

LAB 10 – Meiosis and Tetrad Analysis

Objectives:

- Explain how meiosis and crossing over result in the different arrangements of ascospores within asci.
- Learn how to calculate the map distance between a gene and the centromere of the same chromosome.

Introduction:

All new cells come from previously existing cells. The process of cell division, which involves both division of the nucleus and division of the cytoplasm, forms new cells. There are two types of nuclear division: mitosis and meiosis. Mitosis typically results in new somatic (body) cells. Formation of an adult organism from a fertilized egg, asexual reproduction, regeneration, and maintenance or repair of body parts are accomplished through mitotic cell division. Meiosis results in the formation of either gametes (in animals) or spores (in plants). These cells have half the chromosome number of the parent cell.

Meiosis involves two successive nuclear divisions that produce four haploid (monoploid) cells. Meiosis I is the reduction division. It is this first division that reduces the chromosome number from diploid to haploid and separates the homologous pairs. Meiosis II, the second division, separates the sister chromatids. The result is four haploid gametes (see **Figure 1**).

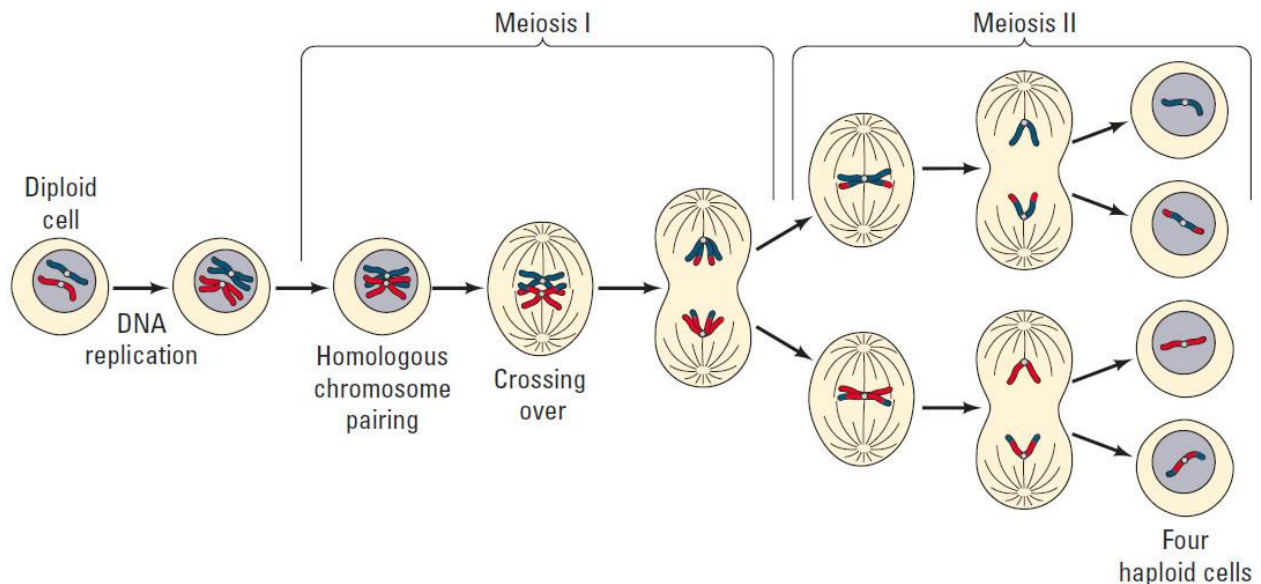


Figure 1: Meiotic Cell Division Emphasizing Chromosome Movement

Each diploid cell undergoing meiosis can produce 2^n different chromosomal combinations, where n is the haploid number. In humans, the number is 2^{23} , which is more than eight million combinations. Actually, the potential variation is even greater because, during meiosis I, each pair of chromosomes (homologous chromosomes) comes together in a process known as **synapsis**. Chromatids of homologous chromosomes may exchange parts in a process called **crossing over**. The relative distance between two genes on a given chromosome can be estimated by calculating the percentage of crossing over that takes place between them.

Sordaria fimicola is an ascomycete fungus that can be used to demonstrate the results of crossing over during meiosis. *Sordaria* is a **haploid** organism for most of its life cycle. It becomes **diploid** only when the fusion of the mycelia (filament-like groups of cells) of two different strains results in the fusion of the two different types of haploid nuclei to form a diploid nucleus. The diploid nucleus must then undergo meiosis to resume its haploid state.

Meiosis, followed by one mitotic division, in *Sordaria* results in the formation of eight **haploid ascospores** contained within a sac called an **ascus** (plural, **asci**). Many asci are contained within a fruiting body called a **perithecium** (ascocarp). When ascospores are mature the ascus ruptures, releasing the ascospores. Each ascospore can develop into a new haploid fungus. The life cycle of *Sordaria fimicola* is shown in **Figure 2** below.

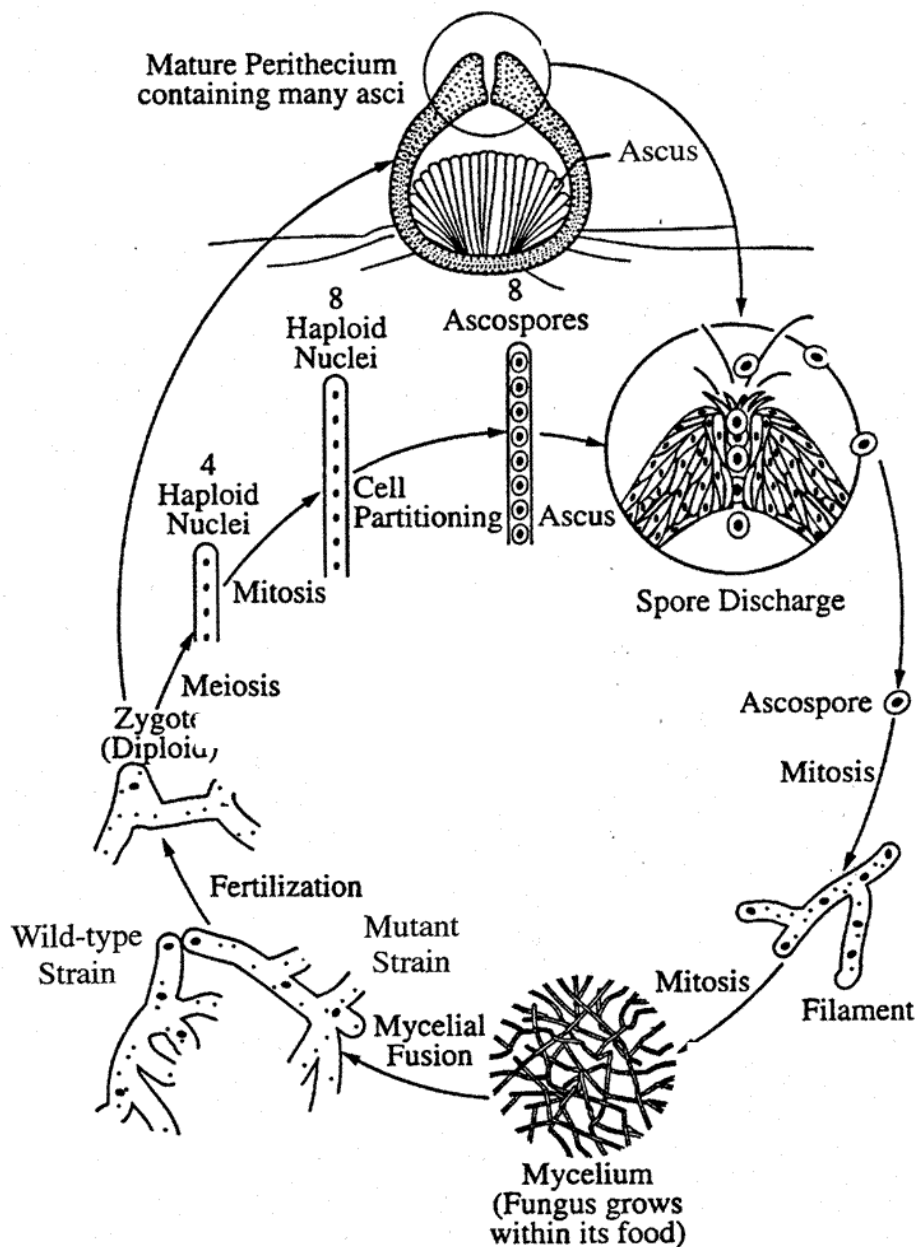


Figure 2: Life Cycle of *Sordaria fimicola*.

The spore color of the normal (wild type) *Sordaria*, is black. This phenotype is due to the production of the pigment melanin and its deposition in the cell walls. Several different genes are involved in the control of the melanin biosynthetic pathway and each gene has two possible allelic forms. The gray spore gene has two allelic forms: the wild type allele (g^+) and a mutant allele (g). The tan spore gene also has two forms: a wild type allele (t^+), and a mutant allele (t). Normal black spores are produced **only if both wild type alleles are present** at the loci of both genes. Thus, black ascospores have the genotype $g^+ t^+$ (remember, spores are haploid). Those with the genotype $g t^+$, are gray, while $g^+ t$ are tan. Ascospores that are $g t$ show a cumulative effect of the two mutations and are colorless – but we won't study that effect in this lab... perhaps it might make a good independent research project though!

To observe crossing over in *Sordaria*, one must make hybrids between wild type and mutant strains of *Sordaria*. The arrangement of the spores directly reflects whether or not crossing over has occurred. In **Figure 3** no crossing over has occurred. **Figure 4** shows the results of crossing over between the centromere of the chromosome and the gene for ascospore color.

To Cross Over or NOT To Cross Over... that is the question.

The following two examples take a look at a tan X wild cross. Two homologous chromosomes line up at metaphase I of meiosis. The two chromatids of one chromosome each carry the gene for tan spore color (t) and the two chromatids of the other chromosome carry the gene for wild type spore color (t^+).

The first meiotic division results in two cells, each containing just one type of spore color gene (either a tan or wild type). Therefore, segregation of these alleles has occurred at the first meiotic division. Each cell is haploid at the end of meiosis I. The second meiotic division results in four haploid cells, each with the haploid number of chromosomes. A **mitotic** division following meiosis simply duplicates these cells, resulting in 8 spores – without any crossing over event. They are arranged in the 4:4 pattern shown below.

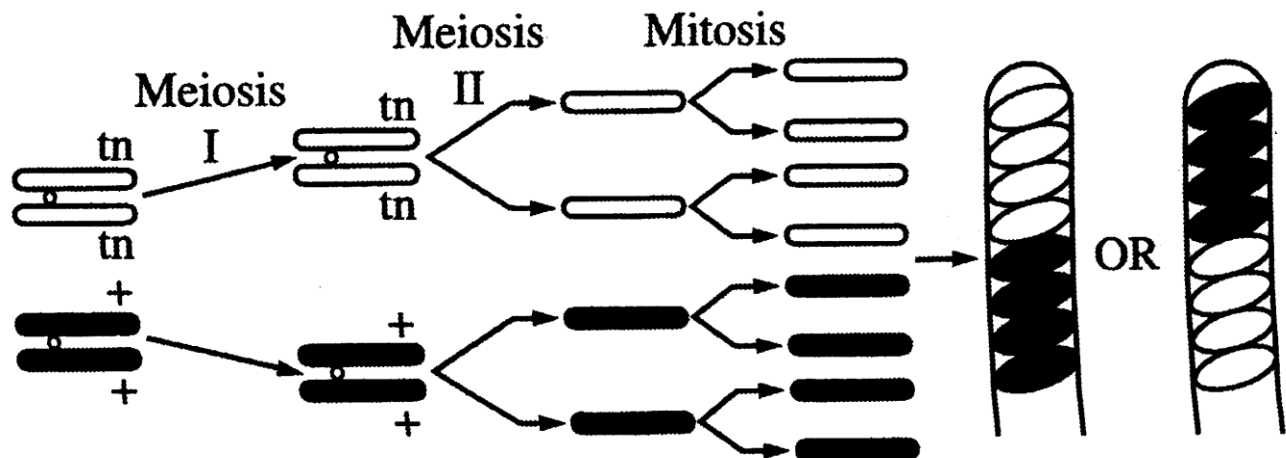


Figure 3: Meiosis with NO Crossing Over

In the following example, crossing over HAS occurred in the region between the gene for spore color and the centromere. The homologous chromosomes separate during meiosis I. This time, because of the genetic shuffling, the first meiotic division results in *genotypically* two different chromosome arrangements – each containing both different alleles (1 tan, 1 wild type); therefore, the genes for spore color have not yet segregated, although the cells are haploid. The second meiotic division results in the segregation of the two types of genes for spore color – resulting in *phenotypically* two different cells.

A **mitotic** division following meiosis results in 8 spores arranged in the 2:2:2:2 or 2:4:2 pattern. Any one of these spore arrangements would indicate that crossing over has occurred between the gene for spore coat color and the centromere.

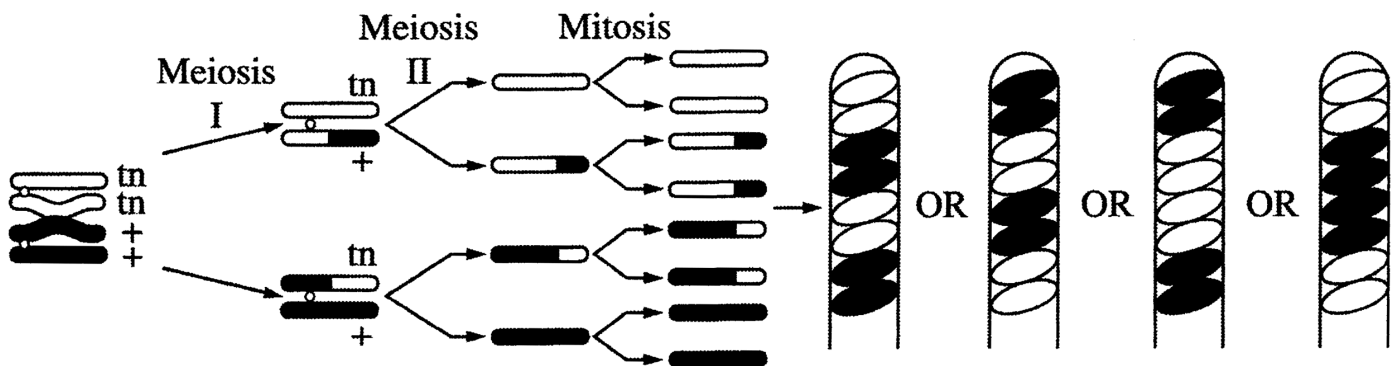


Figure 4: Meiosis with Crossing Over

You should understand that there can be multiple crossing over events, you might sometimes see the 8 ascospores in a 2:1:1:1:1:2 or other 'weird' ratio. These would normally count as a double-crossover event, but realize, you also can have a double-crossover result in a 4:4 if it crossovers *twice* in between the centromere and the gene locus. So we will not count the odd ratios as two – but just one crossover event.

Published gene map locations for the two genes that we are studying are *approximately 27 map units for the tan spore gene* and **33 map units for the gray spore gene** (Olive, 1956). More on mapping genes in the final introduction section.

Mapping Genes on Chromosomes

The exchange of genetic material between homologous chromosomes which occurs during crossing over creates a major exception to Mendel's principle of segregation. Recall that the segregation of alleles from the two parents occurs during anaphase I of meiosis, that is, during the first division of meiosis. If crossing over occurs, however, the alleles rearranged by the crossover are not segregated until anaphase II of meiosis, that is during the second division of meiosis. Thus, it is said that crossing over leads to *second division segregation* of the alleles involved in the crossover. Gene mapping became possible when it was realized that the frequency of second division segregation was related to the physical distance separating the genes involved.

If we assume that crossing over can occur at any point along a chromosome, it is logical that the probability of a crossover occurring between a gene locus and the centromere will be proportional to the locus-centromere distance. In **Figure 5**, we would expect a higher rate of crossover events with Gene B than Gene A.

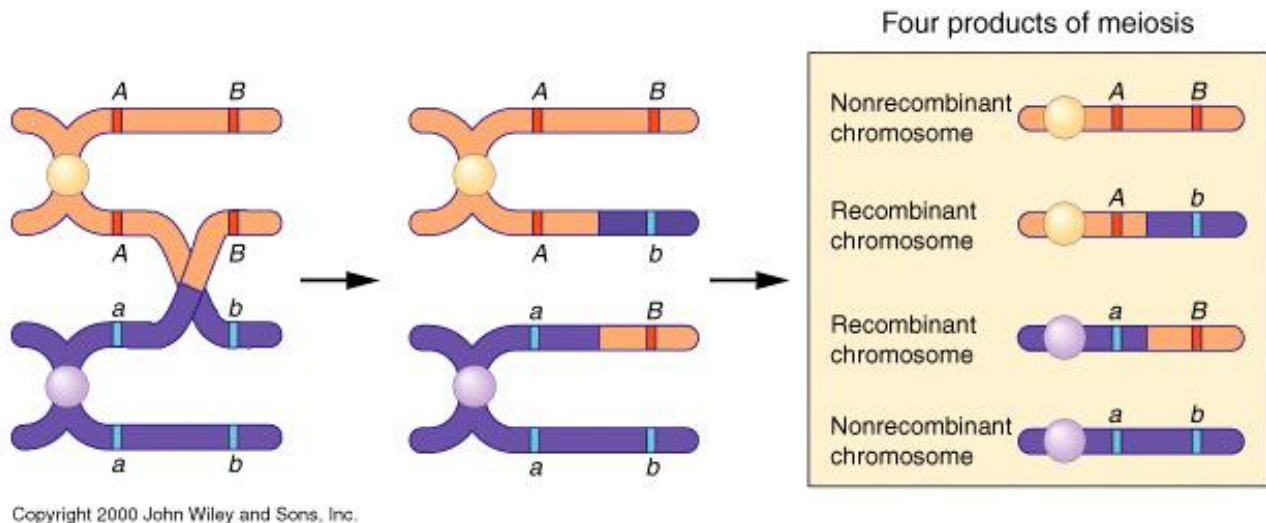


Figure 5: Differential Crossing Over due to Gene Loci Distance from Centromere

Therefore, we can use the frequency (proportion) of crossover-produced ascospores as a measure of the relative distance separating the gene locus and the centromere. Here, the recombinant frequency would be equal to the number of recombinant asci per 100 total asci (both recombinant and non-recombinant).

$$\text{recombinant frequency} = \frac{\text{recombinant asci}}{\text{total asci}} \times 100$$

Geneticists define a crossover *map unit* as the distance on a chromosome that produces one recombinant post-meiotic product per 100 post-meiotic products. The distance is given in the units of centi-Morgans (cM). Given that map units express the percent recombinant asci resulting from crossovers and each single crossover produces 4 recombinant spores and 4 non-recombinant spores (so only 4 out of the 8 are 'different'), the map unit distance for our *Sordaria* cross is **always one half the frequency of crossing over for the gene**. See pages 224-226 in your textbook or Topic 7 in last summer's Math For Life review for more information on gene mapping.

$$\text{map units} = \frac{\text{recombinant frequency}}{2}$$

Using tetrad analysis, geneticists have been able to obtain genetic maps of chromosomes of many organisms. These maps indicate the sequence of genes on chromosomes and the relative location of these genes. However, because a genetic map is based on crossover frequencies, the relative distances between genes DO NOT correspond to real, physical distances. That is, although the sequence of genes is correct, some genes may be closer together and others farther apart than genetic maps indicate. This is because some regions of chromosomes have a greater, or lesser, tendency to form crossovers than other regions. For example, the centromere seems to inhibit crossing over and genes located close to it do not crossover as much as they should based solely on their physical location.

Procedure:

In the example below, two strains of *Sordaria* (wild type and a mutant variety – either tan or gray) have been inoculated on a nutrient plate. Where the mycelia of the two strains meet, **Figure 6**, fruiting bodies called perithecia develop. Meiosis occurs within the perithecia during the formation of the asci.

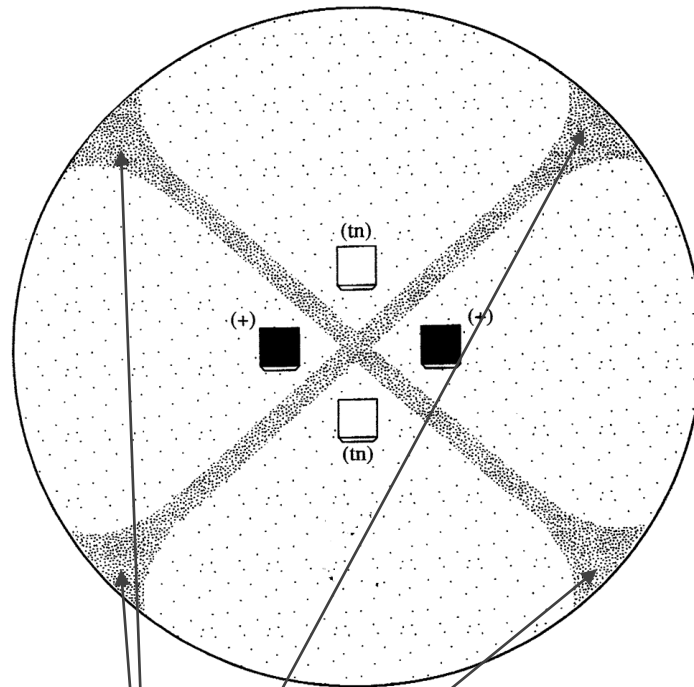


Figure 6: *Sordaria* Cross Plate

1. In each group of 4 students, 2 will set up and work on the wild/tan cross and 2 will set up and work on the wild/gray cross.
2. Use a scalpel to gently scrape the surface of the nutrient medium where the two strains intersect to collect perithecia. At the intersection of the two strains is the region to harvest the perithecia.
3. Place the perithecia in a drop of water on a slide. Cover with a coverslip and return to your work area. Using the eraser end of a pencil (or a toothpick), press down the coverslip gently so that the perithecia rupture but the ascospores remain in the asci – this is tricky to do. You want to rupture the fruiting body, but not each spore case.
4. View your slide using the 10X objective and locate a group of hybrid asci (those containing both mutant and wild ascospores). Try to go to 40X if possible.
5. Count **at least** 50 hybrid asci for each mutant cross and enter your data in **Table 1**.
6. Determine the distance between the gene for spore color and the centromere. Calculate the recombinant frequency by dividing the number of crossover asci (2:2:2:2 or 2:4:2) by the total number of asci X 100. To calculate the map distance, divide the percentage of crossover asci by 2. This is done since only half of the spores in each ascus are the result of crossing over.

Table 1: *Sordaria fimicola* Tetrad Analysis

<i>Sordaria</i> Phenotype Crossed	Number of 4:4 Asci (Non-Crossover Events)	Number of Asci Showing Crossover	Total Asci	% Asci Showing Crossover (Recombinant Frequency)	Calculated Gene to Centromere Distance (cM)
Wild X Tan					
Wild X Gray					

7. Use the chi-square distribution test (**Tables 2 and 3 – for each respective mutant**) to statistically analyze the data using the book value determined by Olive, 1956 for both mutant crosses.
- Enter the number of non-crossover and crossover asci from your mutant cross as observed (o).
 - Calculate the percentage of crossover asci expected from Olive's "book value". (HINT – it is NOT the same as the map distance...) This also will give you the percentage of asci that you WOULD NOT expect to have a crossover.
 - Multiply the percentages by the total number of asci count from your mutant cross; this will give you the expected numbers (e) for this cross based on Olive's map distance.
 - Finish up the chi-square analysis using your calculations and **Table 4** and then answer the questions on page 10.

Table 2: Calculation of the Chi-Square Value for the Tan X Wild Cross.

	o	e	(o - e)	(o - e)²	$\frac{(o - e)^2}{e}$
Crossover Asci					
Non-Crossover Asci					
X² total =					

Table 3: Calculation of the Chi-Square Value for the Gray X Wild Cross.

	o	e	(o - e)	(o - e)²	$\frac{(o - e)^2}{e}$
Crossover Asci					
Non-Crossover Asci					
X² total =					

Table 4: The Chi-square Distribution Table.

degrees of freedom	probability value (p-value)									
	ACCEPT NULL HYPOTHESIS								REJECT	
	0.99	0.95	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	0.001	0.004	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64
2	0.02	0.10	0.45	0.71	1.30	2.41	3.22	4.60	5.99	9.21
3	0.12	0.35	1.00	1.42	2.37	3.67	4.64	6.25	7.82	11.34
4	0.30	0.71	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28

