



LAB THREE MITOSIS AND MEIOSIS

OVERVIEW

In this lab you will investigate the processes of mitosis and meiosis:

1. You will use prepared slides of onion root tips to study plant mitosis and to calculate the relative duration of the phases of mitosis in the meristem of root tissue. Prepared slides of the whitefish blastula may be used to study mitosis in animal cells and to compare animal mitosis with plant mitosis.
2. You will simulate the stages of meiosis by using chromosome models. You will study the crossing over and recombination that occurs during meiosis. You will observe the arrangements of ascospores in the asci from a cross between wild type *Sordaria fimicola* and mutants for tan spore coat color in this fungus. These arrangements will be used to estimate the percentage of crossing over that occurs between the centromere and the gene that controls the tan spore color.

OBJECTIVES

Before doing this lab you should understand:

- the events of mitosis in animal and plant cells;
- the events of meiosis (gametogenesis in animals and sporogenesis in plants); and
- the key mechanical and genetic differences between meiosis and mitosis.

After doing this lab you should be able to:

- recognize the stages of mitosis in a plant or animal cell;
- calculate the relative duration of the cell cycle stages;
- describe how independent assortment and crossing over can generate genetic variation among the products of meiosis;
- use chromosome models to demonstrate the activity of chromosomes during meiosis I and meiosis II;
- relate chromosome activity to Mendel's laws of segregation and independent assortment;
- demonstrate the role of meiosis in the formation of gametes or spores in a controlled experiment using an organism of your choice;
- calculate the map distance of a particular gene from a chromosome's centromere or between two genes using an organism of your choice;
- compare and contrast the results of meiosis and mitosis in plant cells; and
- compare and contrast the results of meiosis and mitosis in animal cells.

INTRODUCTION

All new cells come from previously existing cells. New cells are formed by the process of cell division, which involves both division of the cell's nucleus (**karyokinesis**) and division of the cytoplasm (**cytokinesis**).

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. You will study mitosis in Exercise 3A. **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. You will study meiosis in Exercise 3B.

Where does one find cells undergoing mitosis? Plants and animals differ in this respect. In higher plants the process of forming new cells is restricted to special growth regions called **meristems**. These regions usually occur at the tips of stems or roots. In animals, cell division occurs anywhere new cells are formed or as new cells replace old ones. However, some tissues in both plants and animals rarely divide once the organism is mature.

To study the stages of mitosis, you need to look for tissues where there are many cells in the process of mitosis. This restricts your search to the tips of growing plants, such as the onion root tip, or, in the case of animals, to developing embryos, such as the whitefish blastula.

EXERCISE 3A.1: Observing Mitosis in Plant and Animal Cells Using Prepared Slides of the Onion Root Tip and Whitefish Blastula

Roots consist of different regions (see Figure 3.1a). The **root cap** functions in protection. The **apical meristem** (Figure 3.1b) is the region that contains the highest percentage of cells undergoing mitosis. The **region of elongation** is the area in which growth occurs. The **region of maturation** is where root hairs develop and where cells differentiate to become xylem, phloem, and other tissues.

Figure 3.1a: Median Longitudinal Section

Figure 3.1b: Apical Meristem Tip Close Up

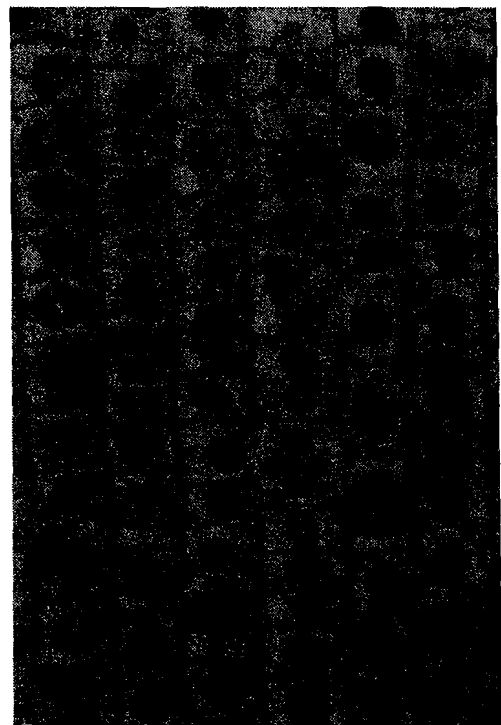
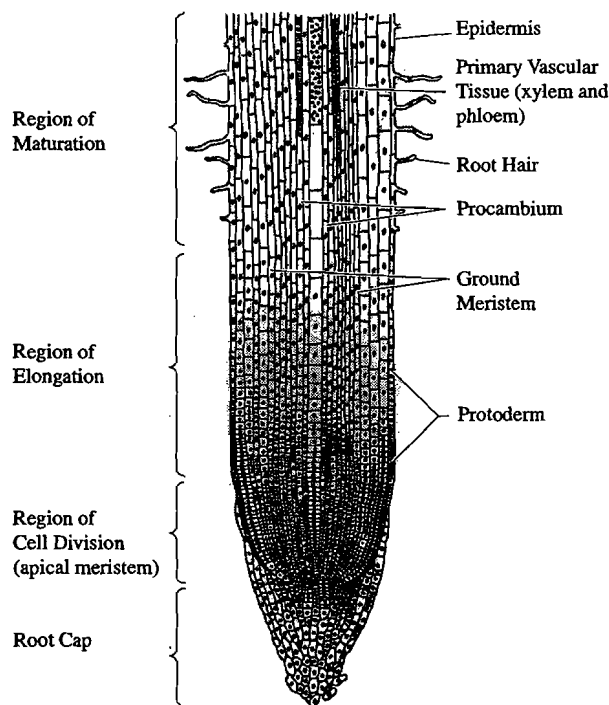
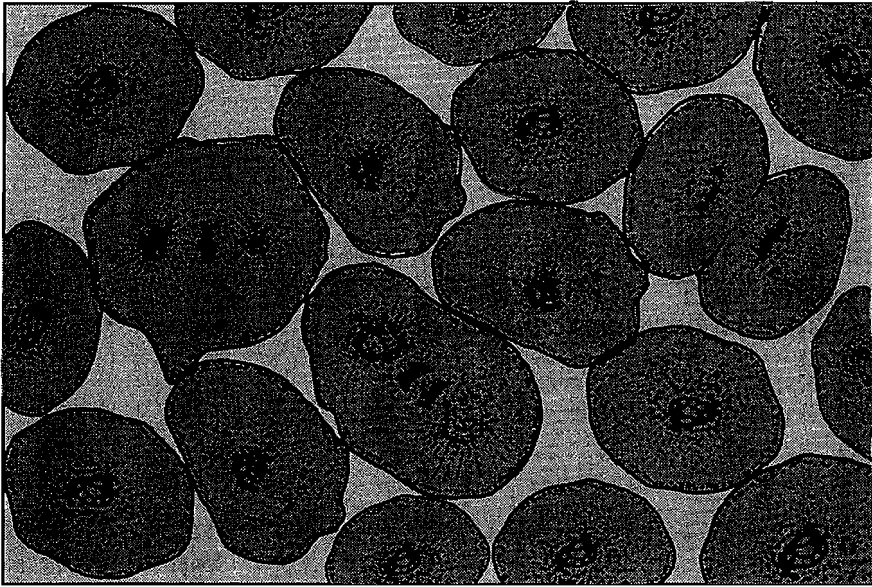


Figure 3.2: Whitefish Blastula

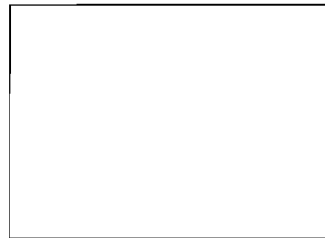


The whitefish blastula is often used for the study of cell division. As soon as the egg is fertilized it begins to divide, and nuclear division after nuclear division follows. You will be provided with slides of whitefish blastula which have been sectioned in various planes in relation to the mitotic spindle. You will be able to see side and polar views of the spindle apparatus.

Procedure

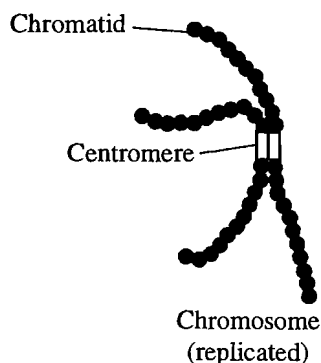
Examine prepared slides of either onion root tips or whitefish blastula. Locate the meristematic region of the onion, or locate the blastula, with the 10X objective and then use the 40X objective to study individual cells. For convenience in discussion, biologists have described certain stages, or phases, of the continuous mitotic cell cycle, as outlined on this page and the next. Identify one cell that clearly represents each phase. Sketch and label the cell in the boxes provided.

-
1. The nondividing cell is in a stage called **interphase**.
The **nucleus** may have one or more dark-stained **nucleoli** and is filled with a fine network of threads, the **chromatin**.
During **interphase**, DNA replication occurs.

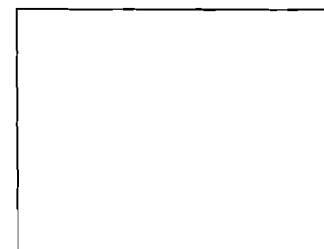


Interphase

Figure 3.3



2. The first sign of division occurs in **prophase**. There is a thickening of the chromatin threads, which continues until it is evident that the chromatin has condensed into **chromosomes** (Figure 3.3). With somewhat higher magnification you may be able to see that each chromosome is composed of two **chromatids** joined at a **centromere**. As prophase continues, the chromatids continue to shorten and thicken. In late prophase the nuclear envelope and nucleoli are no longer visible, and the chromosomes are free in the cytoplasm. Just before this time, the first sign of a spindle appears in the cytoplasm; the spindle apparatus is made up of **microtubules**, and it is thought that these microtubules may pull the chromosomes toward the poles of the spindle where the two daughter nuclei will eventually form.



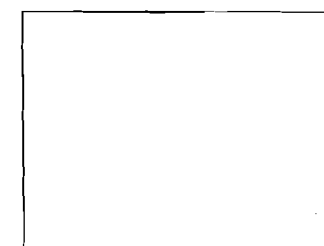
Prophase

3. At **metaphase** the chromosomes have moved to the center of the spindle. One particular portion of each chromosome, the centromere, attaches to the spindle. The centromeres of all the chromosomes lie at about the same level of the spindle, on a plane called the metaphase plate. At metaphase you should be able to observe the two chromatids of some of the chromosomes.



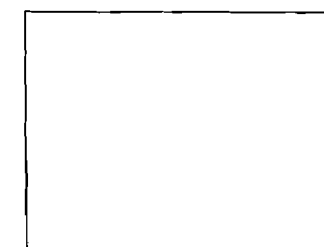
Metaphase

4. At the beginning of **anaphase**, the centromere regions of each pair of chromatids separate and are moved by the spindle fibers toward opposite poles of the spindle, dragging the rest of the chromatid behind them. Once the two chromatids separate, each is called a **chromosome**. These daughter chromosomes continue their poleward movement until they form two compact clumps, one at each spindle pole.



Anaphase

5. **Telophase**, the last stage of division, is marked by a pronounced condensation of the chromosomes, followed by the formation of a new nuclear envelope around each group of chromosomes. The chromosomes gradually uncoil to form the fine chromatin network seen in interphase, and the nucleoli and nuclear envelope reappear. **Cytokinesis** may occur. This is the division of the cytoplasm into two cells. In plants, a new cell wall is laid down between the daughter cells. In animal cells, the old cell will pinch off in the middle along a **cleavage furrow** to form two new daughter cells.



Telophase



Analysis Questions

1. Explain how mitosis leads to two daughter cells, each of which is diploid and genetically identical to the original cell. What activities are going on in the cell during interphase?

2. How does mitosis differ in plant and animal cells? How does plant mitosis accommodate a rigid, inflexible cell wall?

3. What is the role of the centrosome (the area surrounding the centrioles)? Is it necessary for mitosis? Defend your answer.

EXERCISE 3A.2: Time for Cell Replication

To estimate the relative length of time that a cell spends in the various stages of cell division, you will examine the meristematic region of a prepared slide of the onion root tip. The length of the cell cycle is approximately 24 hours for cells in actively dividing onion root tips.

Procedure

It is hard to imagine that you can estimate how much time a cell spends in each phase of cell division from a slide of dead cells, yet this is precisely what you will do in this part of the lab. Since you are working with a prepared slide, you cannot get any information about how long it takes a cell to divide. What you can determine is how many cells are in each phase. From this, you can infer the percentage of time each cell spends in each phase.

1. Observe every cell in one high-power field of view and determine which phase of the cell cycle it is in. This is best done in pairs. The partner observing the slide calls out the phase of each cell while the other partner records. Then switch so the recorder becomes the observer and vice versa. Count at least two full fields of view. If you have not counted at least 200 cells, then count a third field of view.
2. Record your data in Table 3.1.



3. Calculate the percentage of cells in each phase, and record in Table 3.1.

Consider that it takes, on average, 24 hours (or 1,440 minutes) for onion root tip cells to complete the cell cycle. You can calculate the amount of time spent in each phase of the cell cycle from the percentage of cells in that stage.

Percentage of cells in stage \times 1,440 minutes = _____ minutes of cell cycle spent in stage

Table 3.1

	Number of Cells				Percent of Total Cells Counted	Time in Each Stage
	Field 1	Field 2	Field 3	Total		
Interphase						
Prophase						
Metaphase						
Anaphase						
Telophase						
				Total Cells Counted		

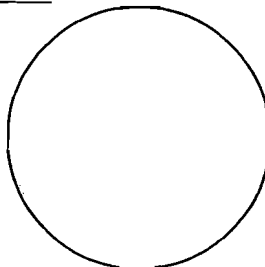
Questions

1. If your observations had not been restricted to the area of the root tip that is actively dividing, how would your results have been different?

2. Based on the data in Table 3.1, what can you infer about the relative length of time an onion root tip cell spends in each stage of cell division?

3. Draw and label a pie chart of the onion root tip cell cycle using the data from Table 3.1.

Title: _____





EXERCISE 3B: Meiosis

Meiosis involves two successive nuclear divisions that produce four haploid 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.

Mitotic cell division produces new cells genetically identical to the parent cell. Meiosis increases genetic variation in the population. 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 different 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.

EXERCISE 3B.1: Simulation of Meiosis

In this exercise you will study the process of meiosis by using chromosome simulation kits and following the directions in Figures 3.4–3.8. Your kit should contain two strands of beads of one color and two strands of another color. A homologous pair of chromosomes is represented by *one* strand of each color, with one of each pair coming from each parent. The second strands of each color are to be used as chromatids for each of these chromosomes.

Figure 3.4

Interphase. Place one strand of each color near the center of your work area. (Recall that chromosomes at this stage would exist as diffuse chromatin and not as visible structures.) DNA synthesis occurs during interphase, and each chromosome, originally composed of one strand, is now made up of two strands, or chromatids, joined together at the centromere region. Simulate DNA replication by bringing the magnetic centromere region of one strand in contact with the centromere region of the other of the same color. Do the same with its homolog.

Summary: DNA replication

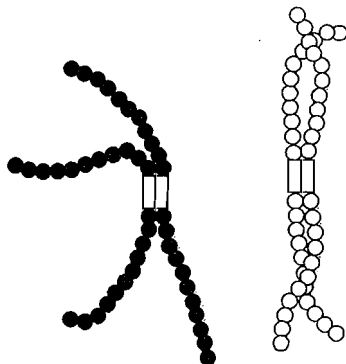




Figure 3.5

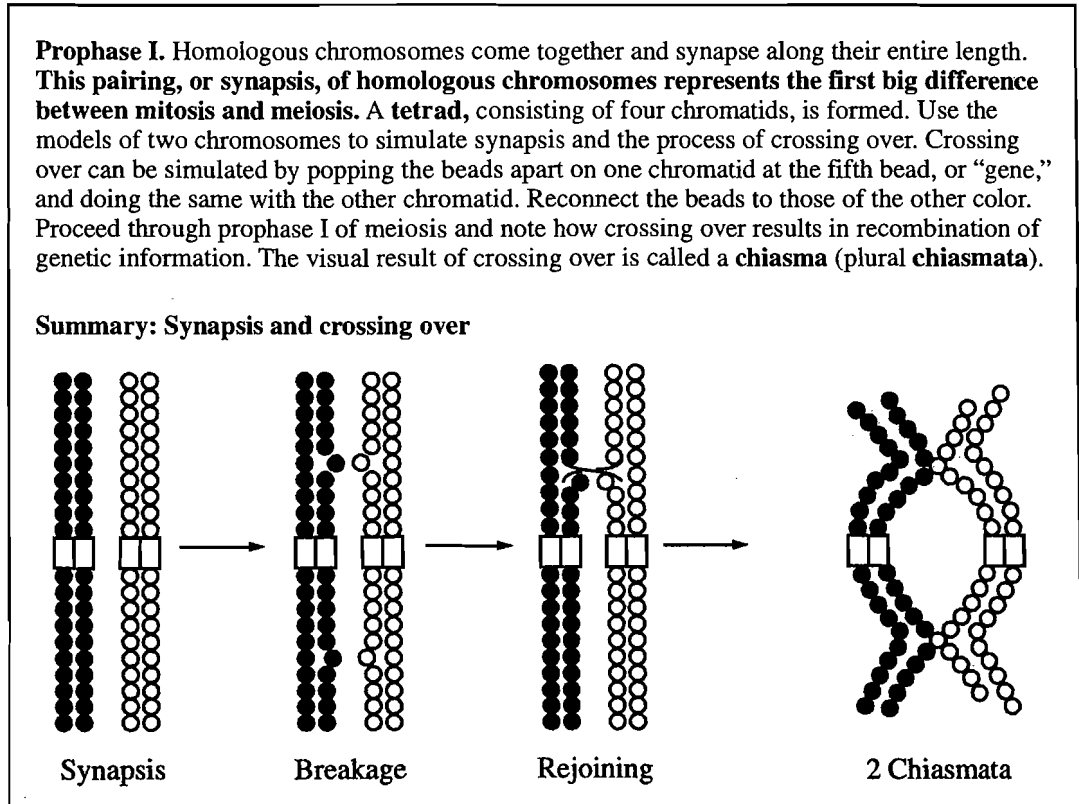


Figure 3.6

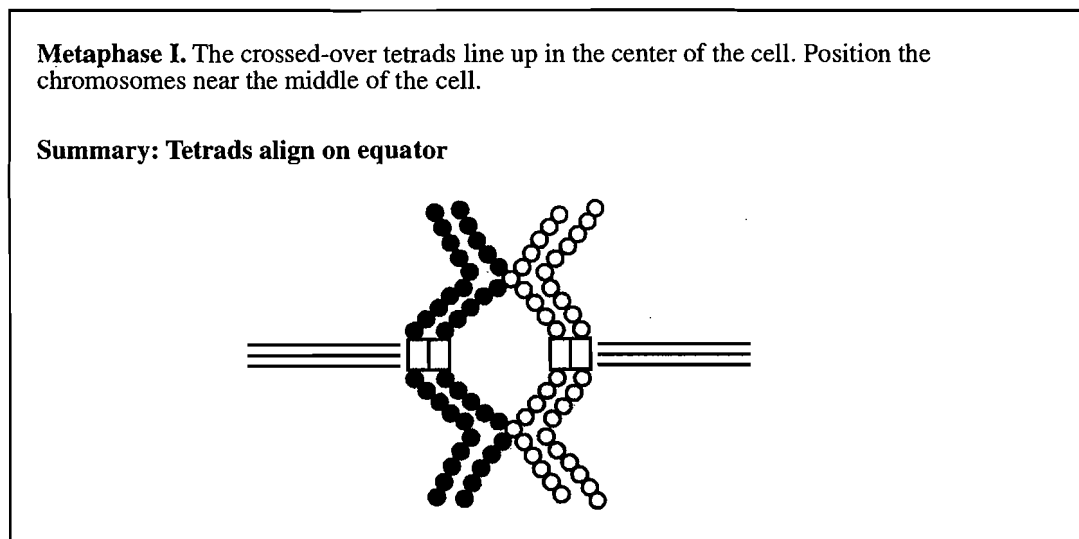


Figure 3.7

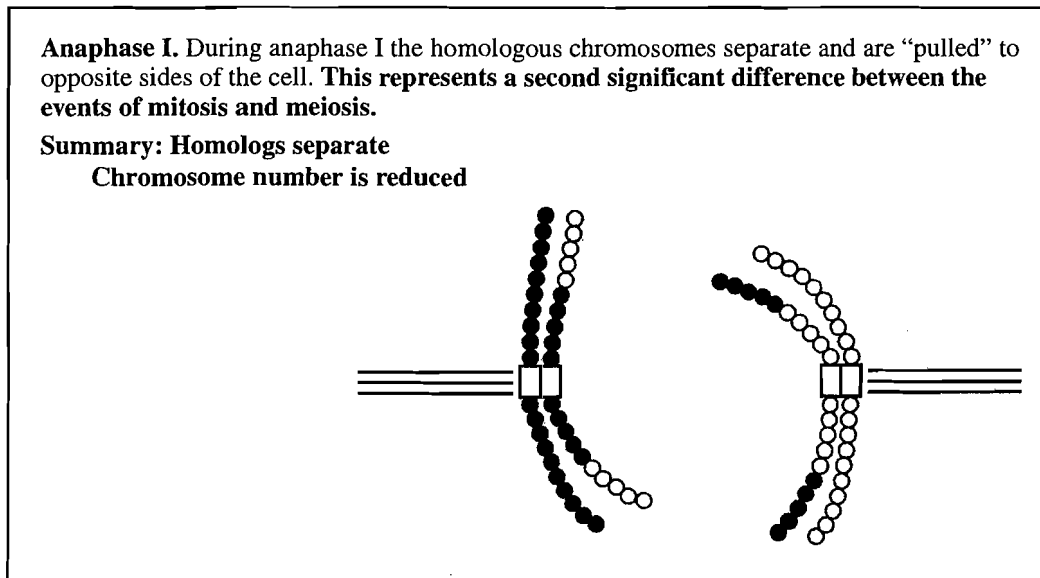
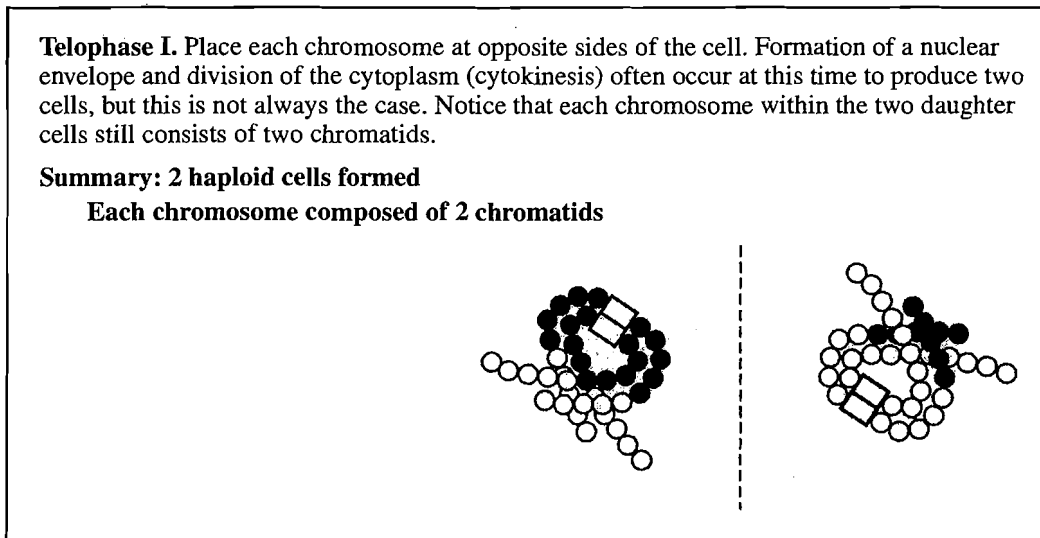


Figure 3.8



Interphase II (Interkinesis). The amount of time spent “at rest” following telophase I depends on the type of organism, the formation of new nuclear envelopes, and the degree of chromosomal uncoiling. Because interphase II does not necessarily resemble interphase I, it is often given a different name — **interkinesis**. **DNA replication does not occur during interkinesis. This represents a third difference between mitosis and meiosis.**

Meiosis II

A second meiotic division is necessary to separate the chromatids of the chromosomes in the two daughter cells formed by this first division. This will reduce the amount of DNA to one strand per chromosome. This second division is called meiosis II. It resembles mitosis except that only one homolog from each homologous pair of chromosomes is present in each daughter cell undergoing meiosis II.

The following simulation procedures apply to haploid nuclei produced by meiosis I.

Figure 3.9

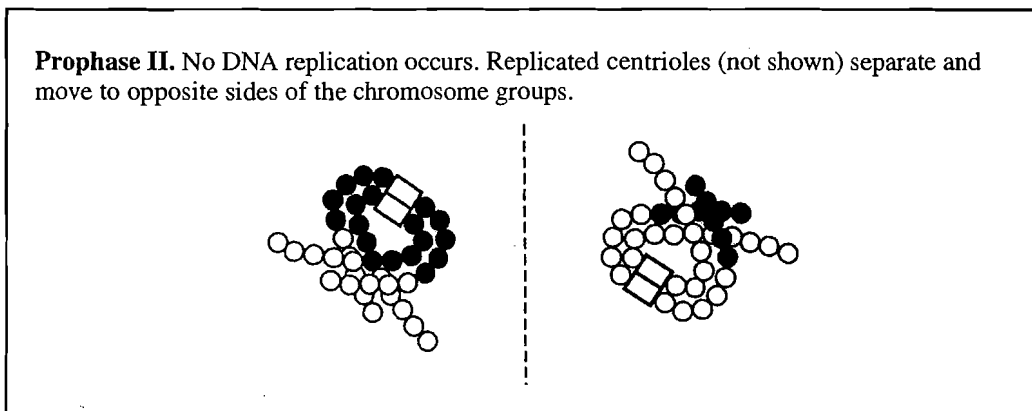


Figure 3.10

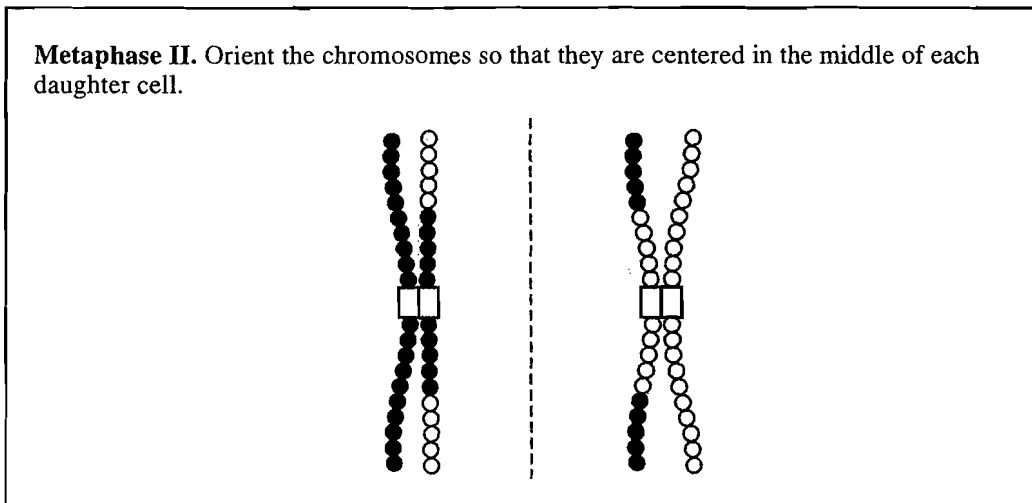


Figure 3.11

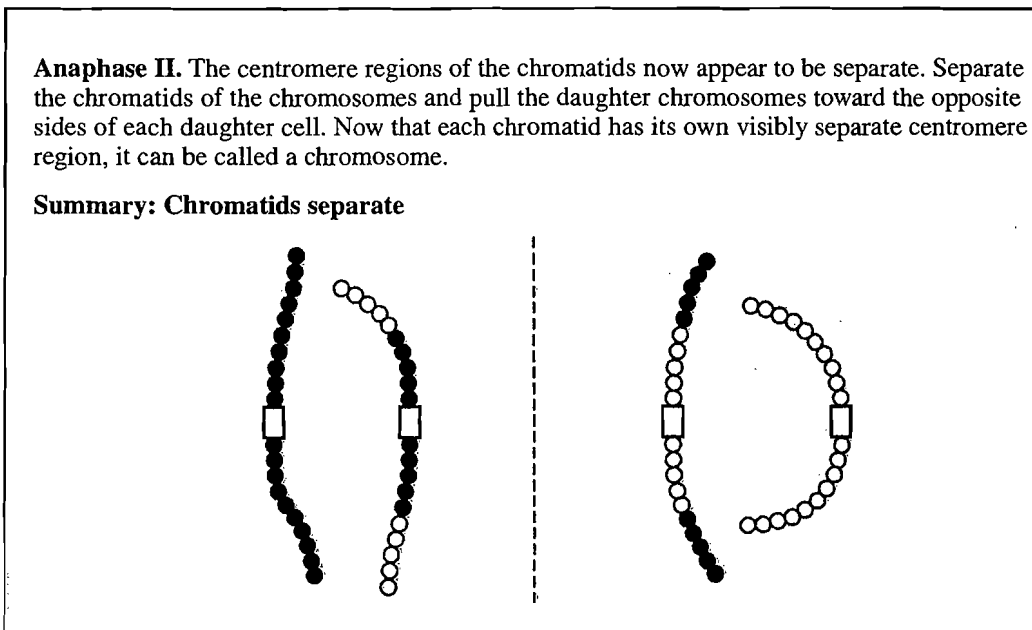
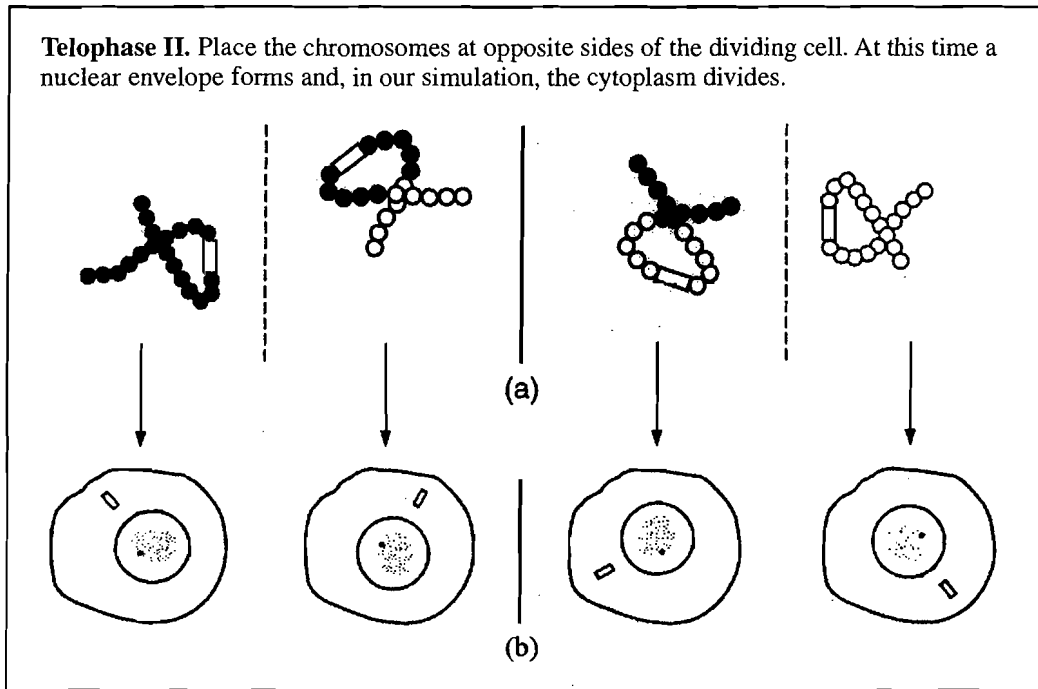


Figure 3.12



Analysis and Investigation

1. List three major differences between the events of mitosis and meiosis.

2. Compare mitosis and meiosis with respect to each of the following in Table 3.2:

Table 3.2

	Mitosis	Meiosis
Chromosome Number of Parent Cells		
Number of DNA Replications		
Number of Divisions		
Number of Daughter Cells Produced		
Chromosome Number of Daughter Cells		
Purpose/Function		



3. How are meiosis I and meiosis II different?

4. How do oogenesis and spermatogenesis differ?

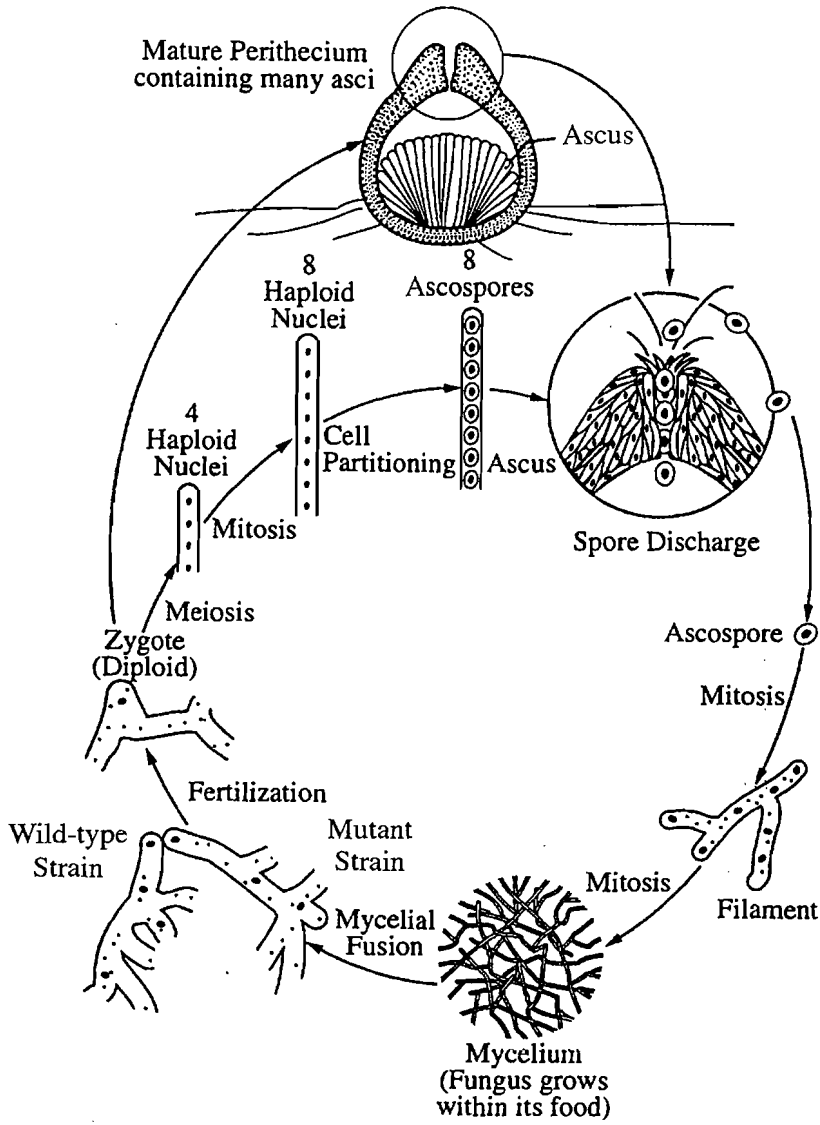
5. Why is meiosis important for sexual reproduction?

EXERCISE 3B.2: Crossing Over during Meiosis in *Sordaria*

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 (filamentlike 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.

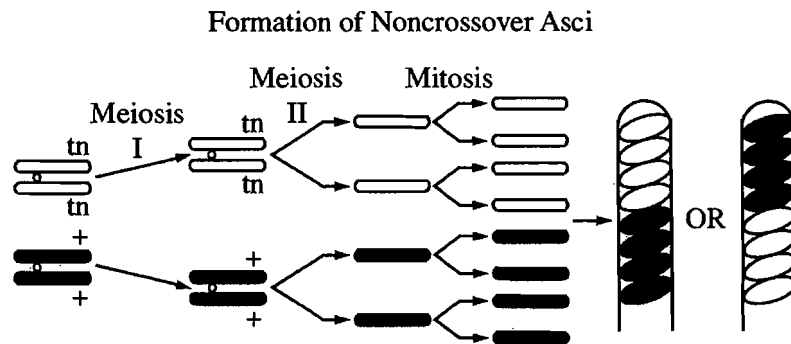
Meiosis, followed by one mitotic division, in *Sordaria* results in the formation of eight **haploid ascospores** contained within a sac called an **ascus** (plural, **asci**). Many asci are contained within a fruiting body called a **perithecium** (ascocarp). When ascospores are mature the ascus ruptures, releasing the ascospores. Each ascospore can develop into a new haploid fungus. The life cycle of *Sordaria fimicola* is shown in Figure 3.13.

Figure 3.13: The Life Cycle of *Sordaria fimicola*



To observe crossing over in *Sordaria*, one must make hybrids between wild type and mutant strains of *Sordaria*. Wild type *Sordaria* have black ascospores (+). One mutant strain has tan spores (tn). When mycelia of these two different strains come together and undergo meiosis, the asci that develop will contain four black ascospores and four tan ascospores. The arrangement of the spores directly reflects whether or not crossing over has occurred. In Figure 3.14 no crossing over has occurred. Figure 3.15 shows the results of crossing over between the centromere of the chromosome and the gene for ascospore color.

Figure 3.14: Meiosis with No Crossing Over



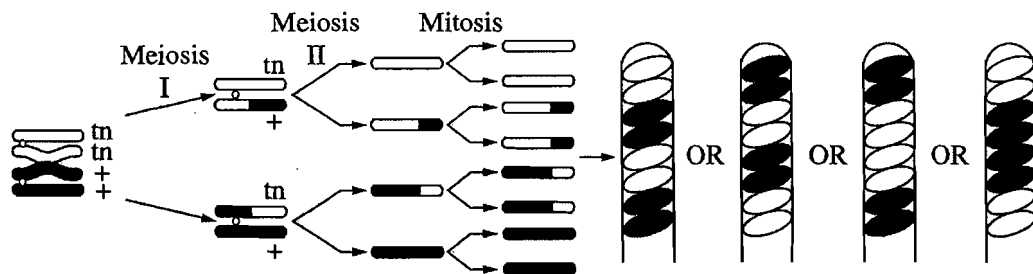
Two homologous chromosomes line up at metaphase I of meiosis. The two chromatids of one chromosome each carry the gene for tan spore color (tn) and the two chromatids of the other chromosome carry the gene for wild type spore color (+).

The first meiotic division (MI) results in two cells, each containing just one type of spore color gene (either tan or wild type). Therefore, segregation of these genes has occurred at the first meiotic division (MI). Each cell is haploid at the end of meiosis I.

The second meiotic division (MII) results in four haploid cells, each with the haploid number of chromosomes (1N).

A **mitotic** division simply duplicates these cells, resulting in 8 spores. They are arranged in the 4:4 pattern.

Figure 3.15: Meiosis with Crossing Over



In this 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 the MI results in two cells, each containing both genes (1 tan, 1 wild type); therefore, the genes for spore color have not yet segregated, although the cells are haploid.

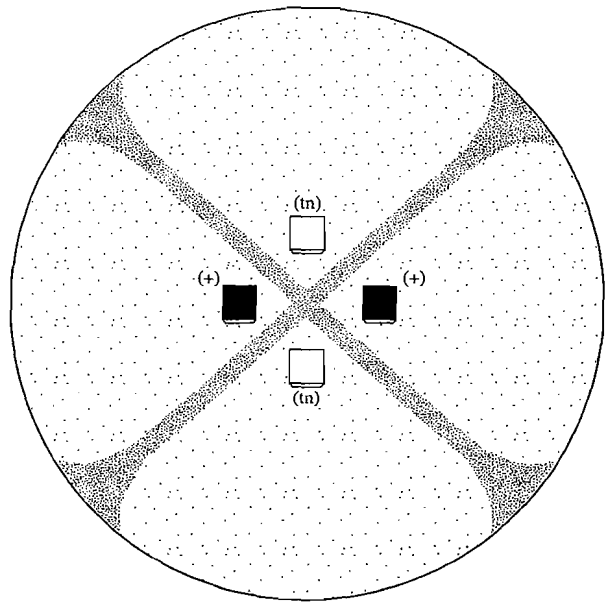
Meiosis II (MII) results in segregation of the two types of genes for spore color.

A **mitotic** division 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.

Procedure

- Two strains of *Sordaria* (wild type and tan mutant) have been inoculated on a plate of agar. Where the mycelia of the two strains meet (Figure 3.16), fruiting bodies called perithecia develop. Meiosis occurs within the perithecia during the formation of asci. Use a toothpick to gently scrape the surface of the agar to collect perithecia (the black dots in the figure below).

Figure 3.16



- Place the perithecia in a drop of water or glycerin on a slide. Cover with a cover slip and return to your workbench. Using the eraser end of a pencil, press the cover slip down gently so that the perithecia rupture but the ascospores remain in the asci. Using the 10X objective, view the slide and locate a group of hybrid asci (those containing both tan and black ascospores). Count at least 50 hybrid asci and enter your data in Table 3.3.

Table 3.3

Number of 4:4 ●●●●○○○○ ○○○○●●●●	Number of Asci Showing Crossover ○○●●○○●● ●●○○●●○○ ○○●●●●○○ ●●○○○○●●	Total Asci	% Asci Showing Crossover Divided by 2	Gene to Centromere Distance (map units)

The frequency of crossing over appears to be governed largely by the distance between genes, or in this case, between the gene for spore coat color and the centromere. The probability of a crossover occurring between two particular genes on the same chromosome (linked genes) increases as the distance between those genes becomes larger. The frequency of crossover, therefore, appears to be directly proportional to the distance between genes.

A **map unit** is an arbitrary unit of measure used to describe relative distances between linked genes. The number of map units between two genes or between a gene and the centromere is equal to the percentage of recombinants. Customary units cannot be used because we cannot directly visualize genes with the light microscope. However, due to the relationship between distance and crossover frequency, we may use the map unit.



Analysis of Results

1. Using your data in Table 3.3, determine the distance between the gene for spore color and the centromere. Calculate the percentage of crossovers by dividing the number of crossover asci (2:2:2:2 or 2:4:2) by the total number of asci $\times 100$. To calculate the map distance, divide the percentage of crossover asci by 2. The percentage of crossover asci is divided by 2 because only half of the spores in each ascus are the result of a crossover event (Figure 3.15). Record your results in Table 3.3.
2. Draw a pair of chromosomes in MI and MII and show how you would get a 2:4:2 arrangement of ascospores by crossing over. (Hint: refer to Figure 3.15).

