

# Advanced Placement Biology Series

## Laboratory Three

### Mitosis and Meiosis

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## Mitosis & Meiosis

## General Introduction

Organisms must have a mechanism for producing new cells in order to grow and maintain system function. The division of existing cells creates new cells. There are two mechanisms for achieving this division: mitosis and meiosis. The type of cell determines which of the mechanisms will be used.

Mitosis is the division of an eukaryotic somatic, or body cell, allowing an organism to sustain life. These cells are reproduced asexually as exact replicas of the parent cells. A mutation is the only situation that will produce a change in the genetic makeup of these cells.

As mitosis begins, the chromosomes are duplicated. The cells are  $4n$  as they proceed into prophase. Each chromosome generates two copies known as sister chromatids. The chromatids from each pair will separate and segregate in opposite directions to the poles of the cell.

When mitosis is finished, each of the daughter cells has received one chromatid from each sister chromatid pair. Each of these is referred to as a homologue. These daughter cells have a diploid, or  $2n$  number of chromosomes and will remain in the diploid state until they prepare to undergo mitosis again.

Meiosis is the process of two successive nuclear divisions followed by cellular divisions that reduces the starting number of  $4n$  chromosomes to  $1n$  in each of four product cells. In animals, these will be gamete cells (either sperm or eggs), and in plant cells they will be sexual spores. In sexual reproduction, this reduction of chromosome material is essential so that two gametes or spores can combine to produce a totally new cell. These new daughter cells have genetic material from each of the parents, and characteristics are acquired in a random manner. This allows for the appearance of new characteristics, the health and continuation of a species, and "survival of the fittest."

### Historical Information:

Two individuals who had an enormous impact on the view of genetics are Gregor Mendel and Barbara McClintock. The list could go on and on. History shows a changing view of how we pass characteristics from one generation to the next, both in plants and animals. As technology advances, our view of genetics moves from visual examination to the molecular level. Genetics has become more refined, moving from the view of external characteristics to a molecular view of the determination of individual base pairs within the genome and the genetic code.

### Gregor Mendel, 1826 - 1884

Mendel's contribution to the area of genetics lies in the evidence that he produced showing that different characteristics in heredity followed specific laws which could be determined simply by counting the diverse kinds of offspring produced from any particular set of crosses. He showed that the hybrid produced from two parental types of garden peas, where only a single characteristic was different, would produce two types of gametes in equal numbers.

Although his work was published in 1866, there is some dispute as to how well-known Mendel's findings were before 1900. By the turn of the century, the light microscope had been invented, and most of the morphological features of the cell had been observed. Many researchers' work had come together to identify chromosomes as the carriers of genetic material, and both mitosis and meiosis had been recorded.

### Barbara McClintock, 1902 – 1992

In 1983, thirty-five years after her discovery of "jumping genes" also known as transposable genetic elements or transposons, Barbara McClintock was the first woman to receive an unshared Nobel Prize for Physiology or Medicine.

Her work with the maize corn plant laid a foundation for modern genetics by providing evidence of transposable genetics elements. The view of DNA was no longer fixed and static.

## Mitosis and Meiosis

The science of genetics has far-reaching implications. The research that is being conducted in the areas of cancer, AIDS (autoimmune deficiency disease), mapping of genomes, and cloning, affects not only those who currently have diseases, but also the future of us all.

Understanding cancer, through research, continues to improve its early detection. Consequently, this improves the overall prognosis of the patients. Genetic research also aids in the detection of types of cancer that can be inherited.

As the epidemic of AIDS escalates, it affects not only our health, but also the economies and politics of the world. At the 13th International AIDS conference in Durban, South Africa, in July 2000, it was reported that within 10 years, more than 30 million children will have lost at least one of their parents to AIDS. AIDS is a disease that in the 1980's hadn't even been named yet. Twenty years later it is being compared to the Bubonic Plague.

The drugs that have been discovered in recent years to combat AIDS, function by interrupting the replication of the virus and allowing the body to replenish the T4 cells that help it fight infections. This research may assist in the treatment of many other diseases.

Mapping the human genome has already found the exact locations of the genes responsible for diseases such as cystic fibrosis, sickle cell disease, Tay-Sachs disease, fragile X syndrome and myotonic dystrophy. The increased resolution of the genetic map has not only medical implications, but forensic pathology applications as well

The process of cloning was used in 1997 to create a famous sheep, named Dolly. Ian Wilmut and his colleagues at the Roslin Institution Edinburgh, Scotland created Dolly. New interest was generated because the original cell, that the nucleus was transferred from, originated from the mammary gland of a 6-year old ewe. Cloning can aid in research, as well as potentially improving livestock strains.

All of these developments have moral and ethical issues that will need to be investigated and safeguarded.

1. Bradley, M. 17 July 2000. ABC Science Online.
2. Shorter, Damon. February, 1999. AIDS. Where are we now?
3. Casey, Denise. 1990. Revised and expanded from the primer contributed by Charles Cantor and Sylvia Spengler (Lawrence Berkley Laboratory) and published in the Human Genome 1989-90 Program Report.
4. Wilmut, Ian, A.E. Schnieke, J. McWhir, A. J. Kind and K.H.S. Campbell. 27 February 1997. Nature. Volume 385. pp. 810-813.

## Mitosis Introduction

## Phases in General

The process of mitosis actually consists of several distinct phases. There are also differences between plant and animal cells as they undergo mitosis. In general, the phases of mitosis that will produce two identical somatic cells from one parent cell are:

### Interphase

$G_1$  – This is usually the longest phase of the cell. The cell is recovering from cell division; it will usually double in size, including the number of organelles, and will perform its assigned functions.

$G_0$  – Cells can exit the cell cycle and enter what is known as the  $G_0$  phase, usually from the  $G_1$  phase. The cell does not duplicate its chromosomes and does not prepare for mitosis.

$S$  – Also called the synthesis phase. During this phase the chromosomes are duplicated. Each chromosome is duplicated, resulting in a doubled chromosome consisting of two chromatids attached at the centromere.

### Interphase Continued

$G_2$  – The final preparations for nuclear division occur. The cell synthesizes proteins and enzymes needed for cell division.

### Prophase

The chromosomes begin condensing, becoming shorter, thicker and visible. The nuclear envelope, that surrounds the nucleus, begins to break down and the spindle fibers become visible.

### Metaphase

The spindle fibers attach to the centromere and align the chromosomes along the region of the equatorial plate of the nucleus known as the metaphase plate. The arms of the chromosomes appear to point towards the poles of the cell.

### Anaphase

The centromere divides, forming two chromosomes. The spindle fibers pull the newly divided chromosomes from the equator to the opposite poles by their centromeres.

### Telophase

Once the chromosomes reach the poles, nuclear membranes reform around the daughter nuclei, and the chromosomes elongate once again, becoming invisible. The spindle fibers disappear. This marks the end of mitosis.

### Cytokinesis

Following telophase, the cell divides its cytoplasm and organelles and forms two daughter cells, completing cell division. Follow these [links](#) to see animations of plant and animal mitosis, or to see micrographs of cells in mitosis.

## Mitosis Introduction

## Plant Phases

### Interphase

Interphase is generally considered to be a “resting phase”. Interphase is made up of three phases:  $G_1$ ,  $S$  and  $G_2$ .

### Interphase, $G_1$

This is usually the longest phase of the cell. The cell is recovering from cell division; it will usually double in size, including the number of organelles and perform its assigned functions.

### Interphase, $S$

Also called the synthesis phase. During this phase the chromosomes are duplicated. Each chro-

## Mitosis and Meiosis

mosome is duplicated, resulting in a doubled chromosome consisting of two chromatids attached at the centromere.

### **Interphase, G<sub>2</sub>**

The final preparations for nuclear division occur. The cell synthesizes proteins and enzymes needed for cell division.

There is a fourth phase which leads to cell death:

### **Interphase, G<sub>0</sub>**

Cells can exit the cell cycle and enter what is known as the G<sub>0</sub> phase, usually from the G<sub>1</sub> phase. The cell does not duplicate its chromosomes and does not prepare for mitosis.

### **Prophase**

During prophase, the chromosomes can be viewed with a microscope. The chromosomes start out as long threads in the nucleus and condense, becoming shorter and thicker. Each chromosome is composed of two longitudinal halves, called chromatids, joined in a narrow area known as the centromere, where the chromatids are not coiled. The centromere, located on each chromosome, divides the chromosomes into two arms of varying lengths. Plant cells do not contain centrioles or asters, yet they do not have sets of microtubules which form spindle fibers that radiate from near the poles, and attach to the centromeres at a point known as the kinetochore.

### **Metaphase**

Once the spindle fibers are attached to the centromere, they align the chromosomes along the equatorial region of the nucleus known as the metaphase plate. The arms of the chromosomes appear to point towards the poles of the cell.

### **Anaphase**

The centromere divides and the two chromatids separate from each other forming two identical daughter chromosomes. The spindle fibers appear to pull the newly divided chromosomes away from the metaphase plate to the poles by the centromere with the arms of the chromosomes trailing behind. The spindle fibers appear to move, but in fact the microtubules are continuously formed at one end of the spindle fiber and disassembled at the other.

### **Telophase**

Once the chromosomes reach the poles, a nuclear membrane forms around each set of daughter nuclei and the chromosomes uncoil and elongate once again becoming invisible. The spindle fibers break down and disappear. This marks the end of mitosis.

### **Cytokinesis**

As mitosis ends, cytokinesis begins, resulting in the formation of two daughter cells. This involves the formation of a membrane-bound cell wall called a cell plate. Vesicles formed by the Golgi bodies fuse together, forming the cell plate where the metaphase plate has been. A new cell wall forms on either side of the cell plate. Once cytokinesis is completed, the original cell will be partitioned into two daughter cells containing identical copies of the cell's chromosomes and approximately half of the mother cell's cytoplasm and organelles. The cell now enters interphase.

## Mitosis Introduction

## Animal Phases

### Interphase

Interphase is generally considered to be a "resting phase". Interphase is made up of three phases:  $G_1$ , S and  $G_2$ .

### Interphase, $G_1$

This is usually the longest phase of the cell. The cell is recovering from cell division; it will usually double in size, including the number of organelles and perform its assigned functions.

### Interphase, S

Also called the synthesis phase. During this phase the chromosomes are duplicated. Each chromosome is duplicated, resulting in a doubled chromosome consisting of two chromatids attached at the centromere.

### Interphase, $G_2$

The final preparations for nuclear division occur. The cell synthesizes proteins and enzymes needed for cell division.

There is a fourth phase which leads to cell death:

### Interphase, $G_0$

Cells can exit the cell cycle and enter what is known as the  $G_0$  phase, usually from the  $G_1$  phase. The cell does not duplicate its chromosomes and does not prepare for mitosis.

### Prophase

Just prior to mitosis, the pair of centrioles duplicates. During prophase, the two pairs of centrioles migrate to opposite poles. Sets of microtubules form spindle fibers which form from the centrioles, and eventually attach to the centromeres. Additional fibers, known as asters, also radiate outward from the centrioles. The chromosomes first become visible, starting out as long threads in the nucleus, and condense, becoming shorter and thicker. Each chromosome is composed of two longitudinal halves, called chromatids, joined in a narrow area known as the centromere, where the chromatids are not coiled. The centromere divides the chromosomes into two arms of varying lengths.

### Metaphase

The spindle fibers enter the nuclear region, extend from the centrioles to the centromere, and attach at a point known as the kinetochore. Once the spindle fibers are attached, they align the centromeres along the equatorial region of the nucleus known as the metaphase plate, and the arms of the chromosomes appear to point towards the poles of the cell.

### Anaphase

The centromere divides and the two chromatids, separate from each other. This forms two identical daughter chromosomes. The spindle fibers appear to pull the newly divided chromosomes away from the metaphase plate to the poles by the centromere with the arms of the chromosomes trailing behind. The spindle fibers appear to move, but in fact the microtubules are continuously formed at one end of the spindle fiber and disassembled at the other.

### Telophase

Once the chromosomes reach the poles, a nuclear membrane forms around each set of daughter nuclei, and the chromosomes uncoil and elongate once again, becoming invisible. The spindle fibers break down and disappear. The cleavage furrow becomes visible. This marks the end of mitosis.

## Mitosis and Meiosis

## **Cytokinesis**

As mitosis ends, cytokinesis begins, resulting in the formation of two daughter cells. In animal cells, a cleavage furrow —an indentation in the cell membrane between the daughter nuclei — develops. The membrane slowly draws together, forming a narrow bridge, then separates the cell into two daughter cells. The cells now enter interphase.

## Mitosis

## Learning Objective

### Mitosis

Prior to performing this exercise, you should:

Have a basic knowledge of mitosis and cell division.

After performing this exercise, you should be able to:

Examine and understand the events taking place during the process of mitosis.

Compare the process of mitosis in plant cells to mitosis occurring in animal cells.

Determine the relative duration of each of the phases of mitosis.

Practice microscope technique and learn a method for preparing a stained sample of garlic root tips.

### Mitosis Experiments

### Time and Material Requirements

#### Time Requirements

- 20 minutes for the examination of the mitotic phases in prepared onion root tip and whitefish blastula microscope slides.
- 20 minutes for the identification and counting of mitotic stages in onion root tip microscope slides.
- 30 minutes for students to prepare and examine their own root tip squash slides.

### Mitosis Experiments

### Time and Material Requirements

#### Per student

- 1 prepared slide of onion root tip
- 1 prepared slide of whitefish blastula
- 1 garlic toe
- 1 bottle 0.5 % Toluidine Blue—per group of four students
- 1 bottle 1M HCl—per group of four students
- 1 microscope slide
- 1 coverslip
- 1 bunsen burner or alcohol burner—per group of four students
- 1 slide holder (clothespin or forceps)—per group of four students
- 1 scalpel or single-edge razor blade—per group of four students
- 1 compound microscope
- paper towels

## Mitosis

## PreLab Preparations

### Preparation of Root Tip Squashes

1-2 days prior to performing the experiment: begin growing your garlic root tips. Either the student or teacher can setup this portion of the experiment.

30 minutes prior to the laboratory time remove the garlic toes from the boxes to expose them to light.

## Mitosis Experiments

## Preparation of Root Tip Squashes

Plant root tips consist of several different zones where various developmental and functional processes of the root are performed. The primary region for the formation of new cells is the apical meristem. The root cap offers protection for the rest of the root, the region of elongation is the area where the bulk of cell growth occurs, and the region of maturation is where tissue differentiation occurs. After examining your own onion root tip preparation, you should compare your findings with those from the prepared slides you examined in the first part of the experiment.

- 1-2 days prior to performing the experiment, begin growing your garlic root tips.  
Hint: In selecting garlic cloves, each individual toe must have root primordia present, or it will not produce root tips.
- Separate a clove of garlic into individual toes and remove the paper-like skin from each of the toes.
- Place a garlic toe in a small vial topped off with water so it rests in the mouth of the vial with the pointed end of the toe up and the blunter end in the water of the vial. Beakers can also be used with toothpicks through the toes for this part of the experiment.

Hint: It is important to "plant" the garlic toes and harvest the root tips at approximately the same time of day (preferably 11 AM to noon) in order to get the greatest percentage of meristematic cells undergoing mitosis. This is because the *Allium* has a mitotic cycle of approximately 12.5 hours. It is also important to grow the root tips in the dark as to ensure the production of roots rather than shoots.

Several viable root tips should grow on each garlic toe. Each student should grow his/her own. In case some of the toes do not yield root tips students can share the root tips.

- Place vial or beaker in a box or dark place for 1-2 days, until the root tips have grown to a length of about 4-5 mm. Remove the toes from the box approximately 1/2 hour before performing the experiment to expose the root tips to light.  
Hint: When exposing the root tips to light, keep them in the vial of water. When cutting the root tips, be sure to dry them off as much as possible. Residual water on the root tips will cause the root tip to be placed on the slide in a drop of water. This will dilute the HCl when it is added and is NOT conducive to optimal results. Do NOT, however, allow the root tips to sit and dry out.
- Using a scalpel or scissors, cut off the end of the root tips to a length of 1-2 mm. Place the root tip on a clean microscope slide.

Caution: Sharp object-handle with care. Use extreme care when handling scalpels and/or razor blades. Be sure to cut away from the body. Adequate care and precautions should be taken in handling and disposal of all glassware, to include beakers, vials and microscope slides.

- Place 2-3 drops of 1M HCl on the root tip. Holding the slide with a clothespin or forceps, pass it through the flame of a bunsen burner for 5 seconds. Be very careful in working with the acid and the flame.

Caution: Gloves and safety goggles should be worn while handling HCl and toluidine blue, as well as lab coats while using organic stains. Use extreme care in lighting and working with bunsen burners and alcohol burners. Remove gloves when working with burners.

- Using a paper towel, blot the specimen to remove the excess HCl. Be very careful not to remove or destroy the root tip. Add a few drops of 0.5% aqueous toluidine blue to cover the

root tip. Use caution and wear a lab coat or smock, safety goggles and gloves while handling the stain. Pass the slide through the flame of a bunsen burner for 1-2 minutes. Do not simply hold it over the flame. Let the slide stand for 1 minute.

Hint: It is extremely important to follow the staining protocol closely to obtain good results. It is important to pass the slides through the flame of the bunsen burner for the periods of time cited in the procedure rather than hold them over it. This will prevent the stain on the slide from boiling and caking to the slide and will also help protect the students from the heat of the burner.

Caution: Toluidine Blue is a mild irritant. Avoid contact with skin and eyes and do NOT ingest. Bottles of stain should be labeled with this information.

- With a paper towel, remove the excess stain. Be careful not to disturb the specimen. Place a fresh drop of toluidine blue and apply a coverslip.

Hint: Instead of blotting off the excess HCl and Toluidine Blue from the preparation it may help to simply stick a corner of the paper towel into the drop on the slide and wick away the moisture without touching the specimen. This may not remove the moisture as effectively, but it will help to preserve the specimen.

- Using your thumb, gently apply pressure to the coverslip to squash and spread out the root tip.

Hint: An alternate method of squashing the specimen involves a pencil eraser. When squashing the root tip under the coverslip, place a paper towel under the slide and another over the top. Then using a pencil eraser apply gentle pressure to the coverslip in a back and forth pattern. The paper towels will help soak up the excess moisture as it seeps out of the edges of the coverslip and not allow it to drain out onto the tabletop, making a mess.

- Blot off stain from the edges of the coverslip. View the slide under a microscope, using a 10X objective to locate the apical meristem and then examine it with the 40X objective. 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 recognizable.

## Meiosis

## Introduction

Meiosis is the cell division process that goes through two meiotic divisions with an interphase between. A cell, in its normal resting state, contains a diploid number of chromosomes equal to " $2n$ ". To prepare for division, the chromosomes are doubled to " $4n$ ". The two meiotic divisions allow for a reduction in the number of chromosomes in each nucleus from " $4n$ " to the number of " $n$ ". This is a necessary factor in sexual reproduction where two spores or gametes combine to form a new cell instead of simply dividing as happens in mitosis.

In the first division the homologues, which are chromosomes carrying the same genetic loci, pair together and each pair moves into a new cell. In the second division however they separate and each homologue moves into its own cell. If there are several pairs in the first cell they will separate or sort independently resulting in random combinations.

### Plant vs. Animal Meiosis

The formation of spores in plants or gametes in animals is the same. The stages of meiosis are similar to those found in mitosis:

### Interphase

During the S phase each chromosome will replicate and is known as a chromatid. An area called the centromere connects these two identical chromatids.

### First Meiotic Division

**Prophase I:** The nuclear membrane breaks down in this phase. The centrioles migrate to the poles. After the chromosomes duplicate they come close, pairing up along their entire length, and can wrap around each other. This is called synapsis and results in a tetrad of four chromatids. Prophase I is where crossing over may occur. Crossing over is a phenomenon where the genetic material in two homologous chromosomes can be transferred between them in this entwined state. At this point there is a chance for one portion of a chromosome to break off and be exchanged for the corresponding portion on the other chromosome. Thus information from the two parents can be intermingled and a brand-new combination of genetic information can occur.

**Metaphase I:** The chromosomes are now lined up in homologous pairs at the equator of the cell. This is a phase that is easy to recognize under a microscope.

**Anaphase I:** Here the homologues separate and migrate toward opposite poles of the mitotic spindle. Each homologue still consists of two chromatids attached by their centromere.

**Telophase I and Cytokinesis:** When the chromosomes reach the poles, the nuclear membranes reform and cell division or cytokinesis occurs, resulting in two separate daughter cells with a diploid chromosome number of  $2n$ . Because of this reduction in chromosome number this process is called "reduction division". The centrioles also replicate in order to facilitate the next meiotic division.

**Interphase:** During this intermediate interphase the cells are again in a resting state, preparing for the second meiotic division. The cells still have a diploid number of chromosomes ( $2n$ ).

**Prophase II:** In this phase meiosis continues, but without the crossing-over that occurs in Prophase I. The two centrioles of each cell again migrate to the poles of the cell and the nuclear membrane disassociates. The chromosomes also seem to shorten and thicken.

### Second Meiotic Division

**Metaphase II:** The chromosomes will again align themselves on the equatorial line, but as single entities this time.

**Anaphase II:** The centromeres split and the chromatids separate and move toward opposite poles of the cell. Each chromatid, with a well-defined centromere, is now a chromosome.

## Mitosis and Meiosis

Telophase II: The chromatids are enclosed within new nuclear membranes and cytokinesis occurs resulting in four cells with a haploid, "n" number of chromosomes which now unwind as the cell enters Interphase. These final daughter cells are known as spores in plants and gametes in animals.

## Meiosis Experiments

## *Sordaria* Life Cycle

*Sordaria fimicola* is an Ascomycete fungus that is haploid for the bulk of its life cycle. The individual fungal filaments, called hyphae (sing. hypha) are haploid, and normally exist in a mass called a mycelium (pl. mycelia) which represents the "body" of the fungus. The ascospores mycelia develop from are also haploid. In fact, the only diploid portion of the lifecycle of *S. fimicola* occurs when the nuclei of specialized hyphae nuclei come together. These hyphae must belong to different strains of the species and fuse to form a zygote. This zygote then undergoes meiosis to produce the haploid ascospores. Meiosis occurs and yields four haploid nuclei that are contained within a sac called an ascus (pl. asci). After meiosis, the four nuclei undergo mitosis and the end result is an ascus containing eight haploid ascospores. Many asci form inside a fruiting body called a perithecium (pl. perithecia).

One source of genetic variability in *S. fimicola* is the color of the ascospores. Most strains show the dark brown, wild type ascospores, although there are variants. Certain strains have tan or gray ascospores. Mating one of these mutant strains of fungus with the wild type offers good illustration of the concept of crossing over. When a tan ascospore strain is mated with the wild type variety, the result is a series of perithecia containing asci with four tan and four wild type ascospores each. How these ascospores are arranged within the ascus is a direct representation of whether or not crossing over has occurred between the centromere and the site for the gene for ascospore color. If no crossing over has occurred, the ascospores will be arranged in a 4:4 manner. If crossing over has occurred, they will in a 2:4:2 or a 2:2:2:2 manner. For more information, see section on ascospore arrangement.

*Sordaria fimicola* is a member of the Kingdom Fungi. The fungi consist of eukaryotic organisms that live by either absorbing dead organic material (saprobes) or by absorbing organic material from the bodies of living organisms (parasites).

True fungi have cell walls like plants and most species are multicellular, but unlike plants, their cell walls are normally made of chitin. Fungi also are not able to produce food via photosynthesis.

The body of a fungus is referred to as a mycelium. The mycelium can take on many different forms in various species, but normally exists as a mass of tiny, hair-like strands of fungus, that are called hyphae (sing. hypha). The cells of these hyphae can be either septate, which means there are cell walls separating the nuclei within the hyphae, or they can be coenocytic. Coenocytic hyphae have a common cytoplasm in which there are many nuclei.

Due to their diversity as a group, classification of fungi is complicated and is accomplished by looking at the mycelium of a species as well as its spores. Septate hyphae the formation of many ascospores, and sexual reproduction characterize the Division *Ascomycota*, which includes *S. fimicola*. Eight of these ascospores are contained in an ascus and many asci are themselves contained in complex fruiting bodies called perithecia (sing. perithecium). There is much variation, however, among the ascomycetes. Many species reproduce both sexually (by ascospores) and asexually (by the formation of conidia and microconidia) while others reproduce solely by the formation of ascospores. *S. fimicola* is an example of a species that reproduces only sexually, producing no conidia at all.

The species is also homothallic and therefore every hypha is self-fertile and does not require separate mating types. When two *S. fimicola* hyphae come together, the two nuclei fuse to form a zygote. The zygote represents the only diploid stage in the *Sordaria* life cycle. The zygote then undergoes meiosis to form 4 haploid spores. These spores then go through mitosis to produce eight ascospores in an ascus. The ascospores mature and the perithecium grows around them. When mature,

the ascospores are released from the asci. One by one the asci project from an opening in the perithecium and rupture, releasing the ascospores.

Once released, at least a few of the thousands of spores released are bound to land on suitable food. When this happens, the spore germinates and a hypha grows from it. As it grows and branches into many hyphae, the resultant mass is again referred to as a mycelium. The hyphae of a mycelium, or the two discrete mycelia, may then come into contact with each other. If this is the case, and if environmental cues dictate, a zygote will again be formed and the lifecycle will repeat itself.

## Meiosis Experiments

## Time and Material Requirements

### Time Requirements

#### Prelab:

- preparation of *Sordaria* colonies 8-10 days prior to experiment (if incubation will be at room temperature allow 11-13 days)

#### Laboratory:

- 45 minutes for the simulation of chromosomal activity in meiosis
- 45 minutes for the examination of *Sordaria* ascospores and crossover genetics. Students should be provided with the cultures, materials and adequate laboratory equipment to perform sterile technique.

#### Required Materials

### Required Materials

#### Per student

- 1 chromosome simulation kit
- 1 compound microscope
- 1 live culture of *Sordaria fimicola* (wild type)—per group of four students
- 1 live culture of *Sordaria fimicola* (tan mutant)—per group of four students
- 3 plates of ascomycetes mating agar
- 1 inoculating loop—per group of four students
- 1 chemical spatula or scalpel—per group of four students
- 1 bunsen burner or alcohol burner—per group of four students
- 1 vial of distilled water—per group of four students
- 3 microscope slides per student
- 3 slide coverslips per student

#### Time Requirements

## Meiosis

## Learning Objectives

### Meiosis

#### Prior to performing this exercise, you should:

Have a basic knowledge of meiosis and cell division.

After performing this exercise, you should be able to:

Understand and demonstrate the events of meiosis I and II using plastic bead chromosome models.

Understand the concepts of segregation, independent assortment, and crossing over, and be able to model them with the plastic bead chromosome models.

Explain how genetic variability arises from sexual reproduction and meiosis.

Understand the roles of mitosis and meiosis in the life cycle of *Sordaria*.

Calculate the distance in map units between a specific gene and the chromosome centromere.

Explain the role played by meiosis in determining the arrangement of ascospores in the asci of *Sordaria*.

## Meiosis

## Prelab Preparations

### Crossing Over Bead Demonstration

The only preparation required for this part of the lab is to have a plastic bead chromosome simulation kit available. Have the beads apportioned to each student according to availability. Ideally, each student should have two pairs of homologous chromosomes, each with 16 beads per chromatid.

### Crossing Over in *Sordaria* Experiment

8-10 days prior to performing the lab activity, you should make mating cultures of the two strains of *S. fimicola*. Follow the protocol outlined in the procedure section of the lab. If you are growing the fungus at room temperature, it is wise to give the mating culture 11-13 days in order to produce mature, hybrid perithecia.

On the day of the lab, the only preparation that needs to be done is providing the students with the cultures, materials and adequate laboratory equipment to perform sterile technique.

### Meiosis Experiments

### Cross Over Bead Demonstration

Using the plastic beads from the chromosome simulation kit, construct four chromatids as shown here, two from red beads and the other two from yellow. Place a magnetic centromere in the middle of each bead strand. Use as many beads as you have available. Ideally, each chromatid should be 16 beads long plus the centromere. Attach the red centromeres together and the yellow centromeres together. This simulates the formation of pairs of homologous chromosomes as the DNA is replicated and the chromosomes begin to condense as the cell prepares to undergo meiosis. If it is available, use acetate covered white paper underneath the beads and use dry erase markers on top of the acetate to designate where the nuclear and cell membranes are as they form and disassociate.

Place these chromatids in the center of an imaginary circle (representing the cell's nuclear membrane), along with two centrioles, each consisting of two of the clear plastic tubes placed at right angles to each other.

In Prophase I, the nuclear membrane breaks down, the centrioles migrate to the poles and the two pairs of homologous chromosomes come close together, pairing up along their entire length.

This is called synapsis and results in a tetrad of 4 chromatids. You can show this by pushing the two pairs of sister chromatids together and entwining their strands in the center of the cell. Also move the centrioles to opposite poles of the cell.

Crossing over is a phenomenon where the genetic material in two homologous chromosomes can be transferred between them in their entwined state. Simulate this by popping off a few of the red beads and replacing them with an equal number of yellow beads and vice versa. In Metaphase I, the chromosomes disentangle and become aligned in the center of the cell in their homologous pairs. To show this, position them in the center of the cell at right angles to the centrioles.

In Anaphase I, the homologous chromosomes separate and are drawn to opposite poles of the cell. Move the chromosomes toward their centriole as they are being drawn by the spindle fibers.

In Telophase I, the chromosome pairs have separated resulting in two daughter cells. The result is essentially two haploid cells with replicated DNA. The centrioles also replicate in order to facilitate the next meiotic division. Pull the pairs of bead strands apart and draw an imaginary line around each daughter cell to represent the newly formed nuclear membrane of each cell. Place another set of centrioles in each cell also to simulate centriole replication.

A second division must then take place in order to separate the chromatids of each daughter cell. The processes of meiosis II result in the formation of four haploid gametes, which, in our model, will have only one chromatid each. In Prophase II, the two centrioles of each cell again migrate to the

poles of the cell and the nuclear membrane disassociates. The chromosomes also seem to shorten and thicken. Simulate this by moving the centrioles to opposite ends of the cell.

In Metaphase II, the chromosomes align themselves between the centrioles in the center of the cell, much like they did in meiosis I. Move the chromosomes into the center of the cell at right angles to the centrioles.

Anaphase II brings about the separation of the chromatids of each chromosome as they are drawn to the centrioles at opposite poles of the cell. Each chromatid, with a well defined centromere, is now a chromosome. To show this, separate the magnetic centromeres of each of the strand pairs and drag them toward the centrioles.

In Telophase II, cell division is completed and results in four haploid daughter cells (gametes). Place each bead strand near its respective centriole and draw an imaginary circle around each new cell.

## Meiosis Experiments

## Cross Over in *Sordaria*

On the bottom of a petri dish of mating agar, place two lines perpendicular to each other through the center of the plate, dividing the plate into four quadrants. Using sterile technique, cut two small agar squares of one of the *Sordaria* strains. Place them on opposite quadrants of the mating culture dish. Place the inocula 4–5 cm apart on the mating culture.

Repeat the procedure with the second variety of *Sordaria*.

The placement of the culture fungus on the mating agar is very important. For the best results the inocula should be placed 4-5 cm apart on the dish. Placing them closer will not result in a faster growing culture and may not yield as distinct a line of hybrid perithecia.

Safety note: Use extreme care in lighting and working with bunsen burners. Refer to the safety considerations section for more information. Exercise caution when handling all glassware. Be sure to dispose of broken glassware properly and in specially designated receptacles.

Label and cover the plate. Incubate it at 30°C. The fungi will also culture at room temperature, but will grow at a slower rate. Perithecia will be visible in about 3–4 days, and reach full maturity around day 10. The hybrid perithecia will occur almost exclusively along the centerline of the plate.

The hybrid perithecia will become apparent at day 3 or 4 in your cultures, but will not mature until around day 9 or 10. Immature ascospores will all have a light color. This will lead to poor results because all of the ascospores will read as tan mutants. If time is a constraint, incubating cultures may show hybrid asci as early as day 6.

Around day 9 or 10, the perithecia should be mature and actively discharging asci one at a time. To extend the sporulation period as long as possible, the plate should be refrigerated.

If you are planning to perform the experiment on more than one day, the mating cultures should be refrigerated. This will extend the fruiting period of the hybrid perithecia to a length of approximately one week.

If you are using the WARD'S demo plate this is where you will begin the experimental protocol. If the Demo Plate arrives a few days prior to usage store at refrigerated temperature to limit further growth of asci.

Using a sterile inoculating loop, place a drop of water on a clean slide. Scrape several perithecia from the centerline of the mating plate and place the specimen on the slide in the drop of water. Avoid picking up agar along with the perithecia, as this will interfere with future observations. (Unlike removing perithecia for culture to the mating agar, it is important not to collect agar from the culture along with the perithecia when removing them for the slide preparation. This may be difficult to do

with an inoculating loop. You may want to try scraping the fruiting bodies off the culture with a small chemical spatula or a scalpel instead. This does, however, complicate the measures necessary to ensure proper sterile technique.)

Place a coverslip over the preparation and press down firmly but gently in the center of the coverslip. The pressure should be sufficient to squeeze the asci from the perithecia, but not enough to crush the asci themselves.

After placing the coverslip on the sample of hybrid perithecia and applying pressure to release the asci, it may be helpful to slide the coverslip around on top of the sample (with slight pressure) in order to spread out the asci a bit, making them easier to observe. Keep in mind, however, that applying too much pressure may increase the chance of rupturing the asci, releasing the individual ascospores.

Place the slide on a microscope stage and observe. Use the low power objective (10X) to locate and observe the asci. It may also be helpful to use the 40X objective to determine the color of some of the ascospores. The slide preparation should show collapsed perithecia and asci clusters (rosettes) with mature ascospores in various arrangements. Immature ascospores will be light colored. Since *S. fomicola* 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 five slides in order to get a sampling of hybrids.

Count approximately 50 hybrid asci from at least 5 discrete fields of view, preferably from different slides when possible. Record your data in Data Table Two.

Using your data, determine the distance in map units from the gene for ascospore color to the chromosome centromere. Calculate the percentage of asci that showed crossover by dividing the number that exhibited crossover by the total number counted and multiplying by 100%. Divide this percentage crossover by two since only half of the ascospores in each hybrid ascus are the result of crossing over. Dropping the % symbol gives you the map unit distance from the gene to the centromere. Record this value in Data Table Two.

$$\% \text{ crossover} = (\# \text{exhibiting crossover} / \text{total \# asci}) \times 100\%$$

## Data Table One

Stage	Number of cells in:					Total cells per phase	% of total cells counted	Time in each phase
	Field 1	Field 2	Field 3	Field 4	Field 5			
Interphase								
Prophase								
Metaphase								
Anaphase								
Telophase								
Totals								

(percent of cells in phase) x (1,440 minutes) = \_\_\_\_\_ minutes of cell cycle spent in phase

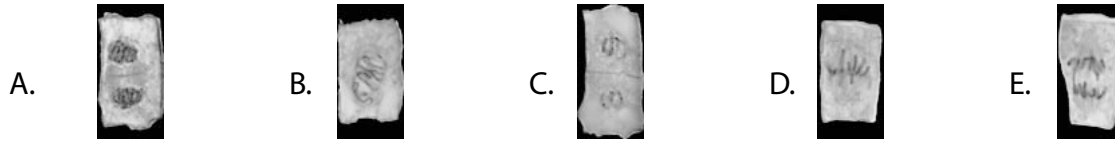
## Data Table Two

Slide #	Slide Number:					Totals
	One	Two	Three	Four	Five	
# of 4:4 asci						
# crossover asci						
total asci						
% asci showing crossover						
gene distance to centromere (map units)						

## Mitosis

## Analysis

1. In the blank following the name of the phase, place the letter of the corresponding picture that shows a cell(s) undergoing that phase.



Prophase \_\_\_\_\_

Metaphase \_\_\_\_\_

Anaphase \_\_\_\_\_

Telophase \_\_\_\_\_

Cytokinesis \_\_\_\_\_

2. Describe the location of the centromere?

3. At what stage do the chromosomes become invisible?

- A. Anaphase
- B. Prophase
- C. Metaphase
- D. Telophase

4. Mitosis results in:

- A. Two daughter cells
- B. Two centromeres
- C. Two sets of organelles
- D. Two identical nuclei

5. Mitosis can most likely be seen in:

- A. Mature sun leaves
- B. Root tips
- C. Animal hair
- D. Fingernails

5. At what stage do the chromosomes replicate?

- A.  $G_1$
- B. M
- C. S
- D.  $G_2$

6. Cytokinesis results in:

- A. Two daughter cells
- B. Two centromeres
- C. Two sets of organelles
- D. Two identical nuclei

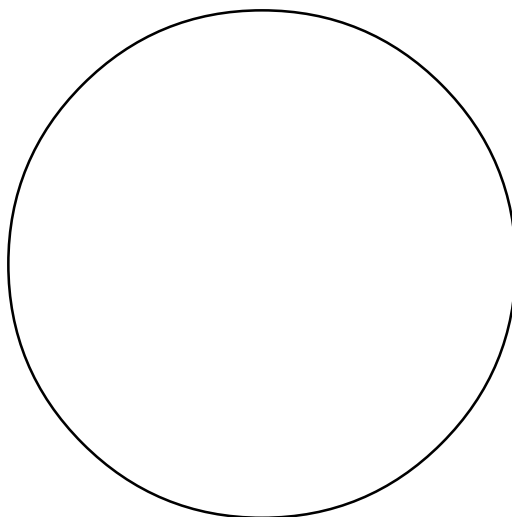
7. The daughter cells:

- A. Contain the identical number of organelles
- B. Contain the identical nuclei
- C. Have twice as many chromosomes as the mother cell
- D. Share the same nucleus

8. Which structure is not found in plant cells:

- A. Chromatin
- B. Centromeres
- C. Centrioles
- D. Cytoplasm

9. 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. Fill in the pie graph to illustrate the percentage of cells in each phase.



10. What is the relationship between the process of mitosis and cytokinesis?

11. What are the primary differences between mitosis in plants and animals?

## Meiosis

## Analysis

1. What is crossing over? During which phase of meiosis does it occur?
2. Describe the evidence for crossing over in *Sordaria*.
3. Draw a diagram showing asci that have shown crossing over and asci that have not.
4. How does meiosis lead to genetic variability within a population?
5. What are the differences between meiosis in plants and animals?
6. What are the differences between mitosis and meiosis?
7. What is occurring during the G phases of meiosis?
8. How does your answer for the gene distance for ascospore color to centromere (or map units) compare to the rest of the class? What can account for the variability?



## Mitosis & Meiosis

## Related Materials

WARD'S offers the following prepared Lab activities to perform this Advanced Placement Exercise:

- AP Biology Lab #3 (36W7102) - this activity contains enough material for 8 lab groups to perform the mitosis section of this lab as well as a *Sordaria* cross demo plate to reduce the time needed to observe crossing over.
- AP Biology Lab#3 (36W7113) - this activity contains the components listed above except it will only cover the amount of supplies needed for two lab groups.
- AP Biology Lab#3 Refill (36W7123) ñ this contains all of the consumable items for the 8-group activity.
- WARD'S Chromosome Simulation Lab Activity (36W1602) - this activity introduces students to the principles of meiosis by creating their own chromosome models using pop beads. There are materials for 30 students.
- WARD'S Plant and Animal Mitosis Lab Activity (36W1212) - this activity involves the preparation and analysis of onion root tips. Students can explore and examine the development of cellular mitosis and make comparisons between plants and animals. There are materials for 30 students.
- The Phenomena Study Set offers a hands-on, 3 dimensional look at cellular production and the maturation process in cells. There are four types of activities available at WARD'S:
  - Mitosis (81W4502)
  - Meiosis (81W4501)
  - Fertilization and first cleavage set (81W4503)
  - Crossover Set (81W4504)

## Mitosis & Meiosis

## Further Investigations

### Mitosis:

Look at samples of other fast dividing tissues, such as: embryos of animals, and the tip of the roots, stems, or leaves in plants.

### Meiosis:

Look at the anthers of lilies and other angiosperms.

Look at microscope slides of the testes of arthropods and mammals, including humans.

*Allium* — A genus of the family *Liliaceae* that includes onion, garlic and leeks.

Apical meristem —The meristematic region near the tip of plant roots. Meristems are those tissues able to retain their ability to divide and produce new cells. Many of these cells mature, differentiate, and become other parts of the plant, while others remain part of the meristem.

Ascomycete fungi —The sac fungi; the largest class of Eumycetes, the true fungi ( of the Phylum *Thallophyta*). Organisms in this group are characterized by possession of a saclike sporangium (ascus) in which ascospores are developed. Includes the yeasts, blue molds, mildews and truffles.

Ascospores —Are the results of meiosis in Ascomycete fungi. Eight ascospores are produced by meiosis followed by mitosis, and they are contained in an ascus. The ascospores are haploid, and have the ability to grow into complete adult individuals after sporulation.

Ascus —The ascus is the sac in which the ascospores of Ascomycete fungi mature and are contained. The asci contain eight ascospores which are the products of meiosis, and are themselves contained in the perithecia.

Asexual reproduction Reproduction through mitosis only, without a reduction in the number of chromosomes. The daughter cells are genetic duplicates of the parent cells.

Asters —The stellate rays forming around the dividing centrosome during mitosis.

Blastula —The blastula is an early stage of embryo development where the cells of the embryo form a hollow ball of rapidly dividing cells. This ball is known as the blastula.

Centrioles —A minute hollow cylinder closed at one end and open at the other, found in the cell center or attraction sphere of a cell. Preceding mitosis it divides, forming two daughter centrioles (diplosomes). During mitosis the centrioles migrate to opposite poles of the cell and each forms the center of the aster to which the spindle fibers are attached.

Centromere —A clear region on a chromosome which marks the junction of its two arms.

Chitin —A white horny substance in the outer covering of body of some invertebrates such as crabs. It is also found in some fungi.

Chromatids —Either of the two bodies resulting from the longitudinal splitting of a chromosome.

Conidia —Asexual spores of fungi.

Crossing over —The process occurring during the synapsis of prophase in meiosis I where the pairs of homologous chromosomes overlap each other and exchange portions of their DNA. This allows for genetic recombination, and is one way sexual reproduction ensures variation among the individuals of a species.

Cytokinesis —The process by which the cytoplasm, as well as the various structures and organelles within it, is divided to form new cells. This action is usually synchronized with the process of mitosis. Cytokinesis usually begins during early anaphase.

Daughter cells —The cells formed as a result of the division of the mother cell.

Diploid —The state of a cell that has double the number of chromosomes (2n) found in gametes. These cells also have paired homologous chromosomes. The somatic cells of most plant and animal species, including humans, are all diploid.

Eukaryotic — An organism composed of one or more cells with visibly evident nuclei.

Equatorial plate —Mass of chromosomes at the equator or middle of the nuclear spindle during the equal division of nuclear material occurring in cell division.

Gametes —A mature male or female reproductive cell; the spermatozoan or ovum.

Genome — The haploid chromosome complement.

Germ cells —Germ cells are those specialized cells that are able to undergo meiosis to form gametes.

Golgi bodies (or Golgi Apparatus) —A lamellar membranous structure near the nucleus of almost all cells. It contains curved parallel series of flattened saccules that are often expanded at their ends. The structure is best seen by electron microscopy. In secretory cells the apparatus functions to concentrate and package the secretory product. Its function in other cells, though apparently important, is poorly understood.

Haploid —The state of a cell that has only unpaired chromosomes, and contains the amount of genetic material found in gametes. This amount of genetic material is equal to half that found in the somatic cells of most plant and animal species. In humans, gametes are the only haploid cells.

Heterozygous —The state of having one or more pairs of genes that are dissimilar. Gametes which are produced will not have the same genes for a given characteristic.

Homologues —Homologues are chromosomes carrying the same genetic loci; a diploid cell has two copies of each homologue, one derived from each parent.

Homothallic —Having only one haploid phase that produces two kinds of gametes capable of fusing to form a zygote.

Homozygous —The state where all genes for a given characteristic are the same. All gametes which will be produced will have similar genes for that given characteristic.

Hyphae—One of the threads that make up the mycelium of a fungus. They increase by apical growth. And they are either coenocytic, which is a multinucleated mass of protoplasm resulting from repeated nuclear division unaccompanied by cell fission, or transversely septate.

Independent Assortment —The principle of Mendelian genetics that holds that the alleles for a gene are distributed among gametes independent of the distribution of the alleles for other genes. For example, assume a tall pea plant with yellow peas is heterozygous for both traits. Whether or not the gene passes on the allele for tallness or shortness to a particular one of its gametes has no impact on the allele the gamete will carry for the color of its peas. Independent assortment is not absolute, however, and is influenced by the location of genes on the same or different chromosomes.

Kinetochores —The kinetochore is the structural feature of the chromosome to which the microtubules of the mitotic spindle attach.

Microtubules—Elongated, hollow structures present in cells. They are important in cellular rigidity and converting chemical energy into work. They increase in number during mitosis.

Mutation —A sudden permanent variation in a characteristic of offspring.

Mycelium —The mass of filaments (hyphae) that constitutes the vegetative body of fungi such as molds.

Mycelium (pl. mycelia) — The body of the fungus. Mycelia are composed of a mass of thread-like, individual strands, called hyphae.

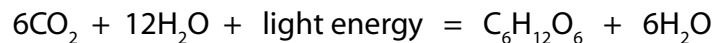
**Nuclear envelope** —This consists of two parallel membranes enclosing a narrow perinuclear space, enveloping the nucleus. Prior to electron microscopy the nucleus was thought to be surrounded by a single, thin membrane.

**Organelles** —A specialized part of the cell which performs a specific function, such as the mitochondria, Golgi apparatus, endoplasmic reticulum, lysosomes, and cell centriole.

**Parasites** —An organism that live either within, on, or at the expense of another organism, referred to as the host, without contributing to the survival of that organism.

**Perithecium** —A spherical, cylindrical or flask-shaped hollow fruiting body in various Ascomycetes fungi. It contains the asci and usually has a terminal pore.

**Photosynthesis** —The process by which plants are able to manufacture carbohydrates by combining carbon dioxide from the air and water from the soil, utilizing light energy in the presence of chlorophyll.



Only plants containing chlorophyll are capable of producing sugars. The red and blue waves of the spectrum are absorbed by the chlorophyll, but other rays are rejected.

**Random** —A random occurrence is one that happens without plan, method or rule. In the case of genetics, a random assortment is one where the chromosomes are combined in the next generation without a guaranteed outcome.

**Region of Elongation** —The region of the plant root where growth actually occurs, making the root longer, and pushing it through the soil. The cell activity in this region is characterized by a great deal of individual cell growth.

**Region of Maturation** —In this zone of the plant root, individual cells achieve their full size and are differentiated into the various types of plant tissue.

**Root Cap** —The very tip of the plant root. It composes an outer covering of cells in order to protect the interior cells from damage as the root makes its way through the soil.

**Saprobies** —An organism that lives on dead or decaying matter, such as a microorganism.

**Segregation** —The principle of Mendelian genetics that states that the paired genes of an organism must separate when forming gametes. This splits up (segregates) the alleles for each gene as the gene pairs are separated, allowing the formation of a pair of genetically different gametes.

**Septate hyphae**—Hyphae in which the nuclei are transversely divided by septa into individual ascospores. Non-septate hyphae are marked by a multinucleate mass of protoplasm.

**Sexual spores** —A reproductive cell, usually unicellular, produced by plants and some protozoans. Usually spores are asexual, but certain fungi form sexual spores (oospores, zygospores and ascospores). Spores generally possess a thick wall enabling the cell to withstand unfavorable environmental conditions.

**Sister Chromatids** — The copies of a chromosome produced by its replication.

**Somatic** —Nonreproductive cells or tissues.

**Somatic Cells** —All the cells of an organism that are not involved in sexual reproduction, and therefore, do not undergo meiosis. Somatic cells only divide by mitosis, yielding daughter cells that are identical to the parent cell.

*Sordaria fimicola* —Is a member of the Kingdom Fungi. The fungi consist of eukaryotic organisms that live either by absorbing dead material (saprobes), or by absorbing organic material from the bodies of living organisms (parasites).

Spindle fibers —These are a bunch of delicate fibrils, containing no chromatin, which connect the two centromeres or asters. The chromosomes arrange themselves on the spindle in an equatorial plate.

Synapsis —The fusion of pairs of homologous chromosomes all along their length as the chromosomes form tetrads in the early stages (prophase) of meiosis. The lining up of the chromosomes during synapsis affords them the proximity to allow crossing over to occur between the homologous chromosome pairs.

Tetrad —The group of four chromatids connected by a centomere that results from synapsis in the early stages (prophase) of meiosis I.

Zygote —The diploid cell produced in sexually reproducing eukaryotic organisms, resulting from the fusion of two gametes. After their formation, zygotes normally undergo rapid cell division and grow into an embryo.