

## Mitosis vs meiosis

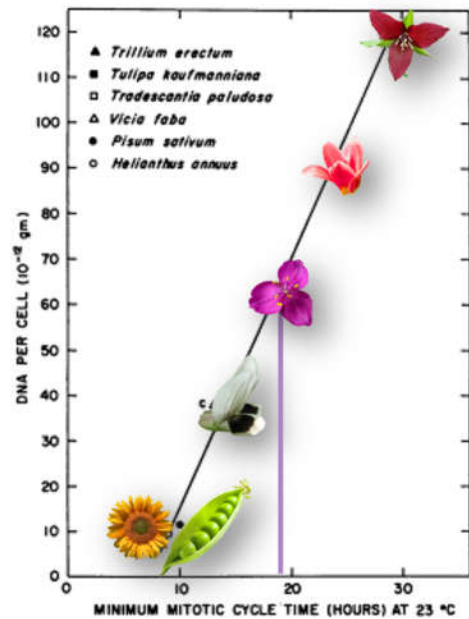


Figure 2, Van't Hopf and Sparrow, 1963

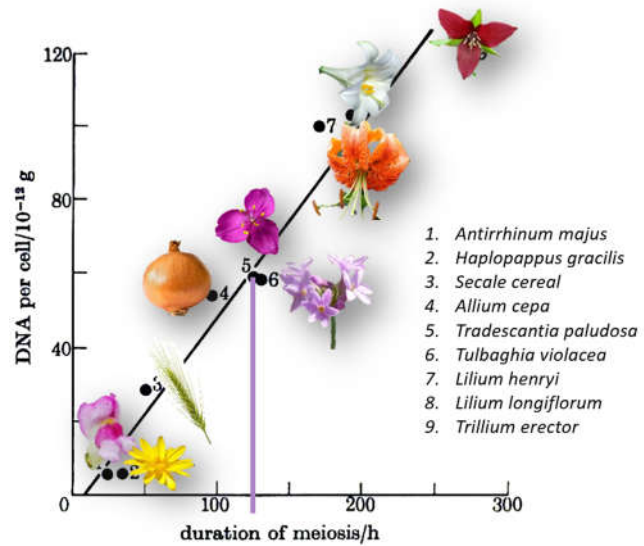
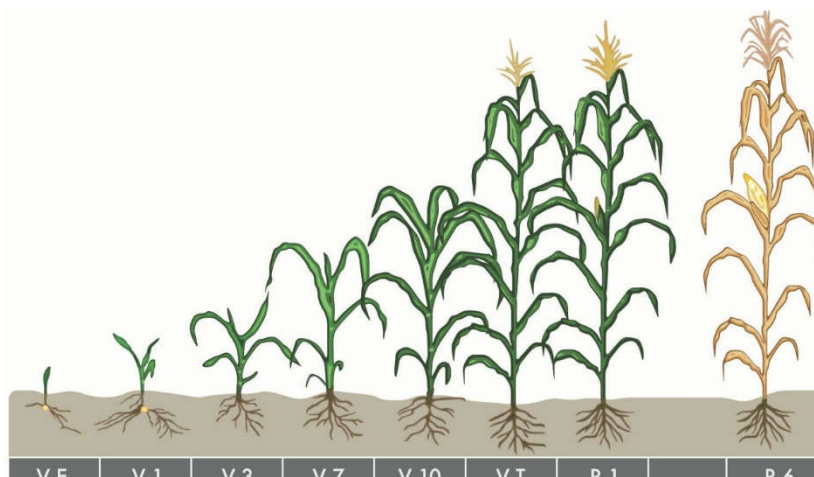


Figure 1. Bennett, 1971

## ♂ Meiosis takes place early

Figure 3. <https://www.dekalbasgrowdeltapine.com>

## Pollen mother cells

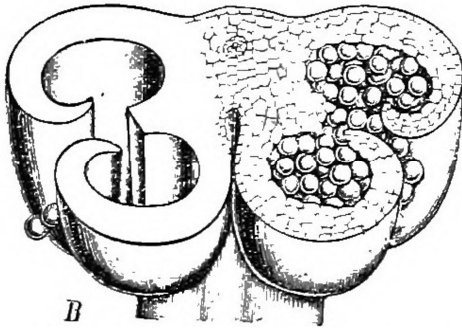


Figure 5. Alamy Stock Photo

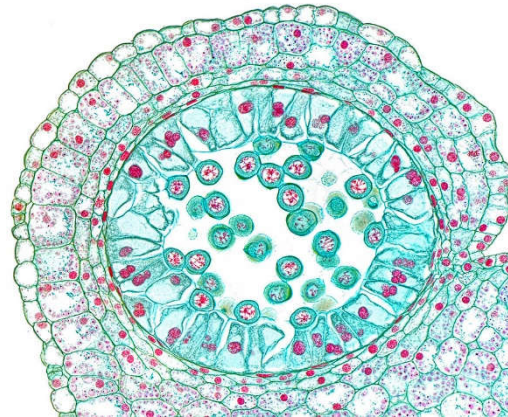


Figure 4.

<https://www.sciencephoto.com/media/707587/view/lilium-ts-anther>

## Preparation of PMCs for meiotic analysis

Flower buds must be collected at the stage during which the pollen mother cells are undergoing meiosis. This normally means that a whole range of sizes will need to be collected and examined in order to determine the optimal collection stage.

- Place flower buds in a fixative (Carnoy's for 24 hours)
  - For *Tradescantia*, use 2-3 days
- Store in 70% EtOH under refrigeration
  - For *Tradescantia*, store in freezer
- Place a microscope slide or watch glass over a dark surface
  - Place add a 70% EtOH to the slide
  - Place a flower bud on it
- Using a dissecting microscope
  - Isolate 3 anthers from each bud
- Blot out the EtOH and add acetocarmine to the slide
  - Macerate the anthers to release the PMCs
  - Remove all the anther debris
  - Blot out excess stain
  - Add a coverslip

- If using acetocarmine, before the final squash
  - Heat the slide over an alcohol lamp, so it feels warm to the touch
  - Do not boil it
- Turn the slide over, add paper towels on top
- Squash with thumb
- Examine the slide under a microscope
  - If the cells are well spread and mitotic figures are apparent
  - Seal the edges with paraffin or nail polish

**Note:** To make permanent slides, remove the excess stain with a tissue, and add a drop of Hoyer's. Mix well to incorporate the anthers into it.

- Place coverslip over material and tap lightly to remove air bubbles
- Heat and squash
- Seal with paraffin if the spread is of suitable quality

*Alternatively,*

1. Remove flower buds from the 70% EtOH, and place in Snow's stain. Leave at least for two weeks under refrigeration.
2. Then dissect out the anthers, and place them on a slide together with a drop of 45% AcOH. Proceed as described previously.

## The study organism

*Tradescantia pallida*

$2n = 2x = 12$

$2n = 4x = 24$



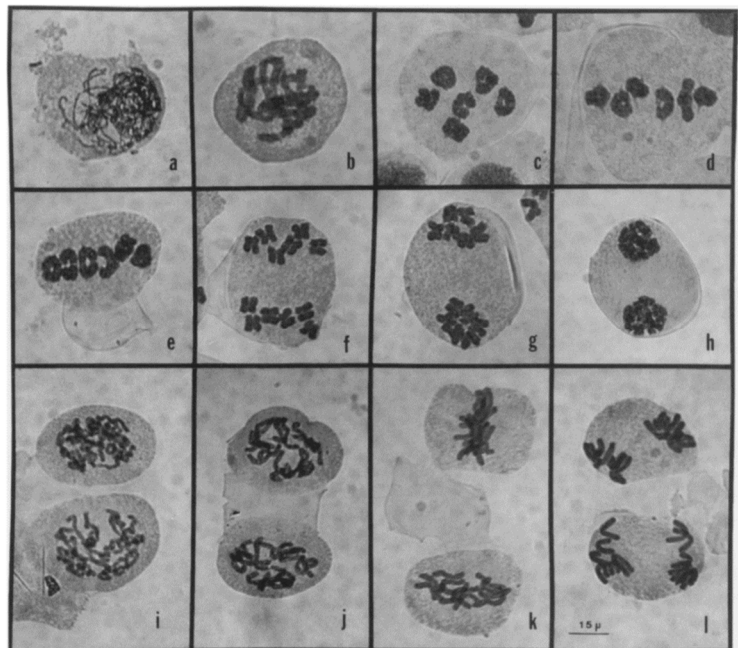
Figure 6. <https://albania.desertcart.com/>

A genus from the western hemisphere

Range of *Tradescantia* spp.



Figure 7. Named after the father/son botanists who described it, John Tradescant the Elder and the Younger. Wikimedia commons



#### Reference

- Hammersmith RL & TR Merton. 1979. Tradescantia: a tool for teaching meiosis. American Biology Teacher, 59: 300-304

## Stains and Solutions

### Carnoy's (6:3:1)

Mix (v/v/v):

- 6 parts 100% EtOH
- 3 parts chloroform
- 1 part of glacial acetic acid

Addition of chloroform can help dissolve fats and oils that would otherwise interfere with observation. However, this mixture is not stable, so it must be used immediately after mixing.

**Acetocarmine**

- 2 g carmine
- 100 ml 45% AcOH

Heat slowly until the color turns. Then boil gently for 5 minutes. Let cool, then filter.

**Hoyer's Mounting Medium**

- 50 ml distilled water
- 30 g gum arabic
- 200 g chloral hydrate (schedule IV)
- 16 ml glycerine

Completely dissolve the gum arabic in the distilled water. This may take up to 24 hours.

In this solution, dissolve the chloral hydrate. This may also take up to 24 hours.

Add the glycerine and mix well.

Due to evaporation, the medium may become too concentrated and cause plasmolysis and tearing of the PMC's and precipitate the stain. Water may be added to thin the medium to the desired consistency. Such a dilution may require 24 hours to be completed.

Use for making anther squashes semi-permanent. Add to anthers on slide together with a drop of stain or 45% AcOH. May also use just the Hoyer's without the stain or AcOH.

**Snow's** (alcoholic hydrochloric carmine, Snow, 1963)

- 15 ml distilled water
- 4 g carmine
- 95 ml 85% EtOH
- 1 ml concentrated HCl

Add carmine and HCl to water. Boil gently for 10 minutes while stirring. Cool, add the EtOH, and filter