Chapter 2

Using Chives to Study Meiosis

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Reprinted from: Robinson, A. D. 1993. Using chives to study meiosis. Pages 17-22, *in* Tested studies for laboratory teaching, Volume 7/8 (C. A. Goldman and P. L. Hauta, Editors). Proceedings of the 7th and 8th Workshop/Conferences of the Association for Biology Laboratory Education (ABLE), 187 pages.

Contents

Introduction

Chives, *Allium shoenoprasum*, can easily be grown in a garden or in a flower pot. Chives in pots can be "forced" to produce flowers in the winter by exposing the plants to increased light which mimics a longer day (or shorter night). The inflorescence of chives is an umbel, and within one floral head one can find buds at different stages of development. While it is possible to find all the representative stages of meiosis within one or only a few inflorescences, it should be kept in mind that within a single anther, all the cells will be in the same stage of meiosis. There are only eight pairs of chromosomes in this species. The chromosomes are relatively large and easily stained with orcein or hematoxylin without pretreatment or fixation (although fixed material may be used if preferred). The buds of chives are large enough so that most students will have little trouble manipulating the material using forceps and dissecting needles.

Materials

Chives plants with immature inflorescences
Compound microscope
Forceps with curved tip
Dissecting needles (bent end preferred)
Microscope slides
Cover glasses
Stains (aceto orcein and/or Wittmann's hematoxylin acetic acid)
Paper towels
Lens paper
Immersion oil

"Quick and Dirty" Method

1. Obtain an unopened inflorescence of chives from the material provided, and remove the paper-like covering to expose the developing buds within (buds may have already been removed from the inflorescence). Transfer a few (three or four) of these buds to a microscope slide. Add a drop of stain (either aceto orcein or Wittmann's hematoxylin). If too much stain is added, simply blot some of it away. Be sure to include one or more very small buds. Larger buds will probably contain only pollen grains because the sporogenous tissue will have completed

meiosis. Microspores and pollen grains should be seen, but it is likely they will be found even in some of the smaller buds, so it is best to start with the smaller buds and include larger buds as needed.

2. Use a dissecting needle or forceps to crush the buds in the stain. This will release microspore mother cells from the anthers. Repeated tapping of the buds with the needle may be necessary to release sufficient numbers of microspore mother cells from the anthers. This is especially necessary with younger buds in which the sporogenous tissue tends to be released as a plug of microspore mother cells.

3. It is now necessary to remove the resulting debris. Use the forceps and/or dissecting needles to remove as much of the debris as possible. Even small pieces of petals prevent the cover glass from lying flat and will lead to a distorted image when viewed with the compound microscope. Many cells undergoing meiosis will be left suspended in the stain, and by the time the debris is removed, the chromosomes within those cells will be adequately stained (perhaps even overly stained). If hematoxylin is used, a drop of 45% acetic acid should be added to destain the cells.

4. Add a cover glass. It may be necessary to add more stain or a drop of 45% acetic acid to the preparation in order to make the cover glass lie flat. Blot away the excess liquid, but be careful to avoid adding very much pressure to the cover glass because the chromosomes are somewhat fragile.

5. Observe the slide at low and high dry magnifications. Do not try to use oil immersion at this time. Once the preparation is scanned with the compound microscope, it may be obvious that some pressure on the cover glass is needed in order to spread the arms of the chromosomes. This will be especially true when viewing cells in the earliest stages of meiosis. If sufficient stain has been blotted away from the edges, the cover glass will be stable enough so that you may now use oil immersion microscopy for detailed observation of good representative stages.

Single Anther ("Elegance") Method

1. Remove a developing bud from the chives plants provided. Place the bud on a slide in a drop of water. Observe the material with a dissecting microscope, and use dissecting needles to remove the petals and sepals which surround the immature anthers. The anthers can be transferred to individual slides or all six can be arranged on one slide. In either case, remove as much of the floral debris as possible since even small pieces of tissue can interfere with the quality of the final preparation.

2. Blot away most of the water using paper towelling. The slide can be tilted to help drain off the water, but care should be used to avoid picking up the anthers on the paper towelling.

3. Add a drop of stain to the anthers and make certain the anthers are where you want them on the slide. Tap the anthers with a dissecting needle to release the sporogenous tissue. Remove the empty anther heads. It may be necessary to add more stain prior to covering the material with a cover glass. Do not squash the material. Excess stain can be carefully blotted away, but the preparation should be viewed with the compound microscope at 100X before undue pressure is exerted on the cover glass. For the early stages of meiosis I (leptotene, zygotene, pachytene), it may be necessary to apply some pressure to the cover glass in order to compress the nuclei enough to distinguish chromosomes. For the later stages, simply blotting away the excess stain will flatten the preparation sufficiently to permit good observation of the chromosomes within the meiotic cells.

4. Once the excess stain has been blotted away and the cover glass appears not to the floating freely, make detailed examination of the meiotic figures with the oil immersion lens.

It may be desirable to preserve some slides for future reference. Temporary preservation can be accomplished a number of ways. One useful technique is to apply a coating of touch-up enamel around the edges of the cover glass. Various fingernail polishes, waxes, and other ringing compounds may also be used, including *KARO* corn syrup! Slides treated carefully this way will last 1 week or more before eventually drying out. Extreme caution should be used if slides treated this way are used for oil immersion microscopy!

Observations

A wide range of cell types may be observed. Some non-meiotic cells will be seen, especially if the "quick and dirty" method is used. Non-meiotic cells will resemble onion root tip cells or other undifferentiated cells. Cells undergoing meiosis will be larger than the cells of the supportive tissue and will contain prominent nuclei or obvious chromosomes. If very small buds are used, pre-meiotic mitotic figures will be observed in what will become microspore mother cells. Meiosis is typically divided into the following stages:

Meiosis I

Leptotene: Chromosomes become visible as threads. Keep in mind that what appears to be a single thread is actually two chromatids because replication took place in the interphase prior to meiosis. It may be necessary to force the cover glass down a bit in order to make individual chromosomes visible.

Zygotene: Pairing of homologous chromosomes occurs. The chromosomes appear thicker and less numerous because pairing occurs. Again, it may be necessary to apply some pressure to the cover glass in order to observe the paired nature of the chromosomes.

Pachytene: The chromosomes become shorter and thicker. The amount of chromosomal material appears to be considerably less because the chromosomes have become much shorter. The fact that the chromosomes are paired should be obvious.

Diplotene: The characteristic feature of diplotene is the presence of chiasmata. The chromosomes are also shorter than in pachytene.

Diakinesis: Repulsion of the centromeres and terminalization of the chiasmata mark this stage. The chromosomes are very short and thick, but they have not yet lined up in a characteristic metaphase figure.

Metaphase I: The chromosomes are lined up on the spindle. The staining procedure used here does not reveal the spindle. A polar view of metaphase I reveals the chromosomes to be organized at one plane in the cell. A non-polar view of metaphase I makes it appear as though the chromosomes are lined up across the middle of the cell.

Anaphase I: Separation of the paired homologs. It should be noted that the chromosomes now appear thinner. Comparisons with metaphase I make it obvious there has been a halving of the genetic material with half going to each pole. Early and late stages of anaphase I will be seen.

Telophase I: Once the chromosomes have moved to the poles in anaphase, they begin to lose their identity and become reorganized into the nucleus. This marks telophase.

Interphase: There is a brief period when the microspore mother cells appear to contain two interphase nuclei. Because this is a brief part of meiosis this interphase may not be seen by every laboratory class. In some species, the chromosomes seem to go directly from telophase I into prophase II.

Meiosis II

Prophase II: Chromosomes become visible as long threads. Note that this occurs in both of the nuclei of the microspore mother cell.

Metaphase II: The chromosomes become aligned on the spindle apparatus. Note that different cells present different configurations depending on the angles from which they are observed.

Anaphase II: Separation of the replicated chromatids. Compare the number of chromosomes and the thickness of the chromosomes with what was observed in anaphase I.

Telophase II: The chromosomes lose their distinctiveness and become organized into nuclei. Note that there will be four haploid nuclei within the confines of what started out as a single diploid cell.

Tetrad: The original microspore mother cell has been divided into four cells. A polar view of these cells resembles an orange with four sections which has been cut through the equator. A non-polar view of the tetrad may be difficult to focus because the "orange sections" appear to be piled on top of each other.

Microspores: The uninucleate haploid tetrads are released, and at this stage are called microspores.

Pollen grains: These result from a mitotic division within the microspores. Stages typical of mitosis can be seen including prophase, metaphase, anaphase, and telophase. Cytokinesis may also be seen. Because of the shape of the microspores, views of metaphase are more often polar than nonpolar, making it possible to count all eight chromosomes, but the same shape makes it difficult to distinguish anaphase from telophase since one generally finds polar views of these stages as well. The resulting pollen grains are bicellular and have distinctive wall markings.

This lab lends itself very well to encouraging students to share what they have found with others. If they have found something worth showing to others, they should announce what they have to share and go see the stages others have found. In this way, a few preparations per student can result in a whole array of meiotic figures for all to see. Students should also compare the stages they have observed with the figures on display.

Preparation of Stains

Aceto Orcein

There are various proportions of dry stain and acetic acid (with or without lactic acid) which can be used to stain chromosomes. The following gives good results with chromosomes of *Allium* sp.: Add 1 g orcein (synthetic), 45 ml acetic acid, and 55 ml water. Boil gently 2–5 minutes, cool, and filter.

There will be some loss of liquid as a result of the boiling, and it may be found that the stain needs diluting. If necessary, the stain may be diluted with 45% acetic acid (45 ml acetic acid and 55 ml water). It may be necessary to refilter the stain to remove dark granules which sometimes precipitate out. There are commercial preparations of aceto orcein which work very well and save the bother of preparing the stain from scratch.

Wittmann's Iron-Aceto-Hematoxylin

Dissolve 2 g of hematoxylin in 50 ml of 45% acetic acid. Add and dissolve 0.5 g iron alum [(ferric ammonium sulfate) Fe (NH_4) $(SO_4)2{:}12H_2O$]. Allow to stand 24 hours, filter, and store in a brown bottle. The stain will last a few weeks but may need to be filtered prior to use.

I have found Wittman's hematoxylin to be a good substitute for aceto orcein. At times, I have had a bad batch of orcein which can lead to a frustrating lab for students. This is a stain from the "old" techniques which requires mordanting for some species. No mordant is necessary for *Allium* and relatives. This stain has never failed me. It is quite likely there will be over staining with this stain, but cells can be destained with 45% acetic acid.

Further Reading

Robinson, A. 1982. Teaching meiosis with chives. Journal of Heredity, 73:379–380.

Robinson, A., and T. Peck. 1990. Postmeiotic mitosis in chives. Journal of Heredity, 81:399–400.

Wittmann, W. 1962. Aceto-iron haematoxylin for staining chromosomes in squashes of plant material. Stain Technology, 37:27–30.