

Meiosis of Normal Diploids

Give the project as much attention as you can in the early weeks will get you a good start on your photos before the end of term where most people find it difficult to find any extra to spend in the cytogenetics lab.

Meiosis is the production of a haploid nuclei from a diploid cell. The joining of two separate haploid nuclei is the basis for sexual reproduction in eukaryotes. Meiosis consists of two separate divisions with only one DNA replication phase. The first division separates homologous chromosomes, and the second division separates sister chromatids.

Plant material for this type of study can be either the microspore phase in this case pollen mother cells from the anther or megaspore cells from the ovary. *Secale cereale* spikes have been collected and dissected from the sheaths of field grown plants. In contrast to the mitotic stages which are continuous, meiosis is synchronised and takes place over a very short period of time. The spikes are harvested in the early summer over a one week time period to get a wide range of material to capture the meiosis event. The lengthiness of this lab is due in part to the fact that the correct anther stage change with the growing conditions in the field which vary from year to year depending on soil moisture and temperature conditions. Once the correct anthers are found, several different stages can usually be seen on one slide.

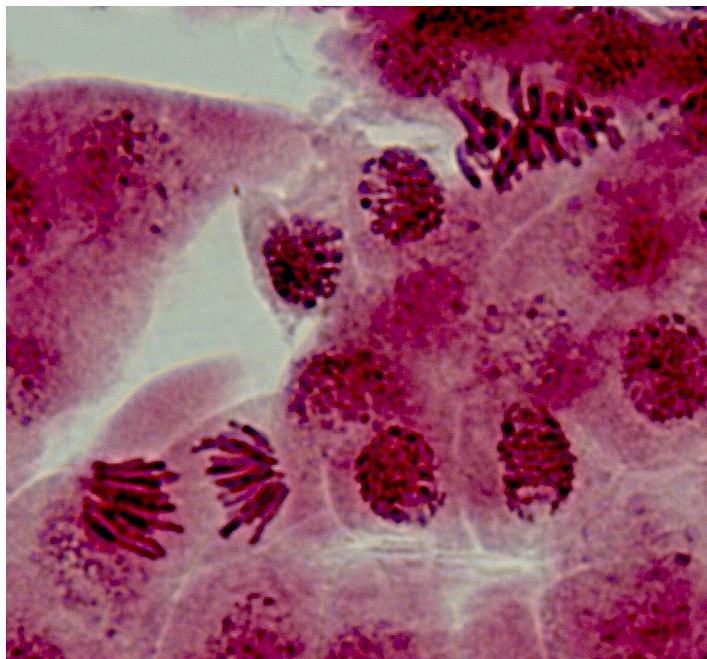


Figure 1: Meiosis of *Secale cereale* Pollen Grain showing several stages of meiosis

Procedure: Staging of the *Secale cereale* (Rye) from previous years indicates that harvesting the spikes or inflorescence when they are in the boot stage, about half to three-quarters of the way up the sheath will give anthers of the right size. These are collected in bulk and placed in Farmers fixative overnight at 4°C. The spikes are then placed in storage solution of 70% ethanol at 4°C.



Figure 2: Prepared rye inflorescences in Petri plate with 70% ethanol. Red triangle indicates a single floret separated from the inflorescence. The opaque anthers can be distinguished from the transparent glume, palea, and lemma.

Anther selection: As was noted in the introduction sampling the right material for observation will take some trial and error. First select a spike that has anthers that are white or pale yellow. You should be able to locate these visually through the papery glumes of the spikelets. Any anthers that appear grey will likely have mature pollen grains. There will also be variation in maturity along the length of the spikes.

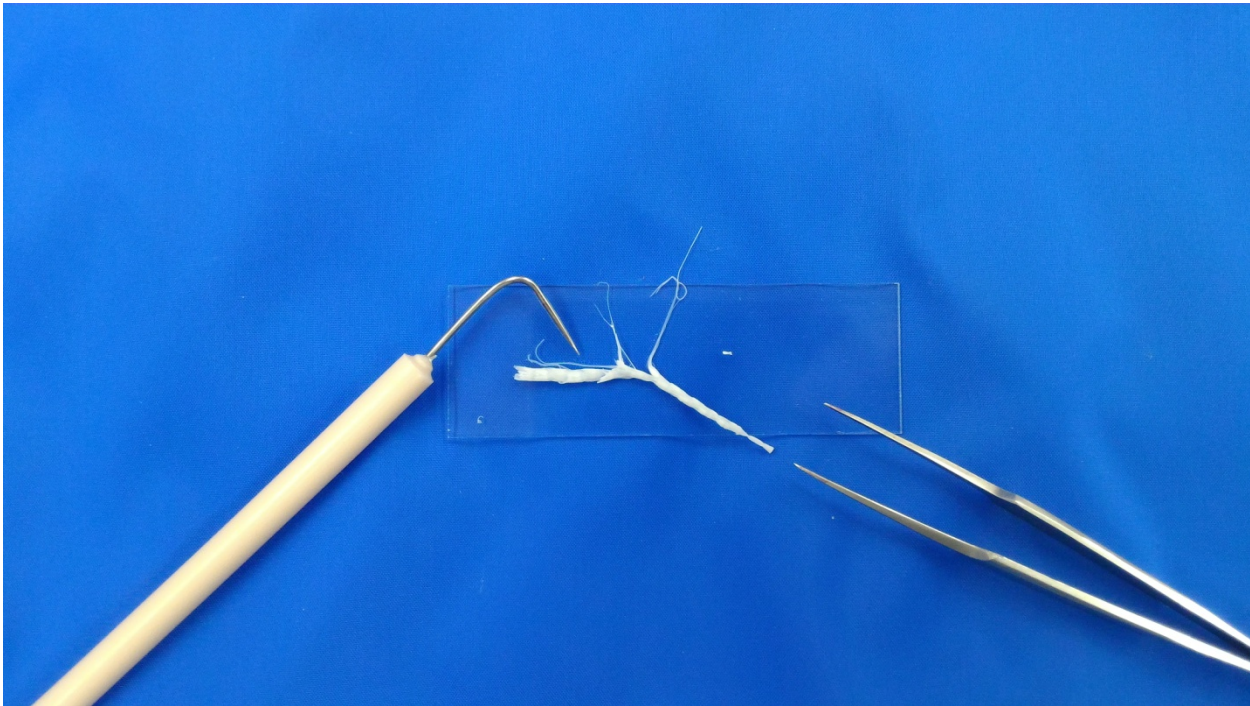


Figure 3: Extraction of the anther from inflorescent. A floret approximately half way up the inflorescence is selected and the anther (red triangle) is removed from the spikelet using a dissecting needle and forceps. The inflorescence can be returned to the Petri plate.

The most mature pollen can be found in the centre and least mature at both the top and the stem end of the spike. Sampling anthers from three or four places along a spike will give you the best range of maturity of the pollen. If you note the length of these anthers, you can use this as a guide when re sampling.

Slide Preparation: To make a good slide for photography, make sure to remove all the large debris from the slide. Any small fragments of a glume or anther wall will affect the focus and therefore the quality of your pictures. Carefully pull the anthers out of the spikelets and collect them on a slide. Add a drop of aceto-Carmine and cut the anthers across the middle. Line several anthers up side by side to save time. The pollen will be released from the anthers with a gentle squeeze from the tweezers. Remove the anthers and any other debris. Apply the cover slip careful so not to add any bubbles, and examine the pollen under low power. Heat the slide gently if you are seeing to movement of the stain under the cover slip. Once you have found good examples of the following stages the lab demonstrator will take the pictures for you. Seal the edges of the cover slip with nail polish or with bees wax if your cell is close to the edge. Prepare a marker slide for transfer to the photomicroscope.

Report: One picture of each of the following labeled appropriately:

1. Diakinesis
2. Metaphase I
3. Anaphase I
4. Metaphase II or Anaphase II

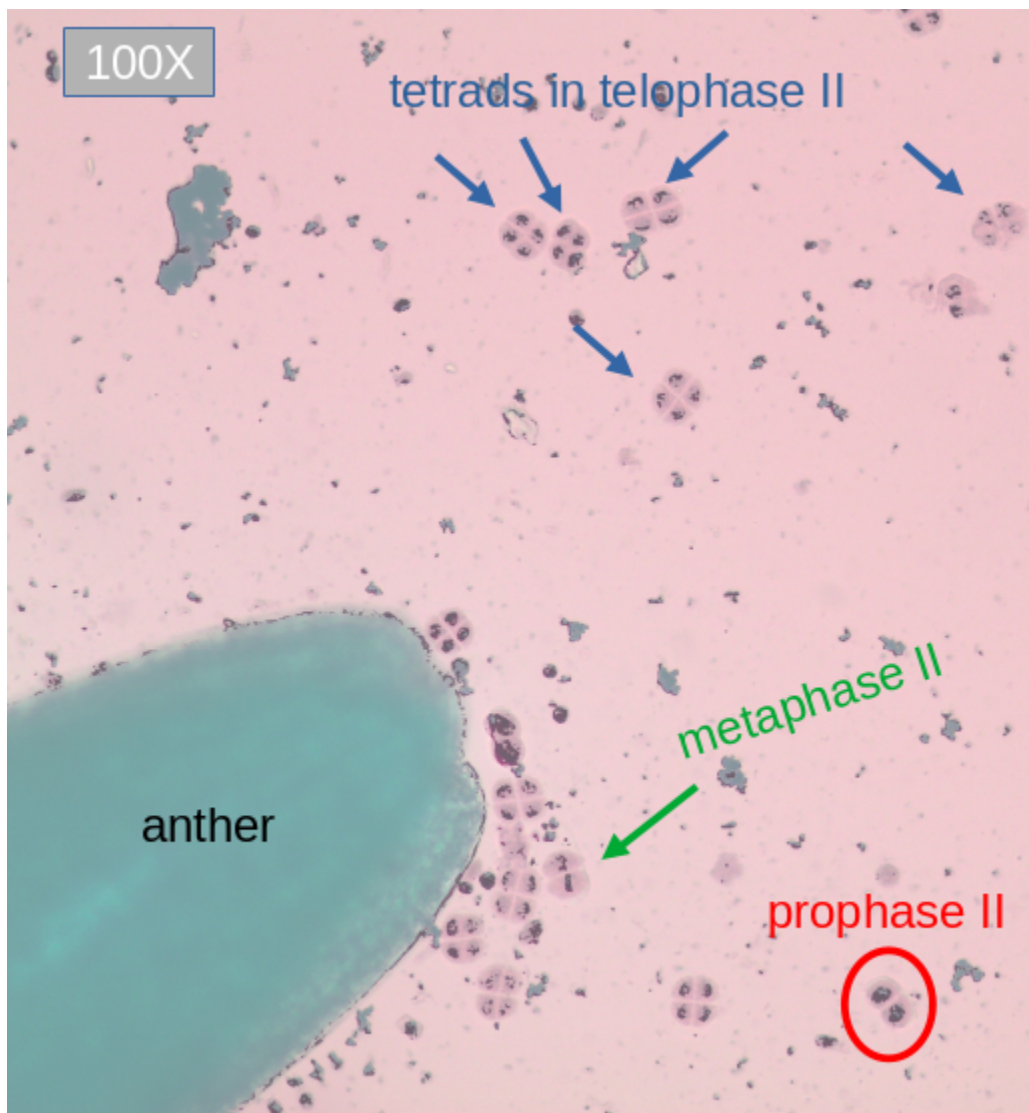


Figure 4: Example of an anther and microspores in various stages of meiosis. Several examples of tetrads in meiotic telophase II are indicated by blue arrows. This anther was a lucky find, in that it had a large number of chromosomes caught in meiosis II. More tetrads are visible in the immediate vicinity of the anther. Metaphase II is evidenced by two groups of chromosomes aligned on the same plane. Similarly, the two sets of chromosomes after the first meiotic reduction division are indicative of prophase II (oval).