

ADVANCED PLACEMENT BIOLOGY
Laboratory 3

Mitosis and Meiosis

74-6450

74-6451



TEACHER'S MANUAL

CAROLINA
World-Class Support for Science & Math

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LABORATORY 3. MITOSIS AND MEIOSIS

Objectives

In this laboratory, students will

- use prepared slides of onion root tips to study mitosis in plant cells
- calculate the relative duration of the phases of mitosis in the meristem of root tissue
- use prepared slides of a fish blastula to study mitosis in animal cells and to compare animal mitosis with plant mitosis
- simulate crossing over and recombination during meiosis using chromosome models
- observe physical evidence of crossing over by studying the arrangements of ascospores in the asci from a cross between wild type and a mutant for tan spore coat color in the fungus *Sordaria fimicola*
- collect data and estimate the percentage of crossing over that occurs between the centromere and the gene locus for tan spore color in the fungus *Sordaria fimicola*

Required Knowledge

Before beginning this laboratory, students should understand

- the function and use of compound light microscopes
- the basics of chromosome structure
- that chromosomes carry the genetic code in the form of DNA
- the life cycles of plants and animals

Expectations

At the completion of this laboratory, students should be able to

- describe the major stages and events of mitosis and meiosis
- compare and contrast mitosis and meiosis
- understand the importance of mitosis and meiosis to the growth, development, and reproduction of plants and animals
- calculate map units from crossover data
- relate the behavior of chromosomes in meiosis to the segregation and independent assortment of genes in Mendelian genetics (**AP® Biology Lab 7**)

Time Requirements

This laboratory requires approximately three 45-minute class periods, but mitosis and meiosis do not have to be taught sequentially. You might wish to conduct this laboratory as two separate exercises. Following is an overview of the activities:

Exercise	Description	Approximate Time Requirement
3A.1	Observe mitosis in onion root tip	45 min total for 3A.1 and 3A.2
3A.2	Observe mitosis in fish blastula	
3B.1	Perform meiosis chromosome crossover simulation	45 min
3B.2	Observe and study <i>Sordaria</i> genetics	45 min

Note that the times given above encompass lab activity time only. They do not include time for data analysis, which may be completed as homework.

Preparation

Photocopy the Student Guide from this manual for your class.

Exercise 3B.1

We recommend the **Chromosome Simulation BioKit®** (RN-17-1100) for this activity.

Ordering the *Sordaria* cross plate

Return the card for shipment of the *Sordaria* cross plate. If possible, allow 2 weeks notice for shipment. Your plate will be shipped on Friday and should arrive the following Monday or Tuesday. Remove the tape and keep the plate at room temperature. Do not put it in a warm incubator.

The cross will have been set up so that the asci are ready for analysis Wednesday–Friday of the week the plate arrives (use dates will be on the plate). However, if the weather has been very cold, the asci may not be mature until Friday. If this happens and you cannot use the plate on Friday, refrigerate it over the weekend and use it on Monday.

Check the culture each day to see if it is ready for classroom use. To determine whether or not the asci are mature, place a few perithecia (the black spheres) on a microscope slide, add a drop of water, and press down on them gently with a coverslip. The perithecia should break open with gentle pressure and you should see asci with black and brownish (tan) spores. If it takes a lot of pressure to break the perithecia, they are not mature. If the spores are a greenish color, they are not mature. If you do not see asci with spores but instead see only dark, sac-like perithecia, the asci are not mature. Incubate them at room temperature, repeating this process until the asci mature.

The best place on the plates to find heterozygous asci is along the X where the two strains come together, near the outside of the plate.

Ordering onion bulbs

Return the card for shipment of onion bulbs if you plan to perform the Optional Activity, *Onion Mitosis, Squash Method*.

The CD

The CD includes images of onion and fish mitosis. You can project these images for simultaneous classroom viewing or set up a computer monitor to display the images for students who are having difficulty recognizing the phases of mitosis. The images on the

Student Materials and Equipment

CD may also be used to visually quiz students' ability to recognize the stages of mitosis. Following is a list of the materials needed for one group of students to perform the exercises in this lab. Prepare as many setups as needed for your class. Materials needed for the Optional Activities, *Onion Mitosis*, *Squash Method* and *Performing Sordaria*

Setup for Each Group

	Exercise 3A.2	Exercise 3B.1	Exercise 3B.2
prepared slide of longitudinal section of onion root	1		
prepared slide of fish blastula	1		
*compound light microscopes	2-4		2-4
*paper towels	as needed		
mature <i>Sordaria</i> cross plate from a wild type × tan mutant cross			1
slides and cover slips			4
transfer pipet and water in a plastic cup			1
*chromosome simulation kit [Chromosome Simulation BioKit® (RN-17-1100)]		1	
*scalpel (for handling perithecia)			1

Crosses, are listed with their respective protocols.

*Items not included in kit.

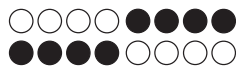
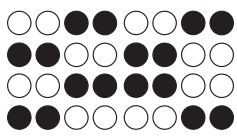
Answers to Questions in the Student Guide**Exercise 3B.2: Crossing Over During Meiosis in *Sordaria***

Students should understand that the map units they calculate are not defined units of length (as are inches or meters). They are relative measures, as "C is farther from A than is B." With the advent of molecular biology techniques, it is possible to determine the actual physical distance between genes in terms of base pairs. This is a different sort of determination than is measuring crossover frequency between two genes. When a chromosome is studied by physical methods and genes are located by restriction sites and numbers of base pairs, the resulting map is called a physical map. When the order of genes and their relative distances apart are studied by performing genetic crosses and analyzing frequency of crossing over, the resulting map is called a genetic map. Genetic maps have been utilized longer than physical maps, because geneticists could perform crosses and analyze the results before physical mapping methods were discovered. In the few cases in which detailed physical maps have become available for comparison with detailed genetic maps of the same organism, the correspondence has been good. The physical length of a map unit has been found to vary between organisms, because overall rates of crossing over vary between organisms.

A frequently asked question is “Why is the percent of crossing over divided by 2 to get map units for *Sordaria*? This is not done when determining map units for *Drosophila*.” In *Sordaria*, one crossover event produces one crossover ascus; that is, all products of the crossover are counted as 1. In *Drosophila*, as in most organisms that have been used to generate genetic maps from crossover frequencies, meiosis results in the production of four gametes from one parent cell. If these gametes are fertilized, they will give rise to two offspring that show in their phenotypes the results of crossover and two offspring that do not. That is, half of the offspring show the results of the crossover event. Therefore, it is necessary to halve (divide by 2) the percent of crossover asci of *Sordaria* to make the results comparable to those determined for *Drosophila*, corn, and most other organisms.

Exercise 3B.2: Crossing Over During Meiosis in *Sordaria*

Table 3.3

Number of 4:4 Asci	Number of Asci Showing Crossover	Total Asci	% Asci Showing Crossover	Gene to Centromere Distance (Map Units)
				

$\% \text{ Asci Showing Crossover} = \text{Number Crossover Asci} / \text{Total Asci} \times 100\%$

$\text{Gene to Centromere Distance} = \% \text{ Asci Showing Crossover} / 2$

Optional Activities

If you wish to have students perform their own *Sordaria* crosses, see *Performing Sordaria Crosses*. If you wish to have students prepare their own slides to view mitosis in onion cells, see *Onion Mitosis, Squash Method*. A blackline master of the Student Instructions for the *Onion Mitosis, Squash Method* activity can be found on page 10.

Performing *Sordaria* Crosses

All needed materials, including parent *Sordaria* cultures, media, and instructions, are in the ***Sordaria Genetics BioKit***[®] (Carolina catalog #RN-15-4847). Alternatively, you can order slant or plate cultures of *Sordaria* wild-type and mutant tan cultures and prepare or buy media. Slant cultures should first be inoculated separately onto plates of culture media, as described in the procedure for Day 1, below. If you order plate cultures, you can take *Sordaria* directly from the plate cultures to crossing agar, starting at Day 5 of the procedure below.

Sordaria culture and crossing media can be ordered (catalog numbers for bottles of prepared media are in parentheses below) or prepared from the following ingredients:

Culture Media (catalog #RN-15-6352)		Crossing Media (catalog #RN-15-6354)	
corn meal agar	17 g	corn meal agar	17 g
glucose	2 g	sucrose	10 g
yeast extract	1 g	glucose	7 g
H ₂ O	1 L	KH ₂ PO ₄	0.1 g
		yeast extract	1 g
		H ₂ O	1 L

Sordaria can be cultured according to the following instructions.

Day 1: Start plate cultures

Aseptically inoculate culture media plates with wild-type and mutant *Sordaria*. You will need about five plates of each variety (wild type and tan mutant). Grow at room temperature for five days.

Day 5: Start cross

When the *Sordaria* cultures have covered the plate, transfer them to crossing media. Make the transfer aseptically, using a scalpel or knife. Cut a plate of wild type and a plate of mutant tan *Sordaria* culture into $\frac{1}{4}$ -inch squares. Before each cut, dip the scalpel in 95% ethanol and flame. Use the scalpel to place squares of the two strains, alternately, $\frac{1}{2}$ inch from the center of the crossing agar plate and $\frac{1}{2}$ inch apart (see Figure 3.21 in the Student Guide). Invert the squares when placing them on the cross plates so that the mycelia are in contact with the agar surface. Maintain aseptic technique throughout the procedure. Incubate the plates at room temperature, away from direct light or sunlight. The asci should be ready 10 to 12 days after the cross was started.

Onion Mitosis, Squash Method**Preparation**

Photocopy the Student Instructions on page 10 for your class.

Rooting the onions

Twenty-four hours before class, peel six small pearl onions, removing the dry, brown membrane and the outer layer of white onion tissue. Cut off any green shoots. Wrap the onions in wet paper towels and place in an open plastic bag. Store them in a warm, dark place and keep the paper towels moist. Use root tips that are about 1 mm long.

Note: The smaller the onion tip, the more mitotic cells your students will find.

Student Materials and Equipment

- 4 pearl onion bulbs (see "Ordering onion bulbs" on pg. 4)
- 1 0.5% toluidine blue*
- 1 1 M HCl*
- 2 plastic transfer pipets
- 8 slides and coverslips
- 1 clothespin (for holding slides)
- 1 razor blade
- Bunsen or alcohol burner**

*Distribute the 1 M HCl and 0.5% toluidine blue in labeled 60-mL cups, one per group. Cups, labels, and solutions are provided.

**Item not included in kit.

LABORATORY 3. OPTIONAL ACTIVITY: ONION MITOSIS, SQUASH METHOD

Preparing an Onion Root Tip Slide

In this exercise, you will prepare your own stained slides of onion root tips and observe mitotic figures. Your teacher has rooted onion bulbs in water. Growth of new roots is due to the production and elongation of new cells. Mitotic divisions are usually confined to the cells near the tip of the root. Follow the procedure outlined below to make your own root tip preparation.

Procedure

1. Obtain an onion bulb that is just beginning to show the emergence of roots. Cut off a root and lay it on a microscope slide. Cut off the first 1 or 2 mm of the root tip and discard the rest of the root. A dot-sized piece of root tip is all you need. The mitotic cells are in the tip, so extra root tissue will only interfere with finding mitotic cells.
2. Cover the root tip with two or three drops of 1 M HCl. Using a clothespin to hold the slide, warm the slide by passing it back and forth over the flame of a Bunsen burner (or alcohol burner) for five seconds. You may smell a faint aroma of cooking onion. If the onion turns brown or the liquid boils away, start over. Remember to wear safety glasses and gloves.
3. Use the edge of a paper towel to blot around the root to remove excess HCl and cover the root tip with 0.5% aqueous toluidine blue (use caution when handling HCl and toluidine blue). Again, pass the slide above the Bunsen burner flame two times, without boiling the liquid. Let the slide stand for one minute.
4. Carefully blot around the root to remove excess stain. Add one drop of fresh toluidine blue stain to the slide and apply a coverslip. Place the slide, coverslip-side-up, between two layers of paper towel on a laboratory bench. Using a pencil eraser or your finger, firmly but carefully apply pressure to the coverslip in order to squash and spread the root tip tissue. **Caution:** Do not break the coverslip.
5. Using a compound light microscope (10×), locate the meristematic region of the root tip. Examine the slide at 40× magnification and identify chromosomes at the various stages of mitosis.
6. Locate cells in prophase, metaphase, anaphase, telophase, and interphase. Look for evidence of cytokinesis. If the slide is not satisfactory, repeat the procedure. Make sketches of these stages of mitosis.

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- compare and contrast mitosis and meiosis
- understand the importance of mitosis and meiosis to the growth, development, and reproduction of plants and animals
- calculate map units from crossover data
- relate the behavior of chromosomes in meiosis to the segregation and independent assortment of genes in Mendelian genetics (**AP® Biology Lab 7**)

Background

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) to form daughter cells.

There are two types of nuclear division: mitosis and meiosis. Mitosis creates two daughter cells that are genetically identical to one another and to the parent cell. Meiosis, on the other hand, results in the formation of either gametes (in animals) or spores (in plants). These cells have half the chromosome number of the parent cell and are not genetically identical to one another or to the parent cell.

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 is accomplished through mitotic cell division. Where can you find cells in the process of 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

almost anywhere as new cells are formed or as new cells replace old ones. Some tissues in both plants and animals, however, rarely divide once the organism is mature.

To study the stages of mitosis, look for tissues where there are many cells in the process of mitosis. Restrict 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 fish blastula).

Exercise 3A: Mitosis

Exercise 3A.1: Prepared Slides of the Onion Root Tip and Fish Blastula

Onion Root Tip

Roots consist of different regions. The **root cap** functions in protection. The **apical meristem** is the region that contains the highest percentage of cells undergoing mitosis. The region of **elongation** is the area in which growth in length occurs. The region of **maturation** is where root hairs develop and where cells differentiate (Figure 3.1).

Figure 3.1: Medial Longitudinal Section of a Root Tip

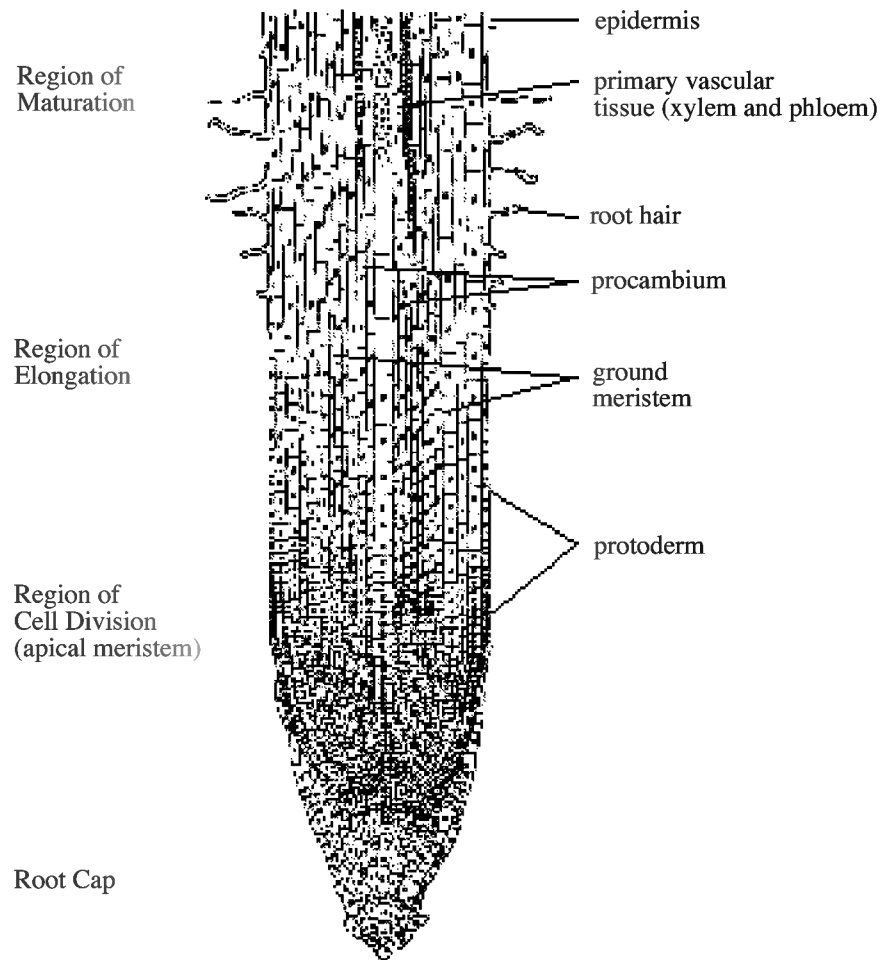


Figure 3.2:
Interphase



Figure 3.3:
Prophase

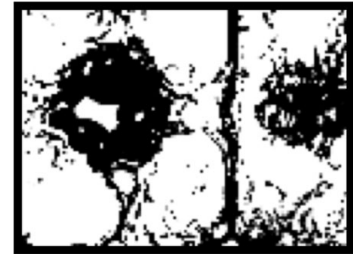


Figure 3.4:
Metaphase

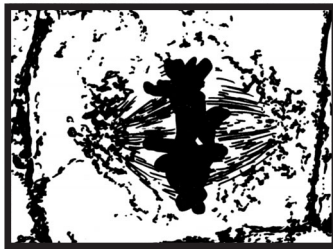


Figure 3.5:
Anaphase



Figure 3.6:
Telophase



Figure 3.7:
Cytokinesis in
Plants

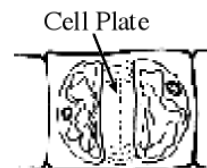
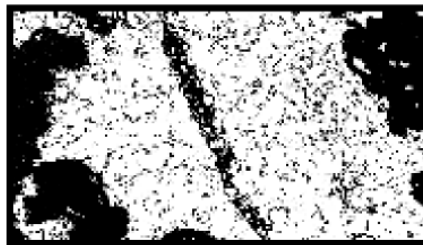
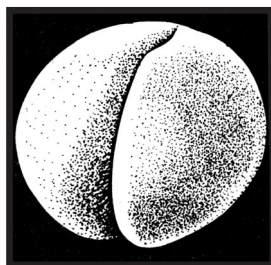


Figure 3.8:
Cytokinesis in
Animals



Fish Blastula

The fish blastula (a stage in embryonic development) is excellent 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 fish blastulae that 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

Mitosis in Plant Cells

Examine prepared slides of longitudinal sections of onion root tips using the 10× objective. Locate the meristematic region. Using the 40× objective, examine individual cells in this region to study the phases of plant mitosis. Find the stages described below. You may not find every stage of mitosis on each individual slide. However, all the stages are present in your class's slide set. You may need to share slides to find them all. Make a drawing of each stage using the space provided to the right of its description. Indicate that it is a plant cell.

Figure 3.2: Interphase



Figure 3.3: Prophase



Figure 3.4: Metaphase

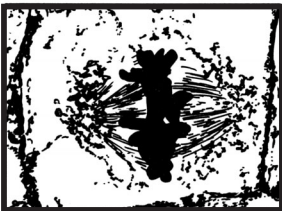


Figure 3.5: Anaphase



Mitosis in Animal Cells

Find the stages of mitosis in the slide of the fish blastula. First, locate the blastula using low power (10×), then observe it under high power (40×). The events of each stage are described below. Make a drawing of each stage using the space provided to the right of its description. Indicate that it is an animal cell.

1. The non-dividing cell is in a stage called **interphase** (Figure 3.2). The nucleus may have one or more dark-stained nucleoli. It is filled with a fine network of threads, the chromatin. Interphase is essential to cell division because the genetic material (DNA) is replicated during this stage.
2. The first sign of a division is **prophase** (Figure 3.3), in which a thickening of the chromatin threads occurs. Thickening continues until it is evident that the chromatin has condensed into **chromosomes**. With somewhat higher magnification, you may be able to see that each chromosome is composed of two **chromatids**. As prophase continues, the chromatids continue to shorten and thicken. In late prophase, the nuclear envelope and nucleoli are no longer visible. The chromosomes are free in the cytoplasm. Just before this occurs, the first sign of a spindle appears in the cytoplasm. The spindle apparatus is made up of **microtubules**. It is thought that these microtubules pull the chromosomes toward the poles of the cell where the two daughter nuclei will eventually form. (Centrioles appear to be basal bodies that give rise to flagella and cilia in animals and lower plants such as mosses and ferns. Centrioles are not found in nonflagellated "higher" plants such as angiosperms.)
3. At **metaphase** (Figure 3.4), 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 an imaginary plane called the metaphase plate. At metaphase, you should be able to observe the two chromatids of some of the chromosomes.
4. At the beginning of **anaphase** (Figure 3.5), 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. The daughter chromosomes continue poleward movement until they form two compact clusters, one at each spindle pole.

Figure 3.6: Telophase



5. **Telophase** (Figure 3.6), the last stage of division, begins when the chromosomes stop moving and are in two tightly compacted clumps at opposite poles. This is followed by the formation of a new nuclear envelope around each group of chromosomes. The chromosomes gradually uncoil to form the fine chromatin network of interphase, and the nucleoli and nuclear envelope reappear.

The division of the cytoplasm, in contrast to nuclear division (mitosis), is called **cytokinesis**. In plants, a new cell wall is laid down between the daughter cells (Figure 3.7). In animal cells, the old cell will pinch off in the middle to form two new daughter cells (Figure 3.8).

Figure 3.7: Cytokinesis in Plants

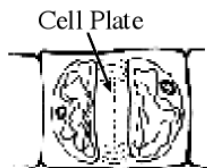
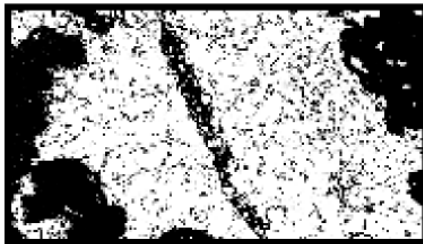
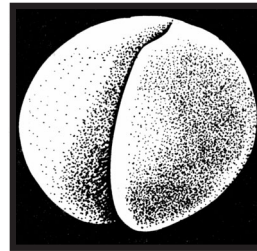


Figure 3.8: Cytokinesis in Animals



Questions

1. The importance of mitosis is that it produces two daughter cells that are each genetically identical to the original cell. Explain how mitosis accomplishes this. Include the relevant events that occur in interphase.

2. A cell has 32 chromosomes. It divides by mitosis to produce two daughter cells. How many chromosomes does each daughter cell have?

Exercise 3A.2: Relative Lengths of Mitotic Stages in the Onion Root Tip

To estimate the relative length of time that a cell spends in the various stages of mitosis, you will reexamine the meristematic region of a prepared slide of an onion root tip.

Procedure

- Using the low-power objective (10×), locate the meristematic region. Shift to the high power objective (40×), and count the number of cells that are in each stage of mitosis (prophase, metaphase, anaphase, and telophase).
- Repeat this count in at least two more non-overlapping fields of view. Record your data in Table 3.1 (on next page).

Table 3.1

	Number of Cells			Total Cells in Phase	Fraction of Total Count*	Estimated Time in Stage
	Field 1	Field 2	Field 3			
Interphase						
Prophase						
Metaphase						
Anaphase						
Telophase						
	Total Count					

*Total Cells in Phase/Total Count

- Calculate the decimal fraction of the total counted for each phase and record your results in Table 3.1 under "Fraction of Total Count."
- Given that it takes on average 24 hours for onion root tips to complete the cell cycle, calculate the average time spent in each phase (as follows) and record your results in Table 3.1.

Fraction of cells in phase × 24 hours = Time spent in the phase in hours

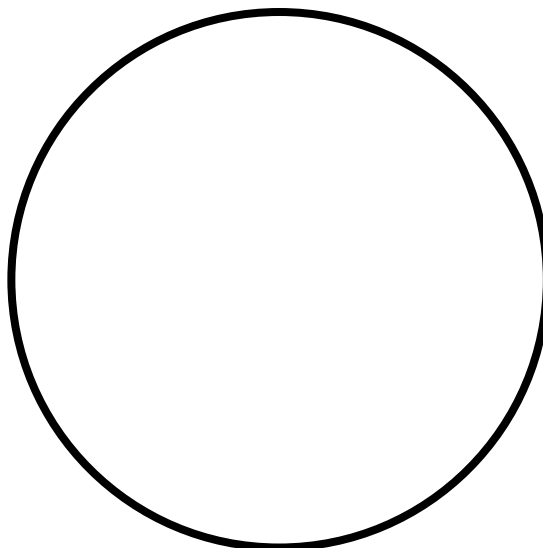
(convert to hours + minutes; e.g. 2.3 hours = 2 hours, 18 minutes)

Questions

- Using the data from Table 3.1, construct a pie graph of the onion root tip cell cycle showing the percent of time spent in each stage. Provide a title and key for your graph.

Pie Graph 3.1

Title: _____



2. Based on your data and pie graph, what do you infer about the relative length of time an onion root tip spends in each phase of mitosis?

3. In Table 3.1, if your counts had included parts of the onion root tip in which cells were not actively dividing, how might this have changed your results?

Exercise 3B: Meiosis

Background

In sexual reproduction, cells (gametes) from two parents fuse, doubling the chromosome number. Meiosis halves the chromosome number; thus, chromosome number remains the same through the generations. Genes, the units of hereditary, are located on chromosomes; thus, all the genes on a chromosome are inherited together, or “linked.” However, events during meiosis can rearrange or disrupt linkage groups. These events include crossing over, which you will study by observing the arrangements of ascospores in the asci resulting from a cross between a wild-type and a mutant for tan spore color in *Sordaria fimicola*.

Consider a sexually reproducing animal with two chromosomes, A and B. An animal of this species will possess two copies of each chromosome. This is because it receives one chromosome A and one chromosome B from each parent. An organism with two sets of chromosomes ($2n$) is said to be **diploid** in chromosome number or, simply, diploid. Reproductive cells (gametes, egg and sperm in animals; spores in plants) result from meiosis, a type of cell division that reduces chromosome number by half. Meiosis accomplishes this reduction in an orderly manner such that our hypothetical diploid animal with chromosomes AA and BB produces gametes that are AB and not AA or BB. Thus, reproductive cells have one set of chromosomes and are **haploid** (n) in chromosome number. (Rare events called chromosomal nondisjunctions can alter this pattern, producing, using our example, gametes with AAB, A, B, or ABB chromosomes.) When haploid gametes unite during fertilization, the resulting zygote is diploid, having received one chromosome of each pair from each parent.

Meiosis involves two successive nuclear divisions that produce four haploid cells. The first division (meiosis I) is the reduction division. DNA replication occurs before meiosis I, just as in mitosis, so the chromosomes enter meiosis I as identical chromatids held together at the centromeres. During meiosis I, the centromeres do not split. Instead, the two members of each chromosome pair are partitioned to the two different daughter cells, so that each daughter cell contains one member of each chromosomal pair. The daughter cells of meiosis I are haploid, even though each chromosome is still

duplicated. (Ploidy is determined by the number of centromeres.) There is no DNA replication between meiosis I and meiosis II. The second division (meiosis II) separates the chromatids.

Mitotic cell division produces new cells genetically identical to the parent cell. In contrast, meiosis increases genetic variation in the population. Each diploid cell undergoing meiosis can produce 2^n (where n is the haploid number) different chromosomal combinations just through random partitioning of members of the chromosome pairs. In humans, the number is 2^{23} , which is more than eight million different combinations.

Meiosis generates even more genetic variation than 2^n chromosomal combinations through a process called **crossing over**. During meiosis I, each homologous pair of duplicated chromosomes comes together in a process known as **synapsis**. The chromatids of homologous chromosomes may then exchange parts. The one-for-one exchange of chromosome segments is called crossing over. The farther apart two genes are on a chromosome, the more likely that crossing over will occur between them. You can estimate the relative distance between two genes on a given chromosome by calculating the percentage of crossing over that takes place between them.

Exercise 3B: Meiosis

Introduction

Exercise 3B.1: Simulation of Meiosis

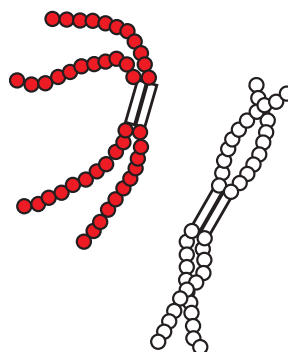
In this exercise, you will study the process of meiosis using chromosome simulation kits. Follow the directions in the kit to assemble two strands of yellow beads connected to magnetic centromeres and two strands of red beads connected to magnetic centromeres. One of the yellow strands represents the chromosome contribution of one parent, and one of the red strands represents the chromosome contribution of the other parent. These two strands represent homologous chromosomes. The second yellow and red strands are to be used as chromatids for each of these chromosomes.

Procedure

Interphase

Refer to Figure 3.9. Place one strand of red beads and one strand of yellow beads 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 the second red strand in contact with the centromere region of the first red strand. Do the same with its homolog, the yellow strand. **Significant events: DNA replication.**

Figure 3.9:
Interphase

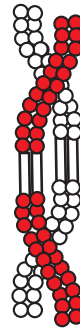


Meiosis I

Prophase I

Refer to Figure 3.10. Homologous chromosomes come together and synapse along their entire length. **The pairing (synapsis) of homologous chromosomes represents the first big difference between mitosis and meiosis.** A **tetrad**, consisting of four chromatids, is formed. Entwine the two chromosomes to simulate synapsis and then simulate the process of crossing over by popping the beads apart on a red chromatid, at the fifth bead or “gene,” and doing the same with a yellow chromatid. Reconnect the red chromatid to the five yellow beads and reconnect the yellow chromatid to the five red beads. Proceed through prophase I of meiosis and note how crossing over results in recombination of genetic information. **Significant events: Synapsis and crossing over.**

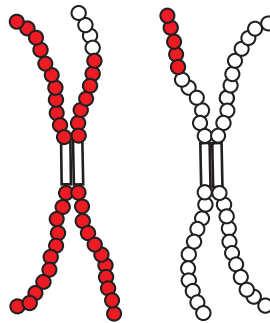
Figure 3.10:
Prophase



Metaphase I

Refer to Figure 3.11. The tetrads line up in the center of the cell. Position the chromosomes near the middle of the cell. **Significant event: tetrads align on equator.**

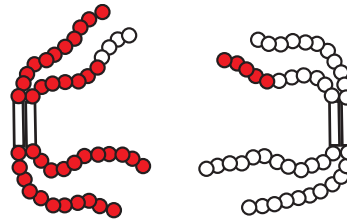
Figure 3.11:
Metaphase



Anaphase I

Refer to Figure 3.12. During anaphase, the homologous chromosomes separate and are pulled to opposite sides of the cell. **This represents a second significant difference between the events of mitosis and meiosis.** Note that each chromosome is still composed of two chromatids joined at the centromere. **Significant events: homologs separate, reducing chromosome number is reduced from diploid to haploid.**

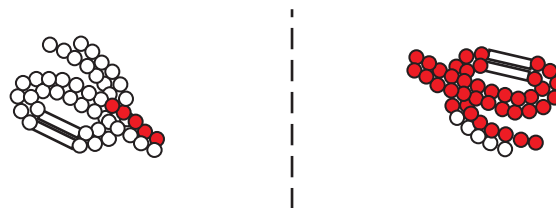
Figure 3.12:
Anaphase



Telophase I

Refer to Figure 3.13. Place each chromosome at opposite sides of the cell. Centriole duplication takes place at the end of telophase in preparation for the next division. Formation of a nuclear envelope and division of the cytoplasm (cytokinesis) may or may not occur at this time to produce two cells. Notice that each chromosome still consists of two chromatids. **Significant events: two haploid groups of chromosomes (or cells) are formed. Chromosomes still composed of 2 chromatids.**

Figure 3.13:
Telophase



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 double-helical 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.

Meiosis II

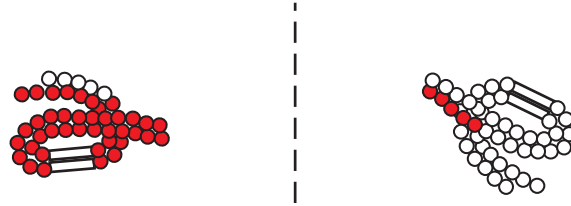
Interphase II (Interkinesis)

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.**

Prophase II

Refer to Figure 3.14. Replicated centrioles separate and move to opposite sides of the chromosome groups.

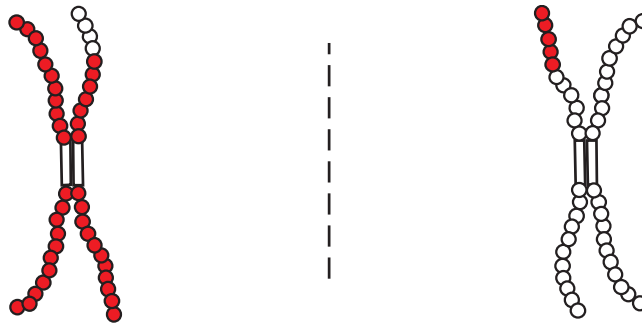
**Figure 3.14:
Prophase II**



Metaphase II

Refer to Figure 3.15. Orient the chromosomes so they are centered in the middle of each daughter cell.

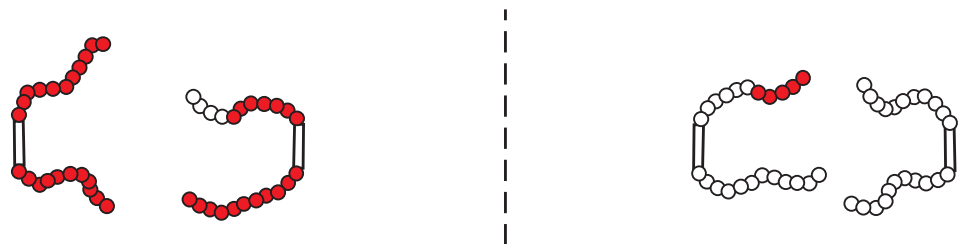
**Figure 3.15:
Metaphase II**



Anaphase II

Refer to Figure 3.16. The centromere regions of the chromatids now appear to be separate. Separate the chromatids of the chromosomes and pull the daughter chromosomes toward the opposite sides of each daughter cell. (Now that each chromatid has its own visibly separate centromere region, it can be called a chromosome.)

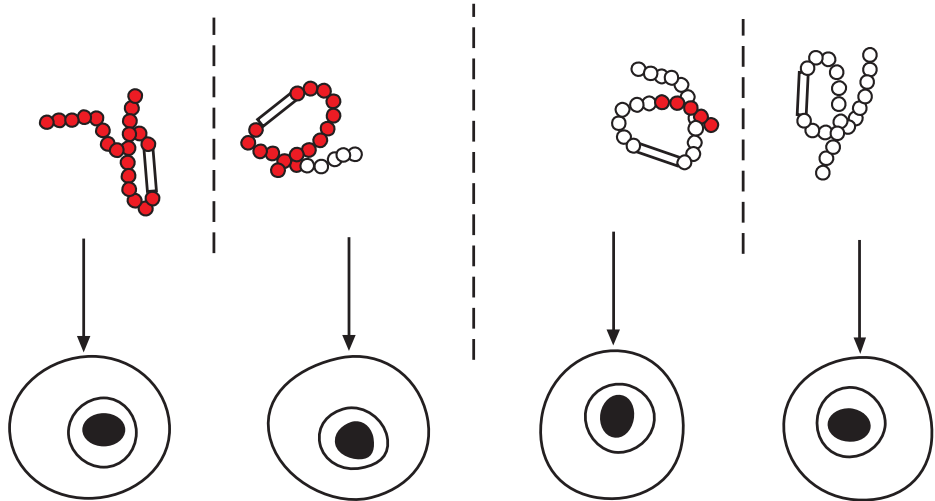
**Figure 3.16:
Anaphase II**



Telophase II

Refer to Figure 3.17. Place the chromosomes at opposite sides of the dividing cell. At this time, a nuclear envelope forms and the cytoplasm divides

**Figure 3.17:
Telophase II**



At the end of Meiosis II, we have 4 daughter cells, each with $\frac{1}{2}$ of the original number of chromosomes. Each chromosome is made up of only one chromatid.

Questions

1. Compare and contrast mitosis and meiosis by completing Table 3.2

Table 3.2

	Mitosis	Meiosis
Chromosome Number of Parent Cell		
Number of DNA Replications		
Number of Divisions		
Number of Daughter Cells Produced		
Chromosome Number of Daughter Cells		
Function of the Division		

2. Using Table 3.2 and reflecting on what you have learned in this lab, list three major ways that mitosis and meiosis differ.

3. How do meiosis I and meiosis II differ?

4. Why is meiosis important for organisms that reproduce sexually?

Exercise 3B.2: Crossing Over During Meiosis in *Sordaria*

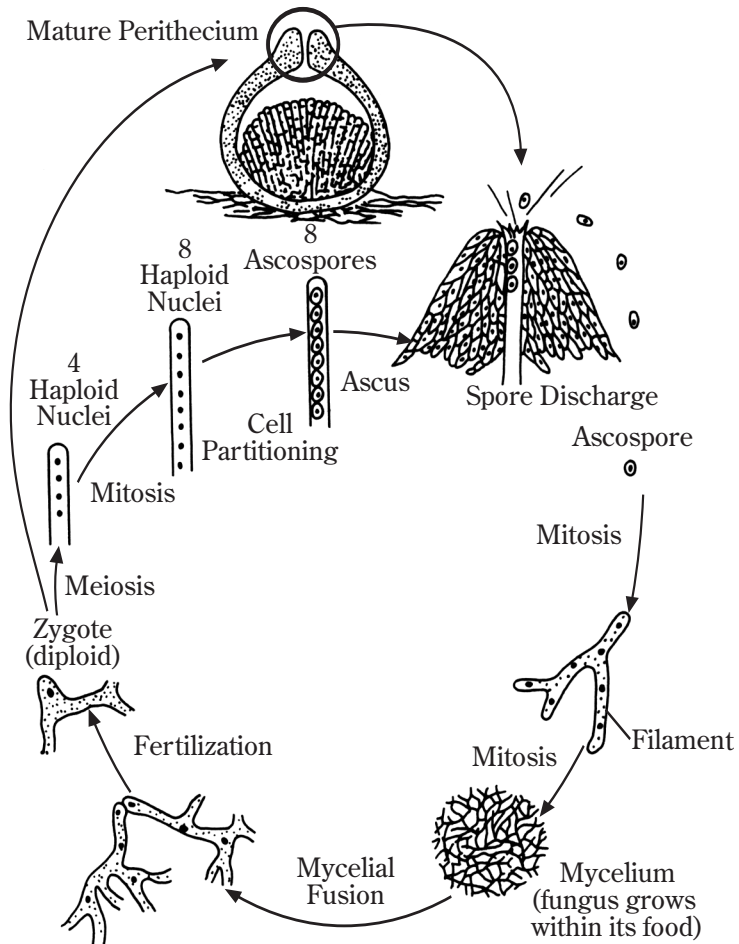
Introduction

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.

We cannot use normal units of length to express distance between genes because we cannot see genes to measure distance. However, we can measure crossover frequency by looking at the phenotypes of offspring from carefully constructed crosses. Since distance and crossover frequency correspond closely, we can compare distances by comparing crossover frequencies. Geneticists invented the arbitrary unit of measure called the **map unit** to talk about distances in this way. Map units are not physical units in that they do not always represent a single, fixed length of DNA. They are relative measures, as “C is farther from A than is B.”

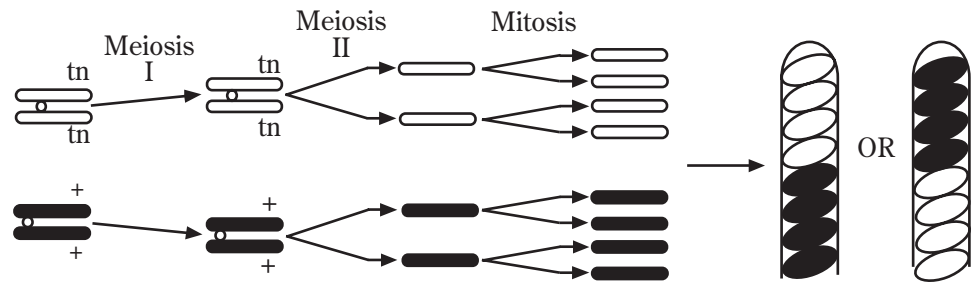
Sordaria fimicola is an ascomycete fungus that can be used to demonstrate the results of crossing over during meiosis. The life cycle of *Sordaria fimicola* is shown in Figure 3.18 (on the next page).

Figure 3.18:
The Life Cycle of
Sordaria fimicola



Wild-type *Sordaria* have black ascospores (+). One mutant strain has tan spores (tn). When the threadlike strands of these two different strains come together on nutrient media, they will fuse. This is followed by the fusion of two haploid nuclei (one from each strain) to form a diploid nucleus. This nucleus undergoes meiosis to form four haploid nuclei. These four nuclei undergo mitosis producing eight haploid nuclei, which develop into spores (ascospores). These divisions take place in a tubular structure, the ascus that is only slightly larger in diameter than the spores, so the spores from a single row inside each ascus. The arrangement of the spores directly reflects whether or not crossing over has occurred. In Figure 3.19, no crossing over has occurred. Figure 3.20 shows the result of crossing over between the centromere of the chromosome and the gene for ascospore color.

Figure 3.19:
Formation of
Noncrossover Asci



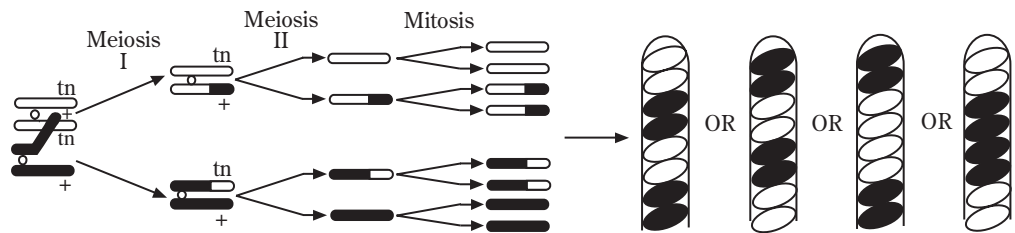
Two homologous chromosomes line up at metaphase I of meiosis. The two chromatids of one chromosome each carry the allele for tan spore color (tn) and the two chromatids of the other chromosome carry the allele for wild-type spore color (+). (An allele is a form of a gene. In this case, the gene is for spore color and there are two forms or alleles of the gene: one for tan spores and the other for dark spores. Dark spore color is called the wild type because it is the most common form of the gene in natural populations of *Sordaria*.)

The first meiotic division (MI) results in two cells, each containing just one type of spore color allele (either tan or wild type). Therefore, segregation of these genes has occurred at the first meiotic division (MI).

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

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

Figure 3.20:
Formation of
Crossover Asci



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 1.

This time, the MI results in two cells, each containing both alleles (1 tan, 1 wild type).

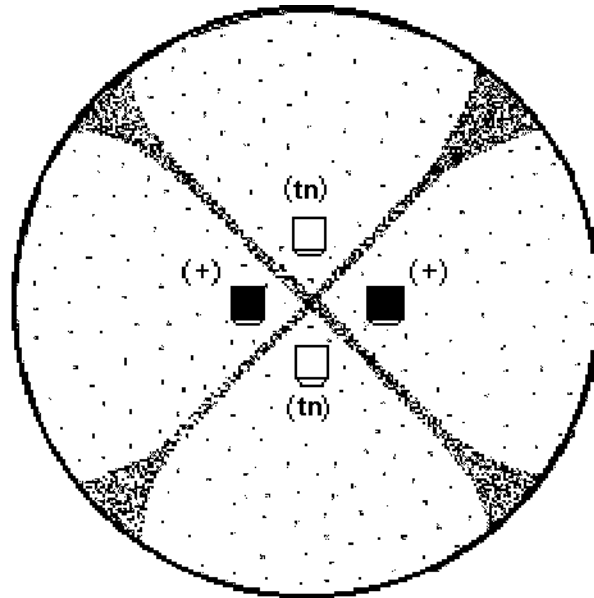
Meiosis II (MII) results in separation (segregation) of the two alleles for spore color.

A mitotic division results in eight 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

For your observation of crossing over, 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 3.21), fruiting bodies called perithecia develop. The perithecia, which look like black dots to the naked eye, contain many asci. Meiosis occurs within the perithecia during the formation of asci. Use a scalpel to gently scrape the surface of the agar to collect perithecia from the region where the brown and black strains meet. The most hybrid asci are usually seen in perithecia from a portion of this X-shaped region that is near the outer rim of the plate.

Figure 3.21:
***Sordaria* Crossover**
Regions



Place the perithecia in a drop of water on a slide. Cover with a coverslip and return to your workbench. Using the eraser end of a pencil, gently press the coverslip down so that the perithecia rupture but the ascospores remain in the asci. You should feel them pop beneath the coverslip. View the slide, using the 10× objective, 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 Asci	Number of Asci Showing Crossover	Total Asci	% Asci Showing Crossover	Gene to Centromere Distance (Map Units)

$\% \text{ Asci Showing Crossover} = \text{Number Crossover Asci} / \text{Total Asci} \times 100\%$

$\text{Gene to Centromere Distance} = \% \text{ Asci Showing Crossover} / 2$

Carolina Biological Supply Company

2700 York Road, Burlington, North Carolina 27215
Phone: 800.334.5551 • Fax: 800.222.7112
Technical Support: 800.227.1150 • www.carolina.com

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