

Laboratory 4.

Meiosis, Mitosis

BRING TO LAB: YOUR TEXTBOOK, WITH DIAGRAMS OF MITOSIS AND MEIOSIS

Eukaryotic organisms all share advanced cell structures you think of as typical. They have:

- 1) a nucleus
- 2) genetic information borne on a set of discrete chromosomes
- 3) other organelles like chloroplasts and mitochondria.

Mitosis is the term for the regular cell division typical of all cells in the bodies of eukaryotes. There are two major events: first, replication (the production of a copy) of each chromosome in the set, and second, cell division, during which the chromosomes are divided into two similar groups, and each group is incorporated into a separate cell along with other cell components like mitochondria.

Eukaryotic organisms go through reproductive cycles that include several distinctive phases: the two landmark events that divide phases in most eukaryotic life cycles are syngamy (the fusion of gametes) and meiosis (the formation of spores or gametes). In the case of syngamy, two gametes, each with a single set of chromosomes, fuse to yield a single cell called the zygote, which has two similar but usually not identical sets of chromosomes. During meiosis, a single cell with two sets of chromosomes goes through two cell divisions, but only one replication of the chromosomes, to yield four cells, each of which has only one set of chromosomes. Eukaryotic life cycles are, in these terms, endless alternations of syngamy with meiosis, that is, endless alternations of cells with one set of chromosomes (called haploid) fusing to yield cells with two sets of chromosomes (called diploid) and diploid cells undergoing meiosis to yield haploid cells.

The idea behind this lab is to see as much as possible of both syngamy and meiosis, and to contrast meiosis with mitosis. Much of the work in this lab requires considerable care – it can be somewhat frustrating, but very rewarding.

I. MITOSIS: FILM LOOP OF THE STAGES OF CELL DIVISION

There is a film loop of mitosis in plant cells for you to look at during the lab. The particular plant chosen for the film loop is so-called African Blood Lily (*Haemanthus catherinae*). The filmmakers removed cells from the growing seeds of these plants and were able to make movies of them as they went through mitosis. The most important thing for you to learn from the film loop is that mitosis, just like meiosis, is a continuous process in which there is a gradual transformation of one cell into two separate cells. The traditional names for the different stages of cell division are for convenience only, they do not represent real, discrete episodes but rather parts of a process.

II. MITOSIS: ONION ROOT TIP MITOSIS PREPARATION

Onions are excellent material for studying mitosis, because lots of roots can be generated in a few days simply by sitting an onion in a dish of water, and it is fairly easy to see mitosis in onion root tips.

Goal:

To see as many stages of mitosis as possible through making mitotic preparations from onion root tips. From these stages understand the flow of mitotic divisions.

Procedure:

In summary, what you need to do in order to see mitotic cells in onion root tips is A) soften up a tiny bit of the root tip in acid, then B) spread the root tip cells apart and stain them with a chromosome stain, and finally C) squash the cells under a coverslip to flatten them and spread the chromosomes apart.

A. Isolating and Softening the Tissue with Dividing Cells

Equipment:

Assemble paper towels, two probes (needles mounted on wooden handles), microscope slides, and coverslips.

1. Get an onion root from the supply.
2. **CLEAN SLIDES AND COVERSLIPS ARE CRITICAL TO MAKING SUCCESSFUL PREPARATIONS.**

Wipe clean a regular microscope slide with a paper towel and water. Put the clean slide on a new piece of clean paper towel. Take a glass coverslip from the supply and place it on a dry paper towel nearby. Remove and dust for hair that you can see on the coverslip. **PLEASE USE YOUR COVERSLIPS AS MANY TIMES AS POSSIBLE; THEY ARE EXPENSIVE! DISPOSE OF BROKEN GLASS APPROPRIATELY (NOT IN THE SINK)!**

3. Place the onion root tip on the slide. With a razor blade, cut just the smallest bit (1mm) of the root tip away from the rest of the root and discard everything but the little tip. Choose just the dense, ivory-colored part of the root tip; if you are unsure ask your teaching fellow for help. The base is clear, grayish, not dense at all.
4. Add a few drops of the acid-alcohol solution, which is in dropper bottles in the lab. (The acid in this solution will partially dissolve the pectin that holds the cells of these roots together, making them easier to spread apart.)
5. Tend your root tip for 15 minutes; add more acid alcohol as it evaporates from your slide. When your time is up, remove the excess acid alcohol with the edge of a paper towel, and throw the paper towel in the wastebasket so you don't get acid alcohol on yourself.
6. Throw away your root tip and start over any time your preparation becomes hopelessly messed up. There are plenty of roots.

B. Staining the Dividing Cells

1. Add a single drop of acetocarmine stain to the root tip on your slide. (Be careful not to get the stain on you!) While looking at the root tip under a microscope, poke and tap at the root tip in the stain to make the cells of the root tip fall apart.
2. Do this sort of thing for about five minutes. Take time to make sure the root tip is really fragmented into tiny pieces. Add a little more stain if your root-tip starts to dry out.
3. Put a clean glass coverslip over your root tip. Look at your preparation under your microscope to see what yo've done so far. Cells with chromosomes should be visible here and there.

C. Spreading the Dividing Cells: The Squash Technique

YOUR TEACHING FELLOW WILL GIVE YOU A DEMONSTRATION OF SQUASHING

1. Now fold your slide in a paper towel on the bench top. The position of the coverslip should be evident from the stain soaking into the paper towel. Carefully place your thumb right on top of the

- coverslip and press down as straight as possible. Don't move your thumb from side to side, or the cells will be ruined.
2. Your preparation is complete. Scan your root tip cells at medium power under the microscope. Under high power, identify as many stages of mitosis as possible, using your text and the diagrams in the lab as guides.
 3. Count 50 cells on the slide. How many are interphase?

III. MEIOSIS: *TRADESCANTIA* MEIOSIS PREPARATION

In this section of the lab, we will be studying meiosis in the anthers of *Tradescantia*, the spiderwort. *Tradescantia* is an ideal plant for studying meiosis, because a single flowering stem contains a lineup of buds, from the smallest at the bottom to the largest at the top. These largest have already gone through meiosis and produced pollen. Young anthers in buds about six down from the top usually contain cells undergoing meiosis to yield microspores.

Goals:

- a. find buds with anthers at the right stage of development to show meiosis;
- b. isolate and stain the cells undergoing meiosis; and
- c. see as many stages in meiosis as possible.

A. Preparation of slides and coverslips:

CLEAN SLIDES AND COVERSLIPS ARE CRITICAL TO SUCCESSFUL PREPARATIONS

Wipe clean a regular microscope slide with a paper towel and water. Put the clean slide on a new piece of clean paper towel. Take a coverslip from the supply and place it on a dry paper towel nearby. Remove any dust or hair that you can see on the coverslip.

Isolating Anther Cells that are Undergoing Meiosis

Work in groups of four.

Each flowering stem of *Tradescantia* is terminated by a group of flowers, below which are two leaf-like bracts. Cut a group of flowers like the one in the drawing below and put it into a beaker of water. Use this drawing as a guide.

GROUP OF *TRADESCANTIA* FLOWERS

1. Remove the bract from one side.
2. Count back six buds from the open flower; choose buds three through six and have each student in your team work with one of the buds. Every team member should follow these directions. Push aside the petals and sepals to reveal the stamens.
3. Carefully remove the six stamens and put them in a small drop of Farmer's fixative on your microscope slide. (The fixative kills everything in the stamens all at once, so that the chromosomes will be in excellent form for observation.)
4. Add a small drop of aceto-carmin stain to the anther contents. Tap and poke at the stamens just as you did the root tips, with the idea of opening up the stamens so that the cells undergoing meiosis will spill out onto your slide.
5. Remove all the big chunks once you're sure that all the anthers are punctured.

6. Carefully cover your preparation with a coverslip. You can reduce the number of bubbles by placing one end of the coverslip in the stain, and letting it fall over the preparation.
7. Look at your preparation under the medium power of your compound microscope. Decide which stages of pollen development you have in you flower from the drawing of pollen development. You may well have more than one stage within a given flower, so be sure to scan the slide carefully.
8. If you see good mitosis or meiosis, you may want to do a squash to spread the chromosomes out. To do a squash, fold your slide between layers of paper towel, place it on a lab bench, and press directly down on the middle of your slide with the bottom of your thumb. Don=t move your thumb from side to side or you will smudge your preparation.
9. Now share your preparation with the other folks in your lab. The goal as a group is to assemble as complete a set of stages in pollen development as possible, but most especially the stages of meiosis. Use your book as well as the drawings here to look for:

premeiosis	meiosis II
microsporocytes	prophase
meiosis I	anaphase
prophase	telophase
metaphase	microspores
anaphase	pollen grains
telophase	

10. Work together to set up slides in a series of microscopes representing as complete a set of stages as possible.