

AP Biology Laboratory 3

Mitosis and Meiosis

Background to Activities A and B

Cells are the basic units of life, and cell division is the basic event that perpetuates life. Individual cells do not live forever; millions of the cells in your body die every day. You keep on living because new cells arising from cell division replace those that die. One-celled organisms reproduce their kind through cell division, while multicellular organisms grow from single cells by repeated cell divisions. This, event—the reproduction of cells—is a fundamental characteristic of life.

A cell's ability to exist and function is largely dependent upon the proteins it is able to make. Each protein molecule consists of a long chain or chains of amino acids. The amino acids used to produce the chains and their sequence in the chains helps determine the structure and function of the protein. One amino acid chain might go into the formation of a molecule of catalase; another into a molecule of tubulin. The amino acids used to produce a chain and their sequence within the chain is determined by the sequence of bases (C, G, A, T) within the cell's DNA. The loss of even a small amount of DNA can be fatal. For example, if a human zygote (the single cell that grows into an embryo) is missing the DNA necessary to make hemoglobin, it will never be able to develop into a fetus. Therefore, when a cell divides, it is essential that it provide each new cell (daughter cell) with a complete set of DNA. To do this, the cell must replicate (produce a duplicate copy of) its DNA before it divides. When division begins, the DNA is packaged into bodies called chromosomes. Because the DNA has been replicated, each chromosome consists of two identical halves, the sister chromatids. As the cell divides, the identical halves of each chromosome are separated. One half goes into one daughter cell and its twin goes into the other daughter cell. As a result, both daughter cells have a complete and identical set of DNA. This process of dividing double chromosomes to achieve an equal distribution of DNA to the daughter cells is termed mitosis.¹

Activity A: Observing Mitosis

Objectives

- Observe mitosis in plant and animal cells

Materials

Prepared microscope slides of onion mitosis and whitefish mitosis, microscopes.

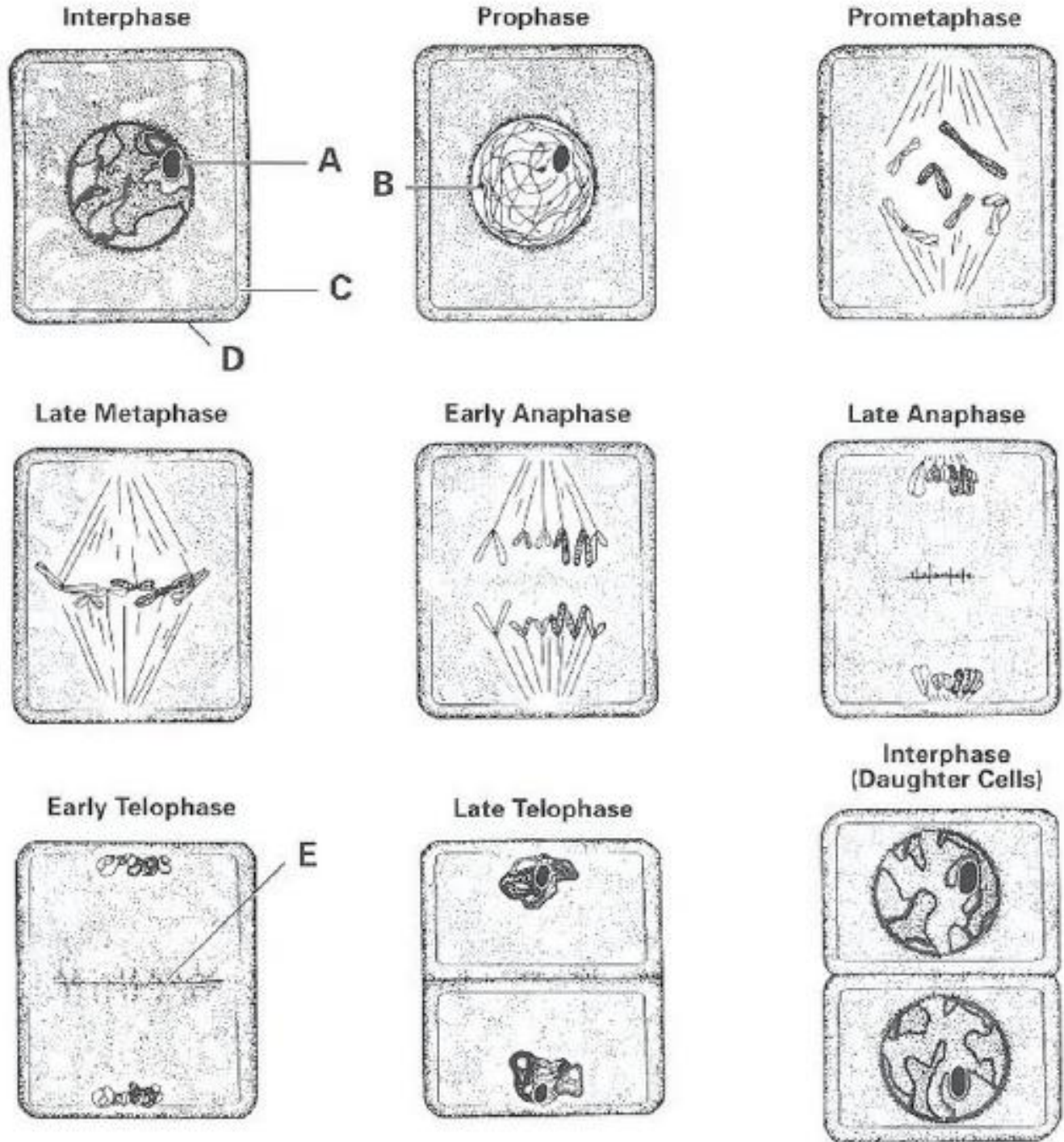
Introduction

In this activity you will observe cells that were undergoing mitosis when they were killed and stained. Although it is really a continuous process, mitotic cell division is usually described in four stages:

prophase, metaphase, anaphase, and telophase. A fifth stage, interphase, describes the nondividing cell.

You will observe mitosis in prepared slides of onion root tips and whitefish blastodisc. You are likely to find mitotic cells throughout the blastodisc. In the root tip, examine the area adjacent to the root cap. These are rapidly growing tissues, and you should see many cells in each stage of mitosis.

Plant Cell Mitosis and Cytokinesis



- A. Nucleolus
- B. Centromere
- C. Cell Membrane

- D. Cell Wall
- E. Cell Plate

Procedure

Use the illustration on the "Plant Cell Mitosis and Cytokinesis" page to help you identify the stages of mitosis that you find on your slides. On the pages following, make your own detailed drawings and make notes that will help you understand what is happening during each stage of mitosis. Remember that the phases mitosis flow into each other, so you will likely see intermediate stages that are not shown in the diagrams. Refer to your textbook or other sources for detailed descriptions of the stages, but remember that your goal is to understand mitosis as a process. Once you understand the process, you can concentrate on the details.

Mitosis Observation: Interphase Cells

In the spaces provided below, on the basis of your observations, draw a plant cell and an animal cell in interphase. Use the space beside each illustration to record notes about what is happening during interphase.

Plant Cell



Animal Cell



Mitosis Observation: Prophase Cells

In the spaces provided below, on the basis of your observations, draw a plant cell and an animal cell in prophase. Use the spaces beside each illustration to record notes about what is happening during prophase.

Plant Cell



Animal Cell



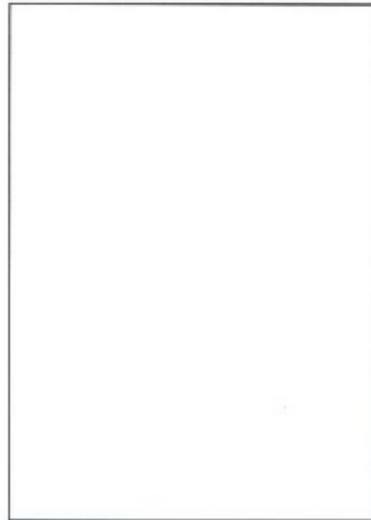
Mitosis Observation: Metaphase Cells

In the spaces provided below, on the basis of your observations, draw a plant cell and an animal cell in metaphase. Use the spaces beside each illustration to record notes about what is happening during metaphase.

Plant Cell



Animal Cell



Mitosis Observation: Anaphase Cells

In the spaces provided below, on the basis of your observations, draw a plant cell and an animal cell in anaphase. Use the spaces beside each illustration to record notes about what is happening during anaphase.

Plant Cell



Animal Cell



Mitosis Observation: Telophase Cells

In the spaces provided below, on the basis of your observations, draw a plant cell and an animal cell in telophase. Use the spaces beside each illustration to record notes about what is happening during telophase.

Plant Cell



Animal Cell



Mitosis Observation: Daughter Cells

In the spaces provided below, on the basis of your observations, draw plant cell daughter cells and animal cell daughter cells. Use the spaces beside each illustration to record notes about the characteristics of daughter cells.

Plant Cell



Animal Cell



Analysis of Results, Activity A: Observing Mitosis

Use your observations of mitosis and your textbook or other sources to answer the following:

1. Mitosis is much the same in the animal cells and plant cells you have examined. What can you infer from this about the evolutionary origins of mitosis?
2. List at least two ways that mitosis differs in the cells of animals and higher plants.
3. Describe what happens to each of the following during mitosis. Indicate the phases in which the changes occur.
 - a. nuclear envelope:
 - b. mitotic spindle:
 - c. chromatin/ chromosomes:
4. List the subphases of interphase and describe the important events that occur during each.
5. List at least two ways that prokaryotic cell division is similar to eukaryotic cell division.

Activity B: Estimating the Relative Lengths of Mitotic Phases

Objectives

- Observe mitosis in plant and animal cells
- Compare the relative lengths of the stages of mitosis in onion root tip cells
- Simulate the stages of mitosis
- Observe evidence of crossing-over in mitosis using *Sordaria fimicola*
- Estimate the distance of a gene locus from its centromere

Materials

Prepared microscope slides of onion mitosis, microscopes.

Introduction

In this activity, you will estimate the relative duration of each phase of mitosis in onion root tip cells. The assumption is that the number of cells observed to be in a phase is related to the amount of time spent in that phase. For example, if phase A lasts two minutes and phase B lasts one minute, the ratio of observed A to observed B would be 2:1.

Procedure

1. Using the low-power objective (10x), locate the area of cell division. Shift to the high power objective (40x), and count the number of cells that are in each stage of mitosis (interphase, prophase, metaphase, anaphase, and telophase).
2. Repeat this count in at least two more nonoverlapping fields of view. Record your data in Table 1.

Table 1: Group Count

	Number of Cells			
	Field 1	Field 2	Field 3	Total 1-3
Interphase				
Prophase				
Metaphase				
Anaphase				
Telophase				

Table 2: Class Data

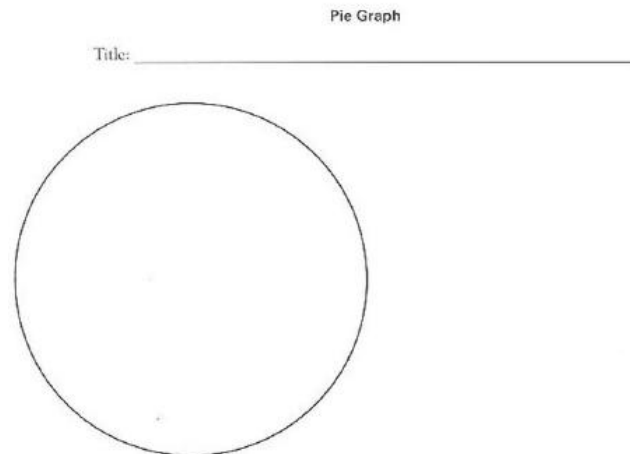
	Class Totals	Decimal Fraction of Total Count	Estimated Time Spent in Phase
Interphase			
Prophase			
Metaphase			
Anaphase			
Telophase			
Total Cells Counted			

3. Record the class totals for each phase in Table 2. Calculate the decimal fraction of the total counted for each phase and record it in Table 2 under Decimal Fraction of Total Count.
4. 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 answers in Table 2.

$$\text{Fraction of cells in phase} \times 24 \text{ hrs} = \text{Estimate time Spent in Phase}$$

Analysis of Results, Activity B: Estimating the Relative Lengths of Mitotic Phases

- Using the data from Table 2, 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.



- On the basis of your data, rank the stages of mitosis in order of time spent in each phase.
 - _____ (most time)
 - _____
 - _____
 - _____
 - _____ (least time)
- On the basis of your observations in Activity A and information on the events of mitosis from your textbook, explain why some phases are longer than others. Refer specifically to each phase.

Activity C: Simulating Meiosis

Objectives

- Observe evidence of crossing-over in mitosis using *Sordaria fimicola*

Background to Activity C

As you have seen, mitosis maintains the chromosome number (and DNA content) from one generation of cells to the next. A second type or nuclear division is required in the life cycles of sexually reproducing organisms.

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. Thus, it would have chromosomes A¹, A² and B¹, B². An organism with two sets of chromosomes (2n) is said to be diploid in chromosome number or, simply diploid. The chromosomes of a pair are said to be homologous; that is, highly similar to each other. If chromosome A¹ has the DNA needed for the production of catalase, chromosome A² will have the same (or highly similar) DNA. Reproductive cells (gametes, egg and sperm in animals; spores in plants) result from meiosis, a type of cell division that reduces chromosome number by separating the homologues. Meiosis accomplishes this reduction in an orderly manner such that our hypothetical diploid animal with chromosomes A¹ A² and B¹ B² produces gametes that are AB and not A¹A² or B¹ B². 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 A¹A²B; A; B; or AB¹B² chromosomes.)

Meiosis involves two nuclear divisions, designated meiosis I (or MI) and meiosis II (or MII). The reduction of chromosome number occurs in meiosis I. Meiosis II is essentially a mitotic division.

Materials

Chromosome sets and Meiosis Student Guide from the *Chromosome Simulation Lab*

Introduction

You will use chromosome models to simulate meiosis. Follow the instructions in the Meiosis Student Guide to complete this activity.

Analysis of Results, Activity C: Simulating Meiosis

- Returning to our example of a diploid organism with chromosomes, A and B ($n=2$), how many different combinations of these chromosomes are possible in the gametes? (If necessary, use the figures below to diagram the division that would give rise to the gametes.)

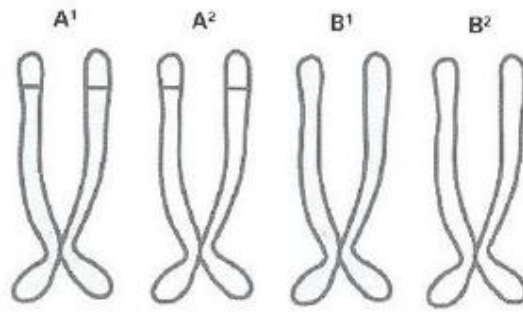


Figure 1

- Using your answer to Question 1 above, and given the following,

n (chromosome number)	Number of possible combinations in the gametes
3	8
4	16
5	32

state a formula for calculating the number of possible chromosome combinations in the gametes based on the value of n .

- For humans, $n = 23$. Using your formula and a calculator, how many possible combinations of chromosomes are there for human gametes?
- For our hypothetical organism with two chromosomes, A and B, when two members of the species reproduce, how many possible combinations of chromosomes are there for the offspring?

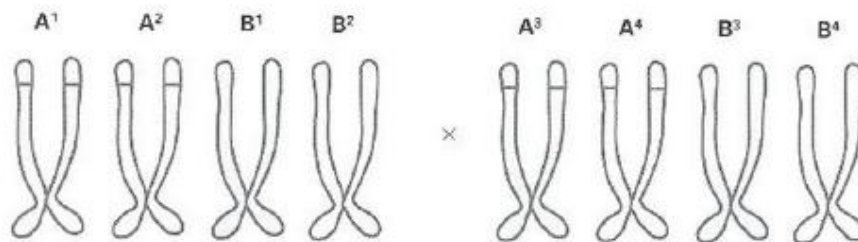


Figure 2

- Looking back at your answers to 1–4, what is the relationship of meiosis to variation in populations (including human populations)?
- List at least three ways that meiosis differs from mitosis.

Activity D: Crossing-Over and Map Units

Objectives

- Observe evidence of crossing-over in mitosis using *Sordaria fimicola*
- Estimate the distance of a gene locus from its centromere

Background to Activity D

When homologous chromosomes pair in prophase I of meiosis, they can exchange parts, which is called crossing-over. When chromosomes exchange parts, genetic material is transferred from one chromosome to another. This alters expected inheritance patterns. For example, the fruit fly, *Drosophila*, has four chromosomes. Chromosome 2 carries a gene for eye color. The normal form of this gene (*Pr*, the “wild type”) produces flies with red eyes, but there is another form or “allele” of the gene (*pr*) that produces purple eyes. On the same chromosome is another gene that effects wing form. The normal wild-type allele (*Vg*) produces normal wings while the alternate form (*vg*) produces vestigial wings, which are smaller and useless for flight. Because these genes are located on the same chromosome, they are considered “linked” and are inherited together. For example, if one chromosome of a homologous pair carries the alleles for red eyes and normal wings and its homologue carries the alleles for purple eyes and vestigial wings, we would expect half the gametes to have the linked alleles for red eyes and normal wings and half the gametes to have the linked alleles for purple eyes and vestigial wings.

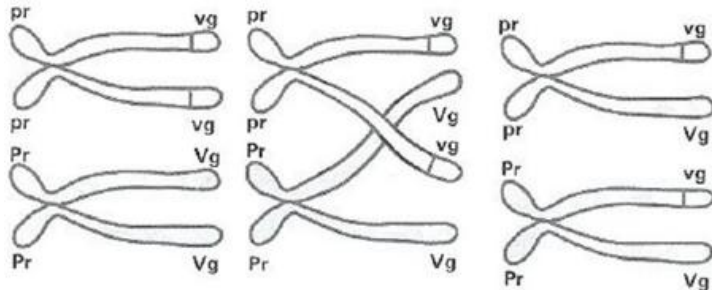


Figure 3. Cross-over involving genes for eye color and wing type

If cross-over occurs between the locations (gene loci) of the genes for eye color and wing type, there will be two new combinations of alleles in the gametes: red eyes with vestigial wings, and purple eyes with normal wings (Fig. 3).

The farther apart two gene loci are on a chromosome, the more likely it is that a crossover will occur between them. By counting the frequency of crossover events between two gene loci, geneticists can determine the relative distance between them. In this way, linkage

maps have been produced for many organisms, including *Drosophila* even humans. In Activity D, you will observe the results of crossing-over for a spore color gene. You will collect data on the frequency of crossover for this gene and calculate the relative distance of the gene locus from its centromere.

Introduction

Sordaria fimicola is a common species of ascomycete that grows on the dung of herbivores. Eight spores (ascospores) are produced in an ascus. Many asci are grouped together within a vase-shaped structure called a perithecium. Two nuclei within a developing ascus fuse to produce a diploid ($2n$) nucleus. This diploid nucleus then undergoes meiosis, followed by a mitotic division to produce eight ascospores in a linear series within the ascus (Figure 4). The order of the ascospores in the ascus reflects the order in which the chromosomes are segregated during meiosis. This can be clearly visualized if the diploid nucleus is a hybrid from two

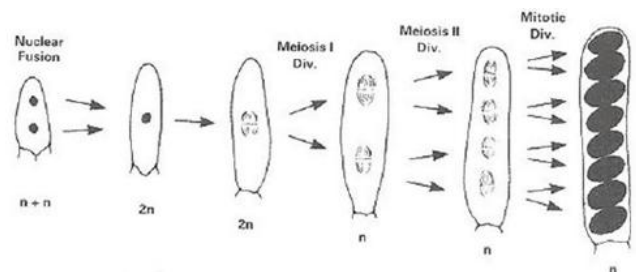


Figure 4. Development of ascospores

different spore colors. The wild-type gene (+) produces a dark spore, while the mutant tan gene (*t*) produces a light spore. If crossing-over does not occur, these genes segregate during meiosis I to produce a 4:4 sequence of ascospores (Figure 5).

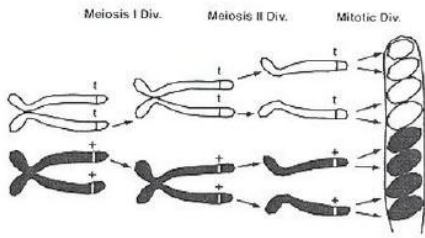


Figure 5. Production of MI asci

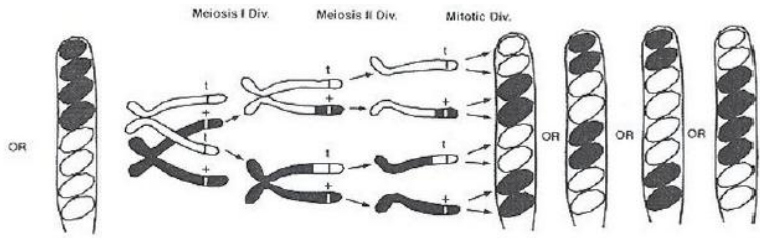


Figure 6. Production of MII asci

However, if crossing-over does occur, the genes do not segregate until meiosis II, producing a 2:2:2:2 or a 2:4:2 sequence of ascospores (Figure 6).

Procedure

You will be using images of already prepared microscope slides of *Sordaria fimicola* perithecia for this activity. To prepare the slides, a scalpel was used to remove several perithecia from a culture of *Sordaria fimicola* spores. The perithecia were placed on microscope slides and gently pressed to release clusters of asci.

1. Use the images provided to search for at least 10 hybrid asci (light and dark spores) and count the number of MI and MII asci. Record the results in Table 3.
2. Calculate the percent MII using the following equation:

$$\% \text{ MII Asci} = \frac{\text{No. of MII Asci}}{\text{Total Asci}} \times 100$$

Record your calculations in Table 3.

3. Calculate the gene-to-centromere distance using the following equation:

$$\text{Gene - to - Centromere Distance} = \frac{\% \text{ MII Asci}}{2}$$

Record your calculations in Table 3.

Analysis of Results, Activity D: Crossing-Over and Map Units

Table 3

	No. of MI Asci (4:4)	No. of MII Asci (2:4:2 of 2:2:2:2)	Total Asci	%MII Asci	Gene-to-Centromere Distance (%MII/2)
Group Data					
Class Data					

1. Does crossing-over increase or decrease genetic variation? Support your answer.
2. A city creates a new lake for its water supply system. The lake is colonized by two water plants, species A and species B. Species A reproduces exclusively by means of buds that grow from rhizomes (asexually). Species B reproduces by budding but also reproduces by seed, which involves sexual reproduction. Given that for both species $n=7$, would you expect to find more genetic variation in the population of species A or species B? Explain your answer.
3. Suppose your Class Data from Table 3 showed 397 MI asci and 0 MII asci. What would you conclude from this?