EXERCISE 7 - LINKAGE, CROSSING-0VER, & 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* **$a^+b^+//ab$**	$a^+\ b^+$ $a^+\ b^+$ $a\ \updownarrow\ b$ $a\ \ b$	$a^+\ b^+$ $a^+\ b$ $a\ \ b^+$ $a\ \ b$	**P** **Recombinant** **Recombinant** **P**
Single crossover: markers in *trans* **$a^+b//ab^+$**	$a^+\ b$ $a^+\ b$ $a\ \updownarrow\ b^+$ $a\ \ b^+$	$a^+\ b$ $a^+\ b^+$ $a\ \ b$ $a\ \ b^+$	**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 **$b^+a^+c^+$ / 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. **b^+a c** (112) and **b a^+c^+** (102) phenotypes indicate crossovers between **b** & **a.**
b. **b^+a^+c** (66) and **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].

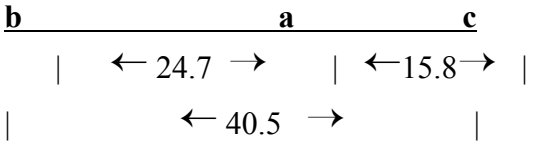
$$\text{Map distance} = \% \text{ recombination} = \frac{(\#\text{ in SCO phenotypes} + \#\text{ in DCO phenotypes} \times 100)}{(\text{total \# progeny})}$$

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

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

(**b↔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

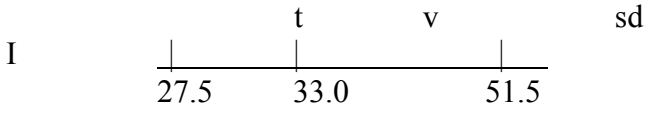
_____ and _____.

Calculated map distance

v) From the gene map 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) 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) 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) Calculate a Chi-Square Test to determine if these result fit the expected amount of crossing over.

EXERCISE 8 - RECOMBINATION IN AN ASCOMYCETE

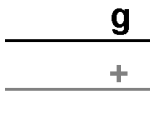
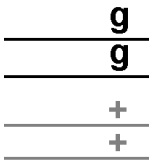
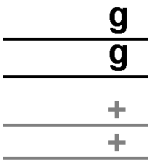
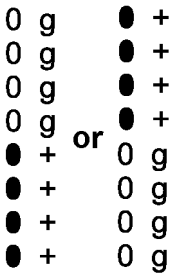
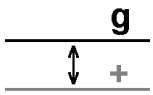
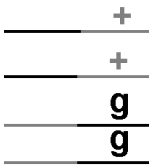
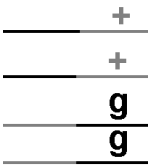
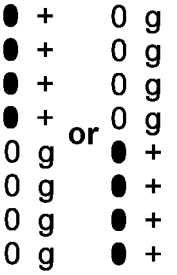
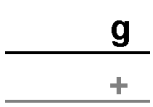
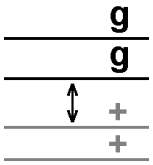
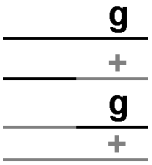
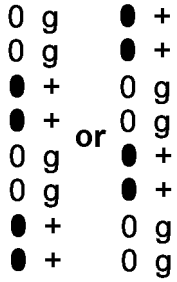
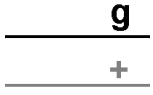
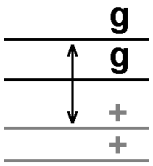
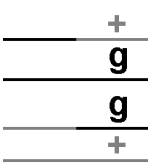
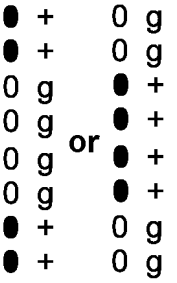
Fungi of the class Ascomycetes are useful for the study of recombination. They produce large numbers of progeny, which makes detection of rare events possible. Most of the life cycle is spent in the haploid condition. Sexual reproduction, which results from the union of "plus" (+) and "minus" (-) mating strains, results in a diploid zygote (the only diploid cell in the life cycle), which produces the **perithecium** (pl. **perithecia**) in which spores are produced in a sac called an **ascus** (pl. **asci**).

Phenotype depends on the genotype. The colour (*i.e.* phenotype) of each haploid spore is determined by the spore colour allele (*i.e.* genotype) it possesses. Segregation of the alleles affecting spore colour can be observed directly from the ascus.

The diploid nucleus undergoes two meiotic division to produce four haploid nuclei, then mitosis takes place in each nucleus so that eight (four pairs of genetically identical) spores are formed in each ascus. These fungi are unique in that each ascus contains all the products of a single meiosis.

Because the cell is narrow, the meiotic spindle is forced to lie along the long axis of the cell, which results in the spores being lined up in exactly the same orientation they had after the completion of the meiotic divisions. Therefore the particular meiotic division at which any genetic exchange (crossing over) occurs can be determined by observing the arrangement of the ascospores.

Linkage & Crossing Over involving one gene in *Sordaria*

Condition	Two-strand stage	Four-strand stage	Chromosomes Following Meiosis	Ascospores in Ascus
No crossover				
Crossover at two-strand stage				
One crossover at four-strand stage				
Alternate crossover at four-strand stage				

First and Second Division Segregation

The pattern of spore arrangement depends on whether or not there was a cross over between the gene and the centromere.

A cross between grey and black strains in the absence of crossing over results in the linear arrangement of 4 grey spores: 4 black spores. This pattern represents **first division segregation** because the two alleles are separated at Anaphase during the first meiotic division.

If crossing over were to occur between the gene and the centromere at the 2-strand stage, the linear arrangement of 4 and 4 would be identical to the arrangement with no crossover, so crossover would not be detected (first division segregation).

If crossing over occurs between two homologous chromatids at the 4-strand stage, the arrangement is interrupted. Depending on which two strands cross over, the arrangement will be either 2:2:2:2 or 2:4:2. These patterns represent **second division segregation** because the two alleles are not separated until Anaphase of the second meiotic division.

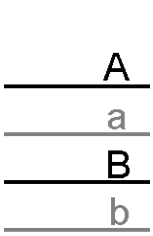
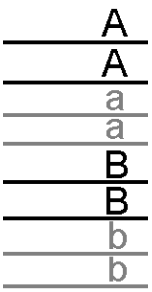
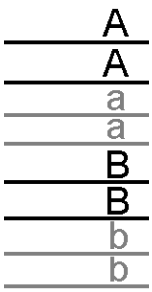
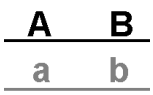
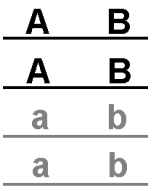
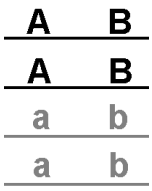
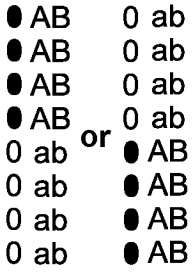
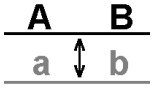
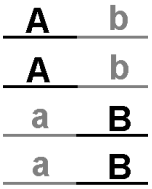
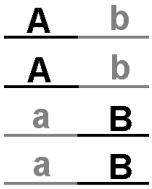
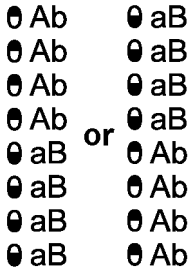
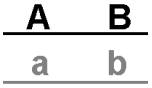
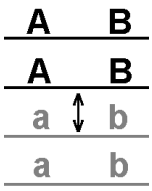
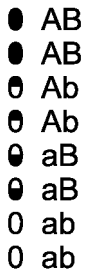
Occasionally asci are found with 5:3, 6:2, 3:1:1:3 ratios. These may be due to an extra chromosome (non-disjunction), epistasis, or mitotic crossing over prior to meiosis. These may also be due to **gene conversion**, where, during a meiotic or mitotic process, one allele or one chromatid converts its counterpart to its own kind, both then being identical.

Gene - Centromere Map distance

Crossing over occurs between any two non-sister chromatids of the 4-strand stage, but only two of the strands will be involved in the exchange in a single crossover, resulting in one half of the strands being parental and the other half being recombinant. Map distance is the frequency of recombination per chromatid, not per tetrad. Therefore, to determine the distance of a locus from the centromere, calculate one-half the percentage of second division segregation for that locus:

map distance = [(½)(# second division segregation asci) / (total # of asci)] x 100

Linkage & crossing-over involving two loci in *Sordaria*

Condition	Two-strand stage	Four-strand stage	Chromosomes following meiosis	Ascospores in ascus
Unlinked				
No crossover				
One crossover at the two-strand stage				
One form of crossover at the four-strand stage				

When two loci segregate the arrangement maybe classified as

PD = two **parental ditypes** only

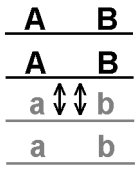
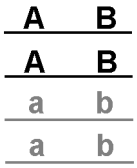
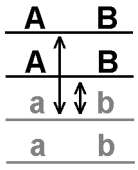
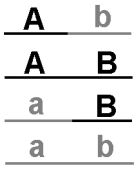
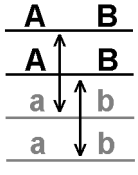
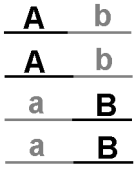
TT = **tetratype**: two parental, two nonparental types
(half the products are recombinant)

NPD = two **nonparental ditypes** only (all the products are recombinant) If no crossing over takes place between the two loci in a dihybrid cross, only parental types will be found in the ascus (first division segregation).

If crossing over were to occur at the two strand stage it would be detected since only recombinant ascospores and no parental types would be found (first division segregation).

If a single crossover occurs between the two loci, then all four possible genotypes are found in the ascus. However the maximum number of offspring found showing recombinant phenotypes is 50%.

Double crossovers involving two loci

Condition	Four-strand stage	Chromosomes following meiosis	Ascospores
Involving two strands			AB AB AB AB 0 ab 0 ab 0 ab 0 ab
Involving three strands			Ab Ab AB AB aB aB 0 ab 0 ab
Involving four strands			Ab Ab Ab Ab aB aB aB aB

Map Distance Between two loci

50% of the asci should show 1st division segregation; 50% should show 2nd division segregation, with varying numbers of each type, the NPD usually occur in much smaller percentages.

If the loci were not linked, but on separate chromosomes they would assort independently and the PD and NPD would be found in equal frequencies. If a crossover occurred between either locus and its centromere, then **TT** would appear, with the number of **TT** depending on the distance from the centromere. The relative frequency of the different types is used to determine the map distances between two linked loci.

Example:

Spore pair				
1	**AB**	**AB**	**AB**	**aB**
2	**AB**	**Ab**	**ab**	**aB**
3	**ab**	**aB**	**AB**	**Ab**
4	**ab**	**ab**	**ab**	**Ab**
Number	117	66	33	2
Classification	PD	TT	PD	NPD
Segregation for A	1st	1st	2nd	1st
Segregation for B	1st	2nd	2nd	1st

1. The presence of a much smaller number (2) of NPD indicate linkage.

2. The presence of the 33 PD asci that show second division segregation for both loci indicate that both loci are on the same side of the centromere.

3. The presence of the 66 TT asci show the locus closer to the centromere undergoing only first division segregation, the locus farther from the centromere undergoing second division segregation.

Distance **A** - centromere = ½ the percentage of second division segregation for A

$$= \frac{\frac{1}{2}(33)}{220} \times 100 = 7.5 \text{ m.u.}$$

Distance **B** - centromere = ½ the percentage of second division segregation for B

$$= \frac{\frac{1}{2}(66 + 33)}{220} \times 100 = 22.5 \text{ m.u.}$$

Map distance between two loci (**A-B**)

$$= \frac{\frac{1}{2}(TT) + \#\,NPD}{\text{Total \# asci}} \times 100$$

$$= \frac{\frac{1}{2}(66) + 2}{220} \times 100 = 15.9 \text{ m.u.}$$

By subtraction 22.5 - 7.5 = 15 m.u. between **A** and **B**. The distances do not correspond exactly since double crossovers are not detected.

PROCEDURE:

Petri dishes with **+** and **g** strains of *Sordaria* have been prepared for you. The + denotes the black or wild-type spore colour. The g denotes gray spore colour. Within both strains, both + and **-** mating strains may be present. Where the two strains meet, sexual reproduction occurs and perithecia are formed.

1. Along the line where the two strains meet scrape off some of the perithecia (black dots) with a dissecting needle and place them in a drop of phloxine on a microscope slide.

2. Cover with a cover slip and fold the slide in a piece of paper towel.

3. Very gently squash the cover slip down with the eraser end of a pencil to break the perithecia and squeeze out the asci. Each ascus contains all the meiotic products or ascospores produced when one diploid zygote underwent meiosis.

4. Examine under the compound microscope. Count the number of asci with different arrangements and fill in the table provided. It may be necessary to make a number of slides to find the various crossing over arrangements.

5. Place all used dissecting needles, slides and cover slips back in the containers of 70% alcohol provided. *NEVER* leave used needles, etc. on the bench. Do not leave dishes open. Although *Sordaria* does not usually become a laboratory pest, it can contaminate the lab space, put future work at risk, and cause discomfort to those allergic to fungi.

EXERCISE 8 - Recombination in Ascomycetes Name: _____

Ascospore Distribution Patterns

Spore Pairs	First Division Segregation	Second Division Segregation				
1	+	+	+	**g**		
2	+	**g**	**g**	+		
3	**g**	+	**g**	+		
4	**g**	**g**	+	**g**		
Number of Asci Your Data						
Totals Class Data						

1. What is the percent second division segregation for
   Your data? _____

   Class data? _____

2. What is the formula for calculating map distance between locus and centromere?

3. Calculate the map distance between the centromere and the g locus for
   Your data: _____

   Class data: _____

4. Is the + allele or the g allele dominant? Explain.

5. Were any ascospore arrangements found other than those listed in the table? What were they and how may they have been produced?

6. A *Sordaria* strain that had both loci a and b was mated to the wild-type strain, and produced the following ordered tetrads.

Spore pair 1	**ab**	**a**+	**ab**	**ab**	**ab**	**a**+	**ab**
Spore pair 2	**ab**	**a**+	**a**+	+**b**	++	+**b**	++
Spore pair 3	++	+**b**	+**b**	**a**+	**ab**	**a**+	+**b**
Spore pair 4	++	+**b**	++	++	++	+**b**	**a**+
Number	60	6	15	9	3	1	6
Classification							
Segregation locus **a**							
Segregation locus **b**							

a. Determine the gene-centromere distance for the two loci.

b. Calculate the map distance between the two loci.

c. Draw the gene map.