MITOSIS AND MEISOSIS

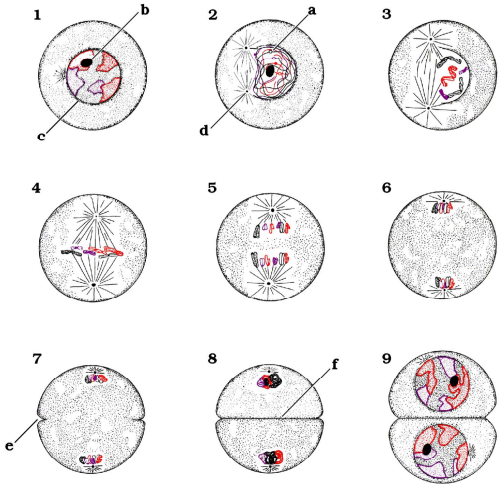
The two labs dealing with cell division are juxtaposed as they are closely related and yet have some significant differences. Learning to compare and contrast mechanisms is very important to the serious student.

Mitosis and **meiosis** are both types of cell division. They both deal with replication of chromosomes. The chromosomes are divided in a similar way and transported to newly formed cells. Some of the stages resemble each other in significant ways. We use these terms to refer to the stages of mitotic divisions; prophase, prometaphase (in some texts), metaphase, anaphase, telophase. Cytokinesis produces cleavage. The result is two daughter cells that are alike. This type of cell division is responsible for growth of the organism and tissue repair. Rapid mitotic division is also a characteristic of tumors and cancers. In the parent cell is diploid the daughter cells will also be diploid (2N). If the parent cell is haploid (N) the daughter cells shall be haploid.

Meiosis involves two separate divisions which result in a reduction of chromosome number. The diploid organism produces cells that are haploid or have one of each chromosome pair, rather than the two in each of the daughter cells of mitosis. These cells are often involved in syngamy or sexual fertilization. We designate the stages the <u>first division</u> of Meiosis I: prophase I, metaphase I, anaphase I, telophase I. Meiosis is often followed by cytokinesis. The <u>second division</u>, meiosis II has stages referred to as prophase II, metaphase II, anaphase II and telophase II. Cytokinesis follows.

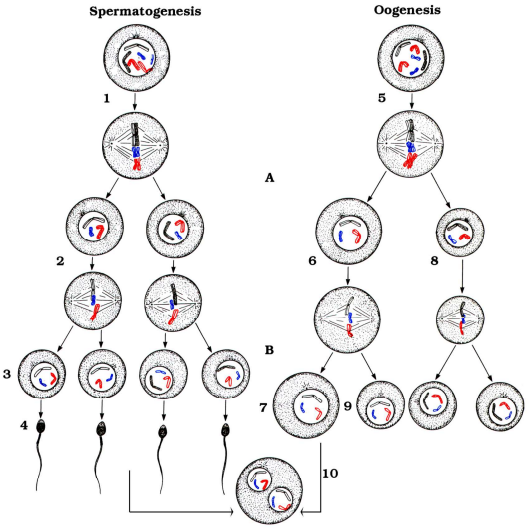
The diagram below compares the two processes. Each parental cell contains 3 pairs of chromosomes to demonstrate the mechanism.

ANIMAL MITOSIS AND CYTOKINESIS



1. Interphase
2. Early prophase
3. Late prophase
4. Metaphase
5. Early anaphase
6. Late anaphase
7. Early telophase
8. Late telophase
9. Interphase
   a. Centromere
   b. Nucleolus
   c. Nuclear membrane
   d. Centriole
   e. Cleavage furrow
   f. Cell membrane

ANIMAL MEIOSIS



A. First meiotic division
B. Second meiotic division

1. Primary spermatocyte (in prophase of meiosis I)
2. Secondary spermatocyte
3. Spermatids (n) haploid
4. Spermatozoa (haploid)
5. Primary oocyte (in prophase of meiosis I)
6. Secondary oocyte
7. Ootid (n) haploid
8. First polar body
9. Second polar body
10. Ovum

Mitosis: Chromosome Structure and Cell Division

Objectives:

The student should be able to:

1. Discuss the levels of organization of chromosomes.
2. Draw and label the four stages of mitosis.
3. Discuss cell changes during interphase.
4. Compare and contrast mitosis in plant and animal cells.
5. Prepare a slide using tissue from an onion root tip.

Key terms:

a. binary fission
b. cell cycle (G1, S, G2, M (mitosis), Cytokinesis)
c. cell plate
d. centrioles
e. centromere
f. chromosome
g. coiling
h. deoxyribonucleic acid
i. diploid
j. euchromatin
k. gametes
l. haploid
m. heterochromatin
n. histones
o. homologous chromosomes
p. interphase
q. kinetochore
r. metaphase plate
s. mitosis: prophase, prometaphase, metaphase, anaphase, telophase
t. nucleosomes
u. spindle apparatus
v. spindle fibers

Organisms grow by cell division. Eukaryotes undergo mitosis for the purpose of growth or for replacement of damaged tissue. During this laboratory you will study the stages of the cell cycle and the major event, mitosis. Mitosis involves replication of the nucleus. The division of the cells after mitosis is referred to a cytokinesis. Each of the new nuclei have cellular contents that have been partitioned into halves of the structure which, upon division, yields two similar cells.

Bacterial reproduce by binary fission. The circular loop of DNA is replicated and each loop becomes associated with the plasma membrane. The division of the cell begins at a point between the two DNA rings.

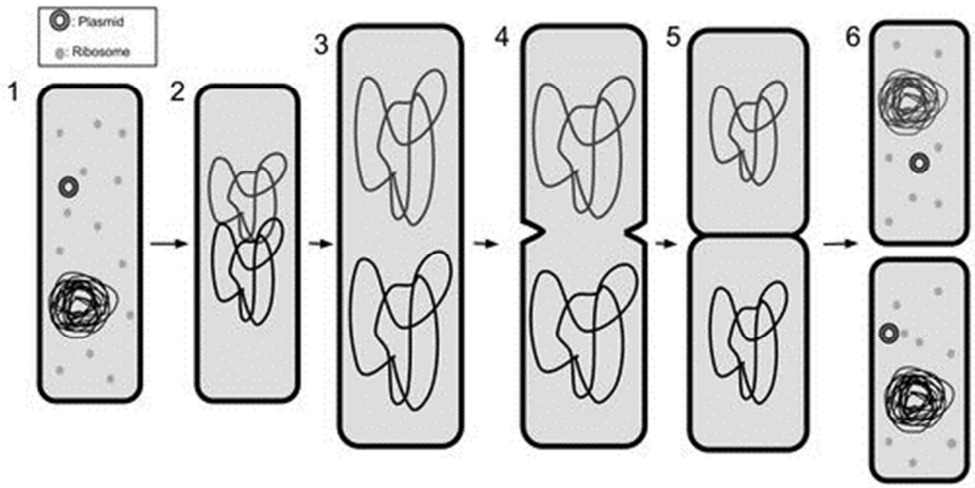


Figure 1. Binary Fission

Organization of the Chromosome

The DNA that constitutes about 40% of the chromosome is a double helix. Each half of the helix is a polymer of nucleotides. The two halves are hydrogen bonded together to create the double helix. A great deal of protein is associated with the DNA. Central histones as well as linker histones exist together with the DNA. The complex of DNA and protein is referred to as chromatin. The DNA is organized into subunits called nucleosomes that contain a central histone and linker histone about which about 200 base pairs of DNA are wound.

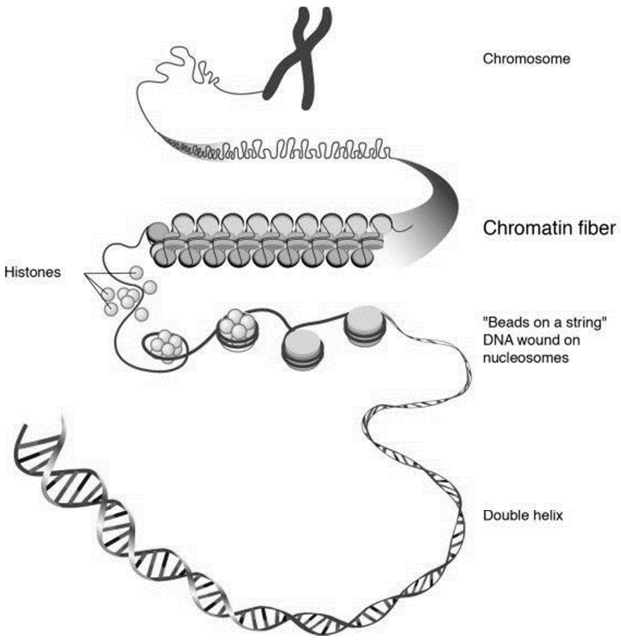


Figure 2

If we examine the structure of a eukaryotic chromosome during cell division, we see a discreet, slightly thickened strand. Each of these strands contains DNA that is very highly coiled. If we were to take a small section of this strand and magnify it, we would see supercoils of chromatin. Further examination would show additional coiling within the supercoil. During interphase this coiling is relaxed and the chromatin is spread out and the individual chromosomes cannot be observed. Some DNA will be always condensed and is referred to as heterochromatin. The DNA that undergoes condensation during mitosis is referred to as euchromatin. Why does this packaging take place for mitotic division? Imagine attempting to move 46 strung out strands in a DNA in a typical human cell. A great deal of breaking and snarling might occur. Nature has designed unique packaging for chromosomes during cells division for the protection of this important polymer. The cells that we will be focusing on are diploid (two copies of each chromosome per cell) while gametes (sex cells) as well as certain other cells are haploid (one copy of each chromosome per cell).

Mitosis

Prior to Mitotic (or M) phase of the cell cycle, Interphase can be divided into stages: Gap 1 (first growth phase), S phase (DNA synthesis) and Gap 2 (growth and replication of organelles) phases. All three phases are associated with cell growth and replication of organelles.

The Mitotic phase is divided into two distinct steps - Mitosis which involves nuclear replication, and Cytokinesis, which results in the division of the cell proper. Mitosis is then divided into five phases: prophase, prometaphase, metaphase, anaphase and telophase. These phases are followed by cytokinesis, which frequently occurs concurrently with the final step of mitosis. It is a very common mistake to consider cytokinesis as a part of mitosis but it should be remembered that cytokinesis is separate from mitosis, the division of the nucleus. We also need to recognize that the differences in the structure of plant and animal cells results in differences in cytokinesis in animal and plant cells. In animal cells the cell membrane invaginates and appears to pinch off creating two cells. In plant cells we observe the formation of cell plate that is the beginning of the formation of the new cell wall.

The Stages of Mitosis:

1. **Prophase:** The nucleolus disappears and the chromatin fibers condense. The chromosomes appear as two sister chromatids that are joined by a central centromere. The mitotic spindle begins to appear. The spindle apparatus emanates from the centrioles which play a role in cell division. Chromosomes that appear to be similar in shape and form and that carry genes for the same traits are called homologous chromosomes. At a given locus or location, alleles for the same trait will be located.

2. **Prometaphase:** It is during this stage that the nuclear membrane breaks down and the microtubules begin to invade the nuclear area where they are destined to attach themselves to the kinetochores, central protein discs, of the centromeres of the chromosomes.

3. **Metaphase:** Chromosomes line up on the metaphase plate, a position equidistant from each of the centrioles.

4. **Anaphase:** During anaphase the sister chromatids are pulled apart by the microtubules that emanate from each pole. The centrioles of the cell move further apart. By the end of this stage we observe the separation of collections of chromosomes which are located near the two poles.

5. **Telophase:** the cell continues to elongate as the daughter nuclei form at the poles. As nuclear envelopes reform and the nucleoli develop the chromatin becomes less tightly coiled and cytokinesis is often well underway at this time.

Procedure for the preparation of onion root tip slides:

1. Obtain an onion root tip. Be certain to cut a few millimeters of the distal end (the end furthest from the bulb) of the root and place it on the slide.

2. Place two drops of 1 M HCL (hydrochloric acid) on the tip. Place it on the light box, a warm light source, to develop. The acid will cause the cells to begin to disassociate from each other.

3. Blot the slide, taking care not to pick up the tissue with the paper.

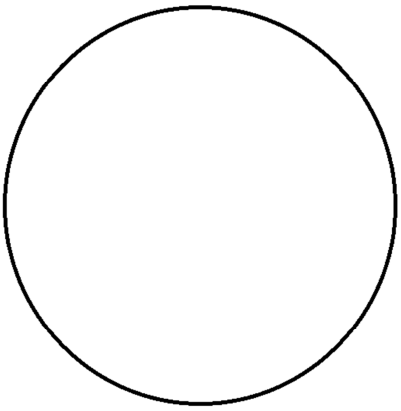
4. Drop two drops of toluidine blue on the tissue and allow it to develop on the light box for five minutes.

5. Blot off the dye and place two drops of toluidine blue on the slide and place a coverslip on the slide.

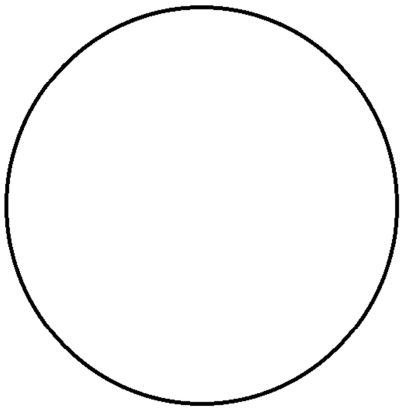
6. With the ball of the thumb apply pressure to the coverslip to obtain a smear of the tissue. Be certain to carry this out on a hard surface.

7. Examine the slide preparation under scanning, low and high power. If the preparation is more than one cell layer thick it will be difficult to observe the stages of mitosis, and probably not enough pressure has been used in the squash. If too much pressure was used, the nuclei will be mechanically damaged and the chromosomes will not be distinguishable.

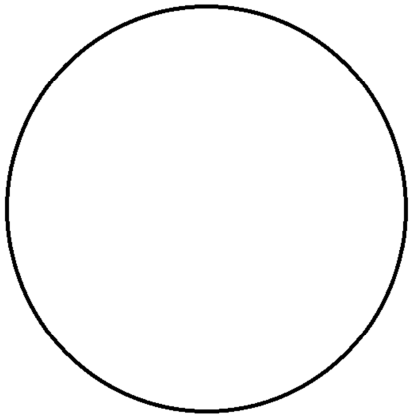
Draw the representative stages of mitosis and label them.



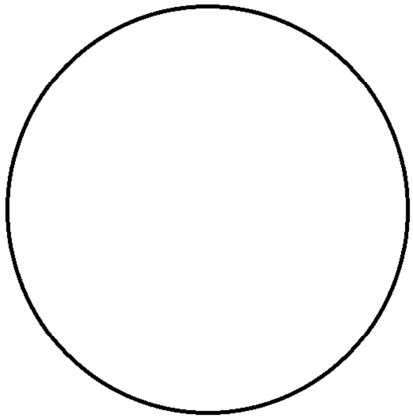
Prophase:



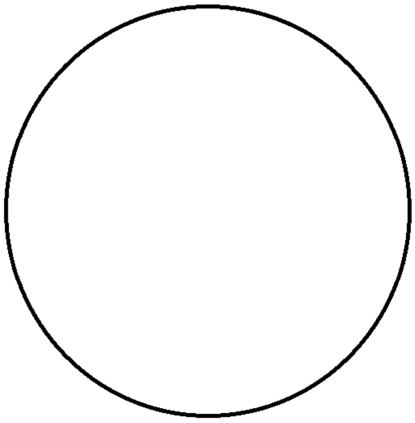
Prometaphase:



Metaphase:



Anaphase:



Telophase/Cytokinesis

Examine animal cells undergoing mitosis and observe the differences between cytokinesis in them. Draw animal cells in late telophase/cytokinesis.

Questions:

1. Briefly describe the events of interphase.

2. Describe the events of the phases of mitosis.

3. If a cell with 16 chromosomes undergoes mitosis, how many chromosomes does it have during anaphase? How many after cytokinesis?

4. When would you expect to find the most mitotic activity in the life of the organism?

5. Why is animal cell cytokinesis different from plant cell cytokinesis?

MEIOSIS

Objectives:

1. At the end of this laboratory, the student should be able to:

2. Demonstrate the stages of Meiosis I and Meiosis II.

3. Demonstrate independent assortment.

4. Discuss the sources of genetic variation and how they, with natural selection, contribute to evolution.

5. Demonstrate crossing over.

6. Compare and contrast oogenesis and spermatogenesis.

Key Terms:

a. alleles
b. asexual reproduction
c. chiasma (chiasmata)
d. diploid
e. haploid
f. independent assortment
g. nurse cell
h. oogonium
i. ovum
j. polar body
k. primary spermatocyte
l. secondary spermatocyte
m. spermatogonium
n. synapse
o. synaptonemal complex
p. syngamy
q. tetrad
r. zygote

Meiosis is a type of cell reproduction that involves reduction of the chromosome number. We observe this process in organisms that sexually reproduce. It is important to halve the chromosome number in the production of gametes or sex cells in order that when they come together in syngamy that the diploid (2N) condition is restored. Meiosis consists of two divisions, meiosis I and meiosis II. We find these two divisions separated by an unusual interphase in which we observe no cell growth and no DNA synthesis.

Phases of Meiosis I

Prophase I:

The first event of meiosis I is Prophase I (Note: Figure 1) in which the chromosomes continue to condense. The homologous chromosomes come together in a tetrad formation held together by a protein lattice. This system is referred to as the synaptonemal complex. (Note: Figure 2) It is while the chromosomes are in synapse that chiasmata occur. This is the "crossing-over" of neighboring arms of chromosomes. (Note: Figure 3) Later, parts of chromosomes are exchanged as the homologs are pulled apart during Anaphase I. They continue to move to opposite poles of the cell and microtubules are spun out between them. The nucleoli disperse and the nuclear membrane fragments but neither of these processes are readily observable. This is often the longest phase of meiosis.

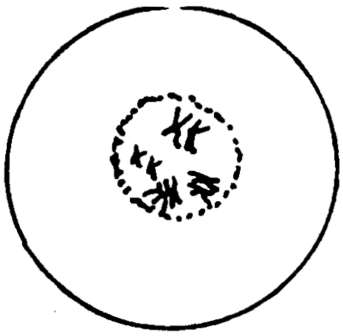


Figure 1. Prophase I

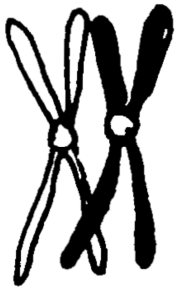


Figure 2. The Synaptonemal complex

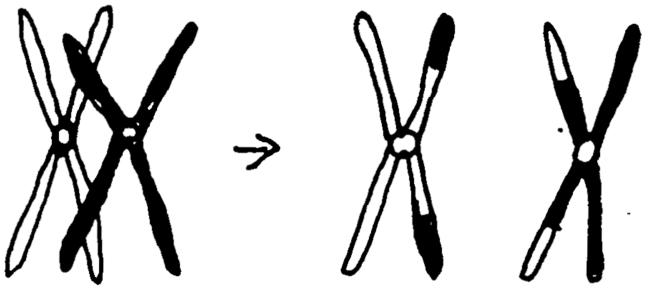


Figure 3. Chiasma

Metaphase I:

Tetrads are now arranged along the metaphase plate. The spindle fibers from one pole are attached to the kinetochore of one homolog while fibers from the other pole are attached to the kinetochores of the other homologs.

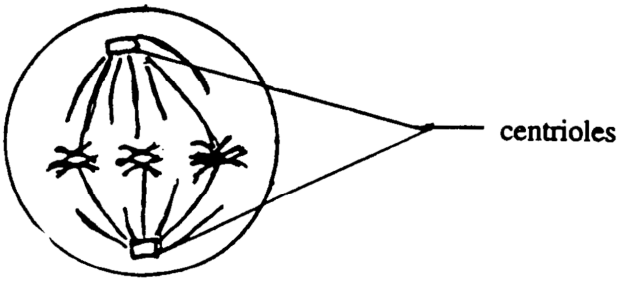


Figure 4. Metaphase I

Anaphase I:

While sister chromatids remain attached at the centromere, the spindle apparatus moves the homologous chromosomes toward opposite poles.

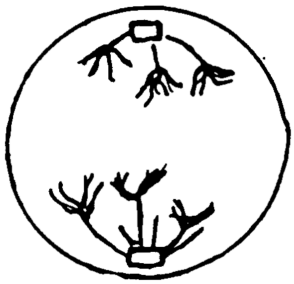


Figure 5. Anaphase I

Telophase I and Cytokinesis:

As cell division proceeds, the spindle apparatus pulls the homologs toward opposite poles creating two haploid chromosomes sets. In some organisms nuclear membranes reform (species specific) and nucleoli reappear. Cytokinesis separates these two haploid cells. Remember that each chromosome still consists of two sister chromatids.

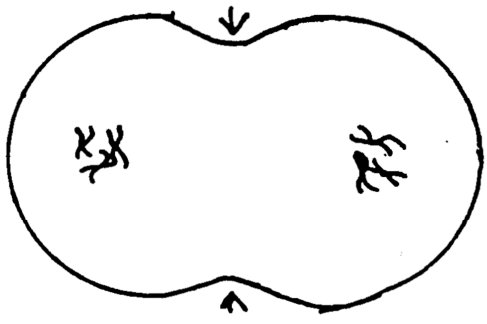


Figure 6. Telophase and Cytokinesis

Phases of Meiosis II

Prophase II:

In Prophase II the spindle apparatus reforms and the microtubules reattach to the kinetochores of the chromosomes.

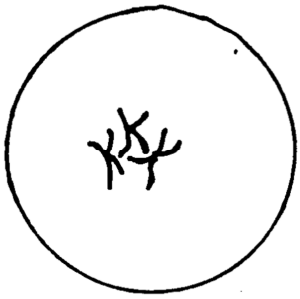


Figure 7, Prophase II

Metaphase II:

This stage resembles mitosis as each chromosome consists of sister chromatids. The chromosomes line up on the metaphase plate, once again with the kinetochores of the centromeres facing the poles.

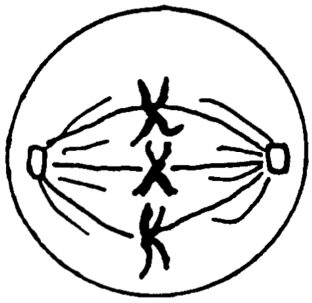


Figure 8. Metaphase II

Anaphase II:

The spindle apparatus pulls the chromosomes toward each pole, separating the sister chromatids.

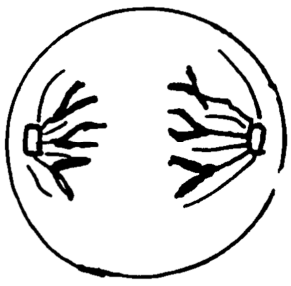


Figure 9. Anaphase II

Telophase II and Cytokinesis:

The haploid nuclei begin to reform at opposite ends of the cells and cytokinesis occurs. We now have four gametes from two meiotic divisions of one cell.

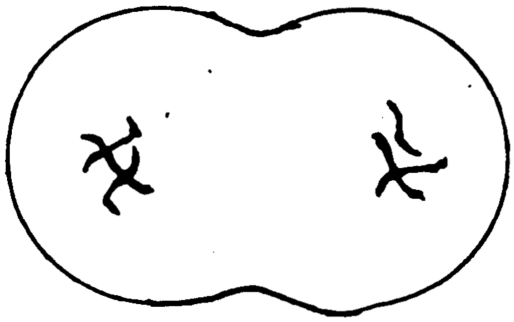


Figure 10. Telophase and Cytokinesis

Chiasma

To demonstrate crossing-over, we will take colored beads and make two chromatids with twenty yellow beads each and hold them together with a twist tie, centromere. Construct two more chromatids of twenty green beads and attach them with a twist tie. Lay them on the table next to each other, as they might appear in synapse. (Figure 2) Cross equal sections of a yellow chromatid with a green chromatid and exchange three beads each. (Figure 3)

Note that we have produced genetic recombination of traits. Originally, each yellow chromatid represented the chromosomal content of one parent, the green chromatid, the other. Now we see that for a simple chiasma, the genes of one parent have been exchanged with genes of another in one chromatid. Genetic reciprocal exchanges are quite frequent and can result in variations that may prove to be deleterious or advantageous to the organism. The number of crossovers has been used historically to map genes, or to locate various genes with respect to each other on the chromosome. Usually, the longer the arms, the more chiasmata are possible.

Modeling Independent Assortment

In this section of the laboratory we will model Mendel's Law of Independent Assortment. We will use pipe cleaners of two colors, with three different sizes of each color.

1. Students in groups of three or four, select two pipe cleaners of each size and each color. Each group should have two large orange, two medium orange, two small orange, two large yellow, two medium yellow and two small yellow pieces of pipe cleaner.

2. Twist the two of each size and the same color together to create a chromosome consisting of sister chromatids.

3. Place the tetrads (one yellow chromosome consisting of sister chromatids next to its homolog, the orange chromosome, of the same size that consists of sister chromatids) side by side. (Figure 11)

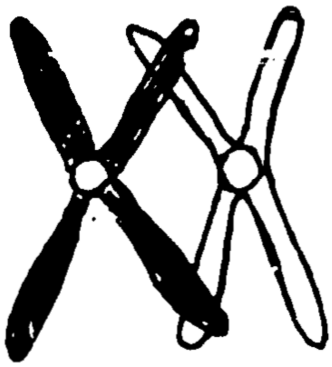


Figure 11. Tetrads

4. Place the centrioles at each of the poles of the cell.

5. Line up the tetrads on the metaphase plate.

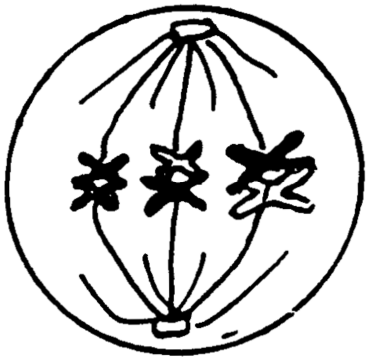


Figure 12. Metaphase Plate

6. Slowly separate the homologs, moving them toward the poles.

7. Note that the reforming nuclei now have all yellow chromosomes, two yellow and one orange or one yellow and two orange chromosomes. Each chromosome still consists of sister chromatids. There are 2^n possibilities for chromosome combinations where n = number of homologous pairs. In this case, there are 2^3 possibilities.

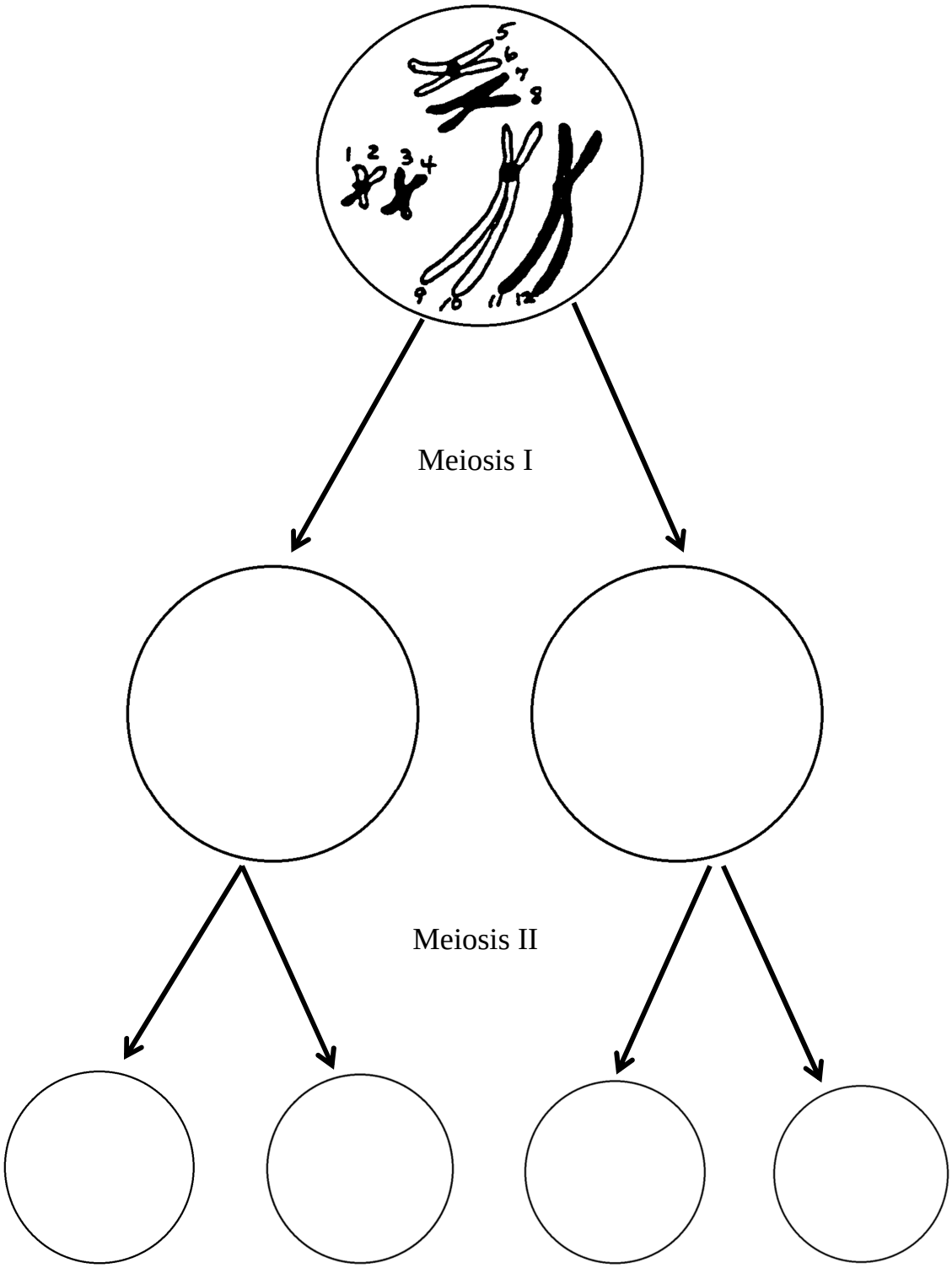
8. To demonstrate meiosis II, take the two cells that you have just produced and prepare each of them to go through the second division by placing the centrioles for each cell at each pole.

9. Line up the chromosomes that consist of two sister chromatids on the metaphase plate of each of the two cells.

10. Separate the sister chromatids and slowly move them to the poles of the cells.

11. You have just produced gametes or sex cells! Note that there are various possibilities for each gamete. The combination of chromosomes can be varied depending on which chromosomes (yellow or orange) move to a given pole.

Practice the concept of Independent Assortment by completing the work sheet on this page.



Independent assortment, chiasmata, and sexual fertilization create new combinations of alleles which may affect the expression of other genes in positive or negative ways. Natural selection then acts to select for beneficial allelic combinations.

Gamete Production in Mammals

Spermatogenesis:

Gametogenesis in males is referred to as spermatogenesis. This typically occurs in the testes in cells lining the seminiferous tubules. (Figure 13) The spermatogonium is a cell that may undergo mitotic division, to simply produce more cells like it or undergo meiosis, which is the reduction/division process required to produce a haploid gamete. In males the meiotic process produces four sex cells for every spermatogonium destined to undergo meiosis.

The secondary spermatocytes that are produced by the meiotic second division are "nursed" by Sertoli cells (Figure 14) or nurse cells that remove some of the cytoplasmic contents. During the differentiation of the sperm, flagella develop and the mature sperm are then released into the seminiferous tubules.

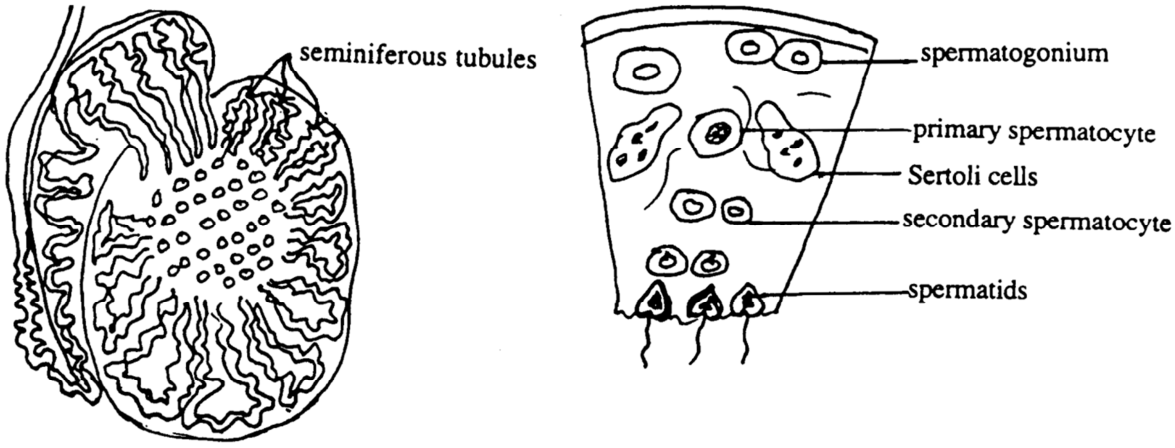


Figure 13, Testes l.s.

Examine a cross section of grasshopper testes and identify a mature sperm. Draw and label the structures seen.

Oogenesis:

In human females, the newborn contains all of the immature ovum that she will ever produce. She has a limited quantity of immature ovum which will be expelled into the fallopian tubes on a regular basis for thirty or forty years between puberty and menopause. Meiosis of the oogonium does not yield four equal germ cells. Each of the two divisions is unequal, ultimately producing one large ovum and three polar bodies which disintegrate.

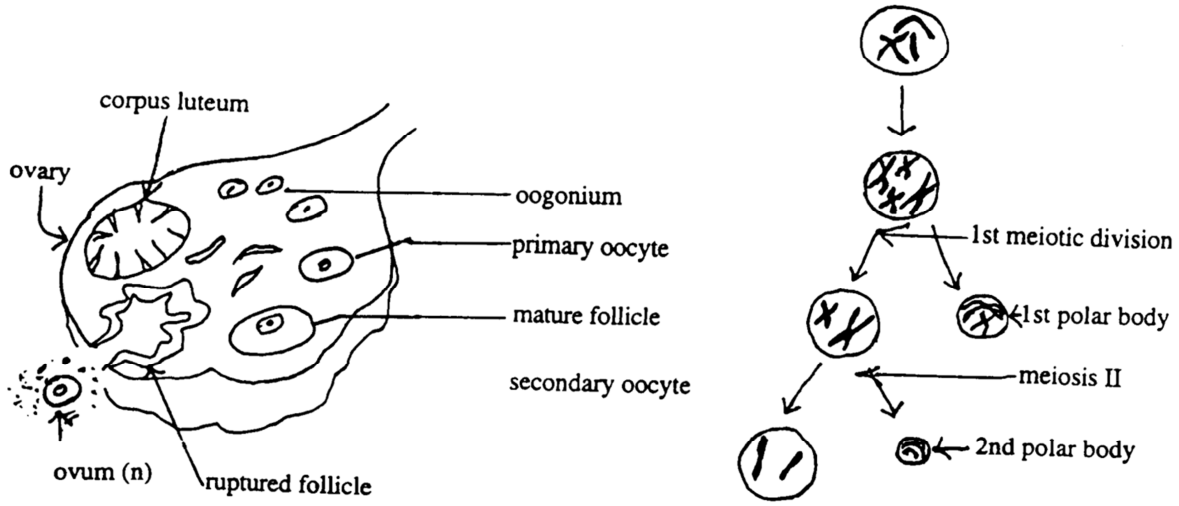


Figure 14. Oogenesis

Gamete Fertilization in *Ascaris*

Examine a cross section of *Ascaris megalocephala*. *Ascaris* is a roundworm which is parasitic and found in the intestine of some mammals. The developing eggs are to be studied in various states of meiosis: sperm entrance, tetrad stage, maturation, pronuclear stage, early cleavage. Just as in the oocytes of other animals the cells, prior to fertilization, are found in Prophase I. Only after penetration by the sperm, which are without flagella, does meiosis proceed.

Ascaris has a diploid number of 4. During Prophase I you will view 8 chromatids, two per chromosome, in each oocyte. During late prophase the tetrads consist of 4 chomatids, two per chromosome. The homologs will be in synapse, or arranged in a synaptonemal complex.

During the first division of the oocyte you will notice that one of each homologous pair, (which still consists of two chromatids) will move to each pole resulting in an unequal division. The smaller cell is the first polar body which will undergo division and who products will ultimately disintegrate. The second division of the larger cell will result in another unequal division and one group of chromosomes will be sequestered in the second polar body as well as in the oogonium. The large cell will become the ovum.

When the ovum is in interphase and the nuclei of the sperm and the ovum have not fused we see two nuclei. These are pronuclei. After fusion of the pronuclei, the egg is referred to as zygote. Division or cleavage begins to occur to create the embryo.

Student Questions

1. What is the synaptonemal complex? When do we observe it in meiosis?

2. What is a chiasma? Demonstrate the result of this chiasma.

3. What are differences between Anaphase I and Anaphase II?

4. Compare oogenesis and spermatogenesis.

5. What is the chief benefit of independent assortment?

6. Discuss the benefits of genetic variation and relate this to environmental pressures of natural selection.