrow$**c**) Map distance = 24.7 m.u. + 15.8 m.u. = 40.5 m.u.

4. We can now draw a map segment showing order and distances among loci. Again, note that the orders **b-a-c** and **c-a-b** are equivalent and that the left/right the orientation of this map is arbitrary]



USING A GENE MAP

The gene map can be used as a table of probabilities to predict the expected amount of

recombination between certain loci.

In a test cross the male contributes only recessive alleles. Recombination occurs in the formation of the female gametes. Therefore whatever alleles present in the female gamete will be expressed in the phenotype of the offspring.

There is a certain probability that a cross-over will form between **a** and **b** loci (= map distance between **a** and **b**) and another independent probability that a cross-over will occur between **b** and **c** loci (= map distance between **b** and **c**). The probability of a double cross-over is the product of these two independent probabilities.

Example 2: Given the map segment **cn** **vg** **sm**

|← 9.5 →| ← 24.5 → |

In a test cross of **$cn^+vg^+sm^+$ // cn vg sm**
Expected DCO = (% recomb. **cn-vg**) (% recomb. **vg-sm**)
= 0.095 x 0.245 = 2.3%

Therefore we expect to find 2.3% of the female gametes to be the results of double crossovers
1.15% **$cn^+vg\ sm^+$**
1.15% **cn vg^+ sm**

Expected SCO (**cn-vg**)

From the gene map 9.5% of the gametes would be expected to have crossovers between **cn** and **vg**, however this includes the 2.3% of double crossovers. Therefore 9.5 -2.3 = 7.2% of the female gametes should have single crossovers:
3.6% **cn vg^+ sm^+** &. 3.6% **cn^+ vg sm**

Expected SCO (**vg-sm**)

From the gene map 24.5% of the gametes would be expected to have crossovers between vg and sm, this includes the 2.3% of double crossovers. Therefore 22.2% of the female gametes should have single crossovers
11.1% **cn^+vg^+sm** & 11.1% **cn vg sm^+**

Total crossovers = 2.3% + 7.2% + 22.2% = 31.7%
Expect 68.3% parental gametes (34.15% of each).

♀ gametes		♂ gametes = 100 % **cn vg sm**
34.15 %	**cn$^+$vg$^+$sm$^+$**	
34.15 %	**cn vg sm**	
11.1 %	**cn vg sm$^+$**	
11.1 %	**cn$^+$vg$^+$sm**	
3.6 %	**cn vg$^+$sm$^+$**	
3.6 %	**cn$^+$ vg sm**	
1.15%	**cn vg$^+$ sm**	
1.15 %	**cn$^+$ vg sm$^+$**	

These percentages can then be used to determine an expected ratio.

wild-type	:	**cn vg sm**	:	**cn vg**	:	**sm**	:	**cn**	:	**vg sm**	:	**vg**	:	**cn sm**
34.15	:	34.15	:	11.1	:	11.1	:	3.6	:	3.6	:	1.15	:	1.15
29.7	:	29.70	:	9.7	:	9.7	:	3.1	:	3.1	:	1	:	1

INTERFERENCE and COINCIDENCE

Crossing over does not occur uniformly along a chromosome. For example, fewer crossovers occur in the area around the centromere than in other areas of the chromosome (making the loci appear closer together than they actually are). Also, the formation of one chiasma typically makes it less likely that a second chiasma will form in the immediate vicinity of the first. This seems to be due may be due to the inability of the chromatids to bend back upon themselves within a certain minimum distance.

This lack of independence is called **interference** and results in the observation of fewer double crossover types than would be expected according to true map distance.

Interference varies in different sections of the chromosome and is measured by the **Coefficient of Coincidence** (C.C.) which is the ratio of observed to expected double crossover types.

C.C. = (observed DCO) / (expected DCO) Interference = 1 - C.C.

To calculate expected DCO, actual distances from gene map should be used when available.

If C.C. = 0 then interference is complete and no double crossovers are observed. In general, double-crossovers do not occur between loci less than 10 m.u. apart.

C.C. values between 0 and 1 indicate partial interference. Generally interference decreases as the distance between the loci increases.

If C.C. = 1 then there is no interference and all the expected double crossovers are observed. With loci more than 45 m.u. apart there is little or no interference. In some cases there may be an excess of double crossovers, *i.e.* negative interference.

In **EXAMPLE 1**: C.C. = (observed DCO) / (expected DCO)
= (33) / (0.247)(0.158)(1000) = 33 / 39 = 0.846

Seeing 84.6% of the double crossovers expected. Interference = 1 - C.C. = 1 - .846 = 0.154 = 15.4%

The coefficient of coincidence can also be used to modify the number of double crossovers predicted from a map.

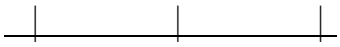
In **EXAMPLE 2**: In the region cn-sm 2.3% double crossover type were expected. However if the C.C. is known to be 70% for this region, then the number of expected double crossovers is modified (.7 x 2.3) = 1.61% and the number of other expected phenotypes are modified accordingly.

EXERCISE 7 Linkage & Crossing Over Name _____
Lab Section _____

1. Female *Drosophila* with cinnabar eye (**cn**) and vestigial wings (vg) were mated to males with roof wings (**rf**). The F_1 were all wild-type. When the F_1 females were test crossed with males homozygous for all three traits the following result were obtained.

382 cinnabar, vestigial	P
401 roof	P
3 cinnabar	DCO
4 roof, vestigial	DCO
59 cinnabar, roof, vestigial	SCO1
67 wild	SCO1
44 cinnabar, roof	SCO2
40 vestigial	SCO2

i) To determine the gene order (**rf** is not given on the gene map) compare the parental and DCO phenotypes.

The correct order of the loci is 

ii) The genotype of F_1 ♀ would have been

iii) The phenotypes "cinnabar-roof-vestigial" and "wild" were formed as a result of a single crossover between

_____ and _____

Calculated map distance.

iv) The phenotypes ("cinnabar-roof" and "vestigial" were formed as a result of a single crossover between

_____ and _____.

Calculated map distance

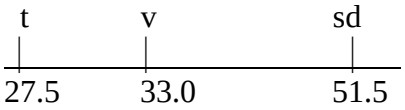
v) From the **gene map** (Appendix B) the locus of **cn** is _____ and **vg** is _____

Map distance **cn - vg** is _____

vi) From these calculations what would be the locus for roof wings? Draw a gene map segment.

2. Tan body, scalloped wing female *Drosophila* were crossed with vermilion eye males.

I



i) From the map segment determine the map distance between gene pairs

Gene pair	Map distance
t - v **v - sd** **t - sd**	

ii) F_1 female phenotype _____
genotype _____

iii) Crossing over occurs only in female *Drosophila*. Using the map distances above calculate the % of each type of gamete produced by the F_1 female.

gamete		%
DCO		
SCO (**t-v**)		
SCO (**v-sd**)		
P		

iv) The loci for tan, vermillion and scalloped are located on the X chromosome. For a male recessive for all three traits, the male gametes formed would be

50% _____ and 50% _____

Would the male gametes influence the phenotype of the offspring? _____Why (not)?

v) What would be the expected F_2 phenotype ratio in a testcross of the F_1 female in (iii) and the male in (iv)?

vi) The testcross result showed

413 tan scalloped	84 tan
380 vermillion	80 vermillion scalloped
18 tan vermillion	2 tan vermillion scalloped
20 scalloped	3 wild-type

Determine the map distance between each pair of loci.

vii) Calculate the Interference

vii) Using the results from (vi) above, calculate a Chi-Square Test to determine if these results fit the expected amount of crossing over.

Title:

Phenotype	Observed (O)	Expected (E)	O-E	$(O-E)^2$	$(O-E)^2/E$
					X^2 =

Interpretation: