OBJECTIVES

- Examine and compare the phases of mitosis in animal and plants cells
- Determine the relative time cells spend in each phase of mitosis
- Prepare microscope slides of mitotic cells using allium root tips
- Follow the processes of mitosis and meiosis in the life cycle of Sordaria
- Examine the arrangement of Sordaria ascospores microscopically to determine the frequency of crossing over
- Calculate the distance, in map units, between a specific gene and the chromosome centromere

MATERIALS

MATERIALS NEEDED PER GROUP

Whitefish mitosis slide
Onion mitosis slide
Compound microscope
Toluidine blue, 0.5%
Hydrochloric acid, 1 M
Garlic root tip
Microscope slide
Coverslip
Compound microscope
Clothespin
Scalpel
Pipet
Bunsen burner
Paper towel
Microscope slides
Coverslips
Inoculating loop

SHARED MATERIALS

Sordaria demonstration cross plate

DID YOU KNOW?

Garlic, also referred to as "the stinking rose", has been used since the days of the Egyptians for medicinal purposes. Modern scientific research confirms the ability of garlic to lower cholesterol and blood pressure, stimulate the immune system, inhibit the growth of intestinal parasites, and protect against a variety of toxins.

PROCEDURE

Part I: Mitosis

A. Observing Mitosis in Plant and Animal Cells

1. Observe the prepared microscope slide of onion root tip mitosis, first at 100X, then 400X. Using the provided Plant Cell Mitosis illustrations as a guide, identify cells that represent each mitotic phase.

2. In the Analysis section, draw each phase of plant cell mitosis that you see. Write a brief description of each phase below each drawing.

3. Observe the prepared microscope slide of whitefish blastula. Using the provided Animal Cell Mitosis illustrations as a guide, identify each phase of animal cell mitosis.

4. In the Analysis section, draw each phase of plant or animal cell mitosis that you see. Write a brief description of each phase below each drawing.

B. Relative Lengths of Phases of Mitosis

1. Examine at least three fields of view of the apical meristem of the onion root tip at 400X. In each view, count the number of cells in the various stages of mitosis. Record this data in Table 1 in the Analysis section.

It is recommended that the magnification of the fields of view be standardized for the entire class to ensure accurate and comparable data for all lab groups. A random or straight-line method may be used; always make sure that none overlap.

2. Calculate the total number of cells counted and the percentage of total cells counted for each stage of mitosis. Record this data in Table 1 as well.

3. Assuming that it takes an average of 24 hours (1,440 minutes) for onion root tip cells to complete the cell cycle, calculate the amount of time cells spent in each phase of the cycle. Use the formula provided below. Enter your results in Table 1.

   Percent of cells in phase x 1,440 minutes = _____ minutes cell spent in phase

DID YOU KNOW?

Researchers at Cornell University have shown how tiny molecular motors carrying target proteins help guide the mitotic spindle that transfers genetic material from the nucleus of a mother cell to a newly-formed daughter cell. If these molecular motors fail, the spindle cannot properly orient itself with the axis of the cell and genetic material cannot be transferred.

C. Preparation of an Garlic Root Tip Squash

As a general laboratory practice, it is recommended that you wear proper protective equipment such as gloves, safety goggles, and a lab apron to avoid staining any clothing or skin.

Due to time constraints, your instructor may have grown garlic root tips in advance. If this is the case, begin with Step 4.

1. Separate a clove of garlic into individual sections. Remove the paper-like skin from each. Run 2-3 toothpicks crosswise through one garlic section and suspend it in a small vial or 100 ml beaker topped off with water, with the root primordia (blunt end) in the water.

The section used must have root primordia present or it will not produce root tips. The root tips will grow within 24 hours for very fresh garlic, or as long as three days for older garlic. Because Allium has a mitotic cycle of approximately 12.5 hours, it is important to "plant" the garlic and harvest the root tips at approximately the same time of day in order to get the greatest percentage of meristematic cells undergoing mitosis.

2. Place the vial or beaker in a box or dark place until the root tips have grown to a length of about 4 to 5 mm. It is important that the garlic grows in the dark to ensure that it produces roots rather than shoots. Several viable root tips will grow on each section of garlic.

3. Remove the garlic from the box approximately one half-hour before performing the experiment to expose the root tips to light.

4. Blot as much excess water from the root tips as possible. Any excess water on the slide will affect your results. Do not allow the root tips to dry out, however.

5. Using a scalpel or razor blade, cut off the end of one of the emergent root tips; the section should be approximately 1 to 2 mm long. Place the root tip on a clean microscope slide and apply two or three drops of HCl to the root tip.

6. Holding the slide with a clothespin or forceps, pass it through the flame of a Bunsen burner for five seconds.

Do not hold the slide over the flame.

DID YOU KNOW?

Scientists have discovered several checkpoint mechanisms that ensure each step in the mitotic process is properly executed before the cell moves on to the next phase.

DID YOU KNOW?
It has been found that many human cancers are likely to stem from defective mitotic checkpoints.

7. Without harming the root tip, blot the specimen with a paper towel to remove the excess HCl.

You may wish to touch a corner of the paper towel to the drop on the slide and allow the paper towel to soak it up. This may not remove the liquid from the slide as well as blotting, but it will not disturb the root tip.

8. Add a few drops of 0.5% aqueous toluidine blue stain, covering the root tip.

Toluidine blue is a mild irritant. Avoid contact with skin and eyes, and do not ingest.

9. Pass the slide through the flame of a Bunsen burner for one to two minutes. Let the slide stand for one minute.

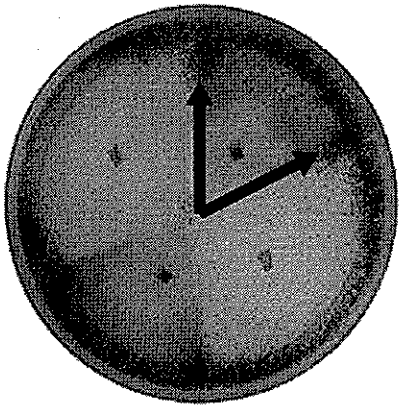
Do not hold the slide over the flame.

10. Without disturbing the specimen, use a paper towel to remove the excess stain.

11. Add a drop of toluidine blue and cover with a coverslip. Using a pencil eraser or other blunt instrument, gently press down on the coverslip to squash and spread out the root tip. Blot off excess stain, if any, that may have come out from under the coverslip.

12. View the slide under a microscope at 100X. Locate the apical meristem. Examine the slide at 400X. Locate cells in the various stages of mitosis, and make sketches of what you find. Keep in mind that since the root tip has been squashed, the meristem may not be readily recognizable.

Figure 3



Part II: Meiosis

To perform the first part of the meiosis lab — creating models of chromosomes with pop beads or other items — in accordance with AP Biology requirements, we recommend WARD'S Chromosome Simulation Kit, available separately (36 W 1602).

1. Place a drop of water on a clean slide with an inoculating loop.

2. With an inoculating loop, scrape several perithecia from the demonstration cross plate. Scrape the perithecia from the interface of two crossing strains (Figure 3) close to the edge of the plate and place in the drop of water on the slide. Avoid picking up agar along with perithecia; it will interfere with results.

DID YOU KNOW?

In 1903, Walter Sutton discovered that chromosomes contained genes and that their behavior during meiosis was random, concepts that later provided the basis for the Chromosomal Theory of Heredity.

3. Cover the slide with a coverslip. Using a pencil eraser or other blunt instrument, gently press down on the coverslip to squash and spread out the perithecia. The pressure should be sufficient to squeeze the asci from the perithecia, but not enough to crush the asci themselves.

It may be helpful to slide the coverslip around on top of the sample, with slight pressure, to spread out the asci and make them easier to observe. Keep in mind, however, that applying too much pressure may rupture the asci, releasing the individual ascospores.

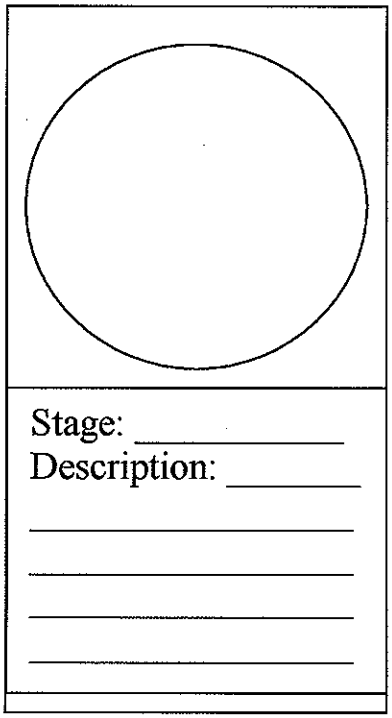
4. View the slide under a microscope at 100X. Locate the asci. You may wish to view the slide at 400X to determine the color of some ascospores. The slide preparation should show collapsed perithecia and asci clusters (rosettes), with mature ascospores in various arrangements. Immature ascospores will all be light colored. Since *S. fimicola* is homothallic, the preparation will show both hybrid and self-fertilized perithecia of both parental types. Hybrid perithecia, however, will not occur very far from the line of contact between the two varieties. Prepare three slides to get an adequate sampling of hybrids, if possible.

5. Count approximately 50 hybrid asci from at least three fields of view, preferably from different slides. Record this data in Table 3 in the Analysis section.

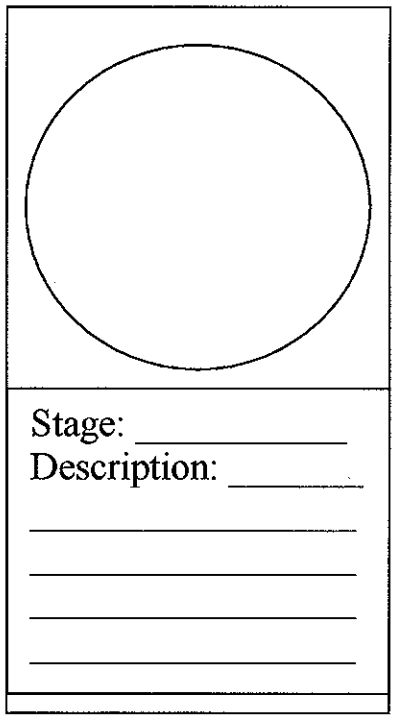
6. Use the formulas in the Analysis section to calculate the frequency of crossing-over and the number of map units between the centromere and the gene for ascospore color.

Name: _____
Group: _____
Date: _____

ANALYSIS



Stage: _____
Description: _____



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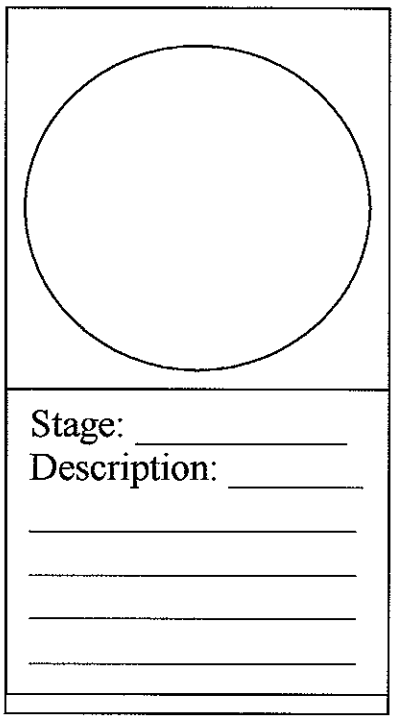

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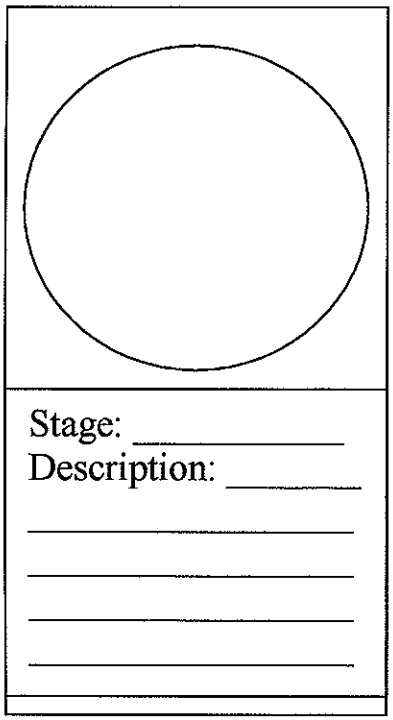

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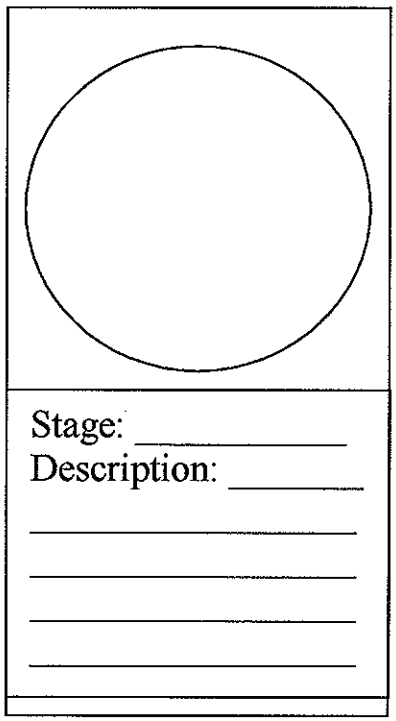
Description: _____



Stage: _____
Description: _____

Table 1
Cells in Each Stage of Mitosis

Stage	# of Cells in Field 1	# of Cells in Field 2	# of Cells in Field 3	Total # of Cells	% of Total # of Cells	Time of Each Stage (min)
Interphase						
Prophase						
Metaphase						
Anaphase						
Telophase						

Total number of cells counted:_____

Table 2

Stage	% of Total # of Cells
Interphase	
Prophase	
Metaphase	
Anaphase	
Telophase	
Total	

Table 3

# of 4:4	# of Crossover	Total	% Asci Showing Crossover	Gene Distance From Centromere

Using the data from the table, determine the distance in map units from the gene for ascospore color to the chromosome centromere. Using the formula provided below, calculate the percentage of asci that showed crossover. This percentage crossover must be divided by two, since only half the ascospores in each hybrid ascus are the result of crossing over. Dropping the % symbol gives you the map distance from the gene to the centromere. Record this data in Table 3.

$$\% \text{ Crossover} = \frac{\text{\# showing crossover}}{\text{total counted}} \times 100\%$$

$$\text{Gene Distance from Centromere} = \frac{\% \text{ Crossover}}{2}$$

Name: _____
Group: _____
Date: _____

ASSESSMENT

1. Referring to the percentage of total cells counted in each phase of mitosis, determine which phase takes the longest for the cell to complete, and explain why. Sketch a pie graph of the percentage of cells in each phase to illustrate. Be sure to include a title and key for your graph.

2. What is the relationship between the processes of mitosis and cytokinesis?

3. Which of the following is significantly different between plant and animal cell mitosis?

   a. metaphase
   b. anaphase
   c. cytokinesis
   d. prophase

4. How does meiosis lead to genetic variability within a population? Use *S. fimicola* as an example.

5. How does this represent an adaptive advantage for organisms that reproduce sexually?

6. Define the following terms:

   somatic cell –

   germ cell –

   chromatin –

   centromere –

   diploid –

   haploid –

   zygote –

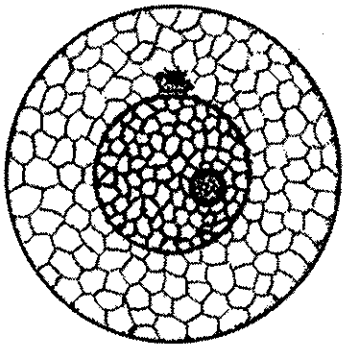
7. Create a Venn diagram showing at least two similarities and two differences between mitosis and meiosis.

8. Why is *S. fimicola* an ideal organism for the demonstration of crossing-over?

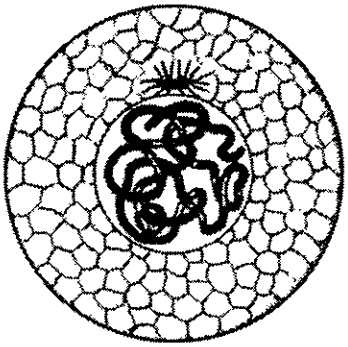
9. When eukaryotic cells undergo mitosis, it results in two identical daughter cells, each with a nucleus. Prokaryotic cells are cells that do not contain a distinct nucleus. Research cell division in prokaryotic cells and compare and contrast it to division in eukaryotic cells.

10. A classmate of yours was absent on the days the lab was performed. Come up with a list of materials (excluding pen and paper) you might use to visually demonstrate the concepts of mitosis, meiosis, and crossing-over.

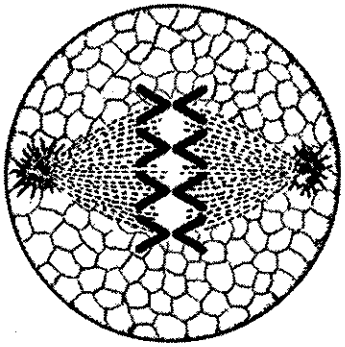
Animal Cell Mitosis



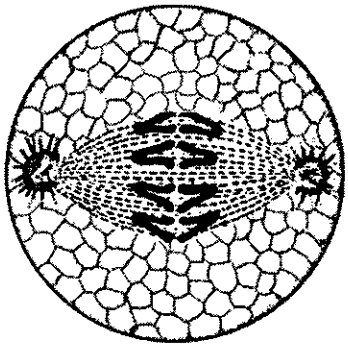
Interphase



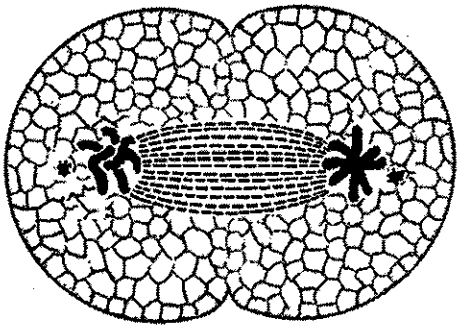
Prophase



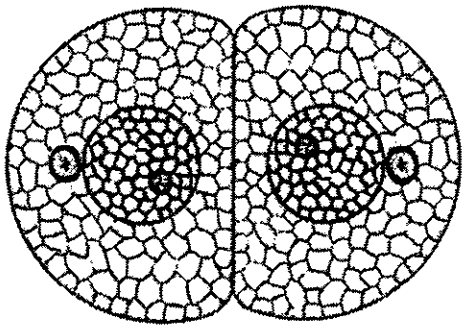
Metaphase



Anaphase

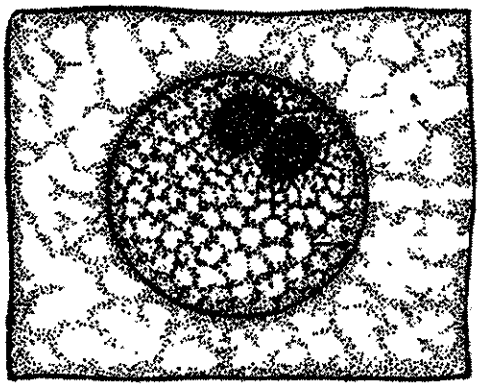


Telophase

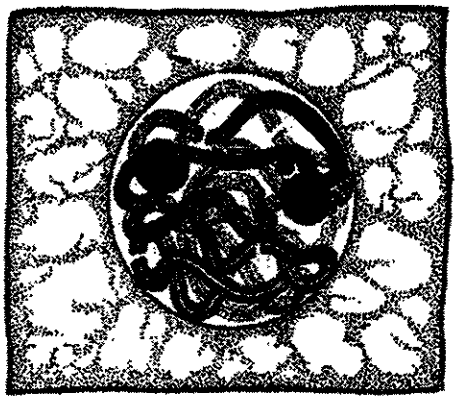


Cytokinesis

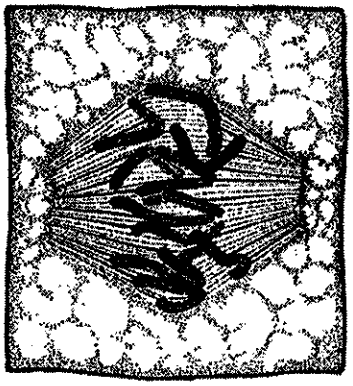
Plant Cell Mitosis



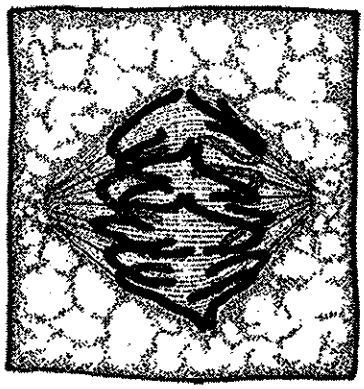
Interphase



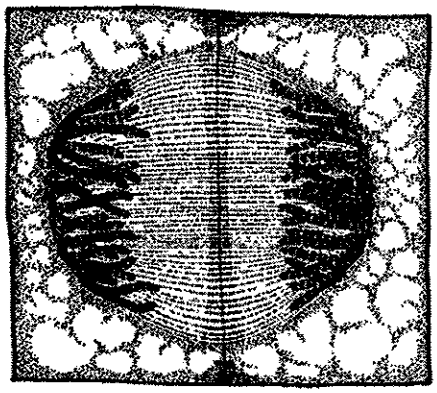
Prophase



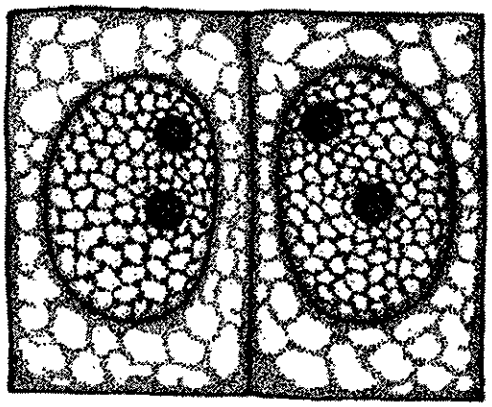
Metaphase



Anaphase



Telophase



Cytokinesis