LABORATORY EXERCISES # 15 and #16

WHAT LAND PLANT ADAPTATIONS FOR NUTRITION AND REPRODUCTION ARE DERIVED FROM ALGAE AND FUNGI?

INTRODUCTION

For years, Taxonomists have argued about the number of Kingdoms into which living things are classified. Plant and Animal Kingdoms initially seemed adequate for many, but other people saw the logic of introducing one or more new Kingdoms because organisms which did not fit into either original group very well existed. Monera, Protist, and Fungi Kingdoms were then introduced to resolve some of these inconsistencies. Scientists then decided to further classify Monera as two separate kingdoms, Archaebacteria and Eubacteria. We now use this SIX-KINGDOM system, which includes the Prokaryotes ARCHAEBACTERIA and EUBACTERIA, and the Eukaryotes PROTISTA, PLANTAE, FUNGI, and ANIMALIA.

As Protista evolved, they developed the ability to self-nourish via photosynthesis. Photosynthetic Green, Brown, and Red algae (water-dwelling) are considered protists, but are precursors to modern land plants. Today, you will be looking at the green algae Spirogyra and Volvox. The land plants are grouped as Thallophytes and Embryophytes. Embryophytes show more differentiation of tissues and form embryos in the female reproductive structures. Examples of these are the mosses, liverworts, ferns, and the seed plants. Today, you will observe Volvox and Spirogyra as representatives of colonial green algae. Previously, fungi (mushrooms, molds, and yeasts), were called Thallophytes because they possessed a cell wall. However, fungal cell walls consist of cellulose and chitin, whereas the cell walls of plants are composed of cellulose and pectin. In addition, fungi are heterotrophic, rather than autotrophic like plants, and their reproductive methods are also quite different from those of plants. Thus, these organisms were given their own kingdom, the KINGDOM FUNGI. Today, you will also observe a member of the Kingdom Fungi—the bread mold Rhizopus.

Just as in animals, the tissues of the leaf, stem, and roots of plants are organized to carry out life functions. The organs come from the branch of the Plant Kingdom called TRACHEOPHYTES. As the name implies, these plants have tubes like your trachea but instead are used to transport materials such as water and dissolved minerals. A subgroup of the Tracheophytes are the FERNS, the cone bearing plants called GYMNOSPERMS (“naked seeds”), and the fruit bearing plants called ANGIOSPERMS (“covered seeds”). We will be looking at structures from the two subgroups of the angiosperms called MONOCOTS and DICOTS. Monocots have non-branching stems and parallel leaf venation (examples: corn and other members of the grass family). Dicots have branching herbaceous (soft) or woody stems and branching leaf venation (examples: lilacs and other woody flowering trees).

An important and unique life function that plants perform is to derive their energy from the sun’s rays, capturing it in the **BONDS** of **GLUCOSE** molecules manufactured in the process of **PHOTOSYNTHESIS**. The plant must be able to regulate the uptake and release of both water vapor and carbon dioxide, which are needed as raw materials. In order to accomplish this, root hairs and pores called **LENTICELS** along the stems of the plants absorb and release gases through the process of **GASEOUS DIFFUSION**. In addition, the lower epidermis of leaves contain openings called **STOMATES**. The size of the stoma (*stoma* is singular, *stomates* is plural) opening is regulated by the chloroplast containing **GUARD CELLS** which surround it. Gas exchange through the stomates is advantageous because the amount of exchange can be controlled by the opening and closing of the guard cells. This can prevent the plant from losing too much water in dry conditions.

The guard cells are specialized epithelial cells. These cells are bean-shaped. The inner edge has a thicker cell wall than the outer edge. During the day, as photosynthesis occurs, these cells fill up with water and become **TURGID**, causing them to change shape [**See diagram below**]. Because of the difference in the thickness of the cell wall, the cells separate and form the stoma opening. During the night, the cells lose water and become **FLACCID**. The stoma opening grows smaller. Thus, when the plant needs carbon dioxide during the day, the stomates are open. Many scientists have been interested in how the guard cells regulate the size of stoma. There is evidence that **LIGHT-DEPENDENT ACTIVE**

TRANSPORT is used to bring potassium ions into the guard cells during the day. This causes a change in osmotic balance and water will rush in, causing the cells to become turgid. At night, the active transport no longer occurs and the potassium ions diffuse out. The water will then also diffuse out, causing the cells to become flaccid. Regulation of the behavior of guard cells to control transpiration pull utilizes feedback regulatory mechanisms in plants that are similar to chemical feedback mechanisms in animal endocrine and nervous systems.

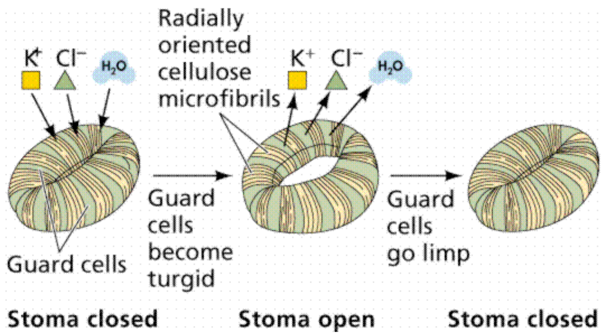


Fig. 18.1 GUARD CELLS control stomata openings

STUDENT OBJECTIVES

1. Observe and identify structures of several types of algae and fungi.
2. Relate the structures of the algae and fungi to reproductive function.
3. Draw and label sketches of algae and fungi.
4. Examine the stomates on the underside of a lettuce and scallion leaf.

PRE-LAB QUESTION:

You will be observing Green Algae, believed to be the precursors to modern land plants, in today's exercise. What two adaptations were necessary for these photosynthetic algae to make the transition from aquatic (water) to terrestrial (land) habitats? Answer this question at the beginning of your summary questions and bring drawing sheets with you to the lab session along with these instructions.

MATERIALS

Compound and dissection light microscopes, forceps, scalpel, lens paper, microscope slides & coverslips, cultures of Spirogyra, Volvox, Scallion, fresh lettuce, water, dropper, Lugol's Solution, paper towels, charts and textbooks. Also, prepared slides of sexual reproduction in Spirogyra and Rhizopus (bread mold). (Note: Those students with fungal or mold allergies should consult their teacher ahead of time.) At lab, use charts, botany atlases, & embedded diagrams in this procedure to help in labeling your drawings.

PROCEDURE

Work in pairs. You will be observing the following items: Live spirogyra, prepared slides of spirogyra, live Volvox, prepared slides of Volvox and prepared slides of bread mold (Rhizopus) under the light microscope, and will mount your own slides of stomates from lettuce (dicot) & scallion (monocot) leaves.

IMPORTANT CAUTIONS: PREPARED SLIDES ARE FRAGILE AND VALUABLE. LAST YEAR, WE LOST AN INSANE AMOUNT OF THEM TO CARELESSNESS ON THE PART OF MANY PEOPLE. IT IS ALSO IMPORTANT THAT WE MAINTAIN TABLE INVENTORIES AS WELL AS OUR GENERAL INVENTORY. FIRST, CHECK YOUR TABLE TO MAKE SURE YOU HAVE ALL PREPARED SLIDES THAT YOU NEED TO DRAW. DO NOT THROW, CRUNCH THE COMPOUND LENS UPON, OR MISHANDLE OUR SLIDES IN ANY WAY. THEY ARE GLASS & PLASTIC, HELD TOGETHER LOOSELY WITH GLUE AND ARE NEVER, EVER TO BE WET.

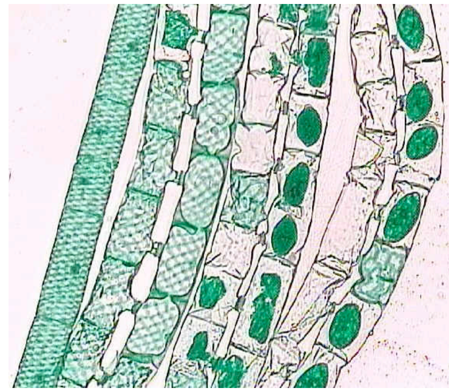
I. Observation of Spirogyra

1. Use forceps to place several filaments of Spirogyra on your slide. Add several drops of water. Place a coverslip on the slide. Observe it under low-power. It looks like green threads but actually consists of

many long, narrow cells attached in a line. Each cell has a long spiral CHLOROPLAST and a NUCLEUS. Turn to high-power and observe more closely.

2. Located in the chloroplast are numerous starch storage structures called PYRENOID BODIES. Try to find them under high-power. To help you more easily identify the pyrenoid bodies and the nucleus, place several drops of Lugol's Iodine Solution next to the coverslip and then draw the solution under the coverslip by placing a piece of paper towel the opposite edge.

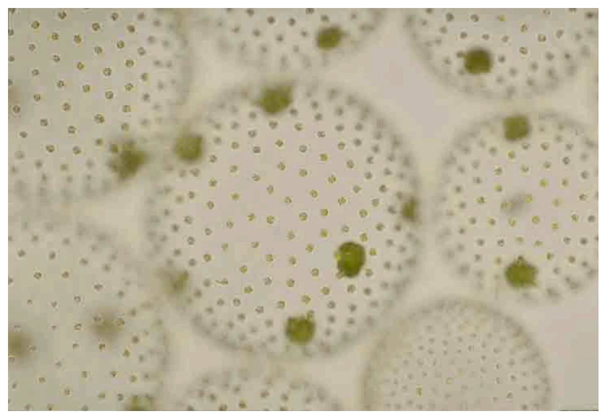
3. REPRODUCTION IN SPIROGYRA:
Spirogyra can reproduce asexually by fragmentation and by conjugation. Conjugation (sexual reproduction) occurs between two opposite mating type filaments, designated active (+) and passive (-). The strands lie next to each other. Conjugation tubes are formed from two cells which are opposite each other. The contents from the (+) type cell's conjugation protoplast passes through the tube into the (-) cell. These gametes are called isogametes. The materials fuse and a zygospore (zygote) is formed. It has 2N chromosomes. Just before the zygospore germinates, it undergoes meiosis and four 1N nuclei are formed. Three die and the remaining 1N nucleus becomes the nucleus for the next generation of filament cells.



Observe the PREPARED SLIDES of spirogyra conjugation. Find two cells undergoing conjugation. Try to locate the conjugation tube, the conjugation protoplast, the zygospore, and the empty cell.

II. Observation of Volvox

Volvox is an example of a colonial algae. Many identical, double-flagellated cells (500-50,000), called chlamydomonas, are held together by slender cytoplasmic strands. Use low-power to locate a large green sphere made up of small, green flagellated cells. Switch to high-power and observe more closely. The sphere of algae cells is able to swim in a coordinated manner. In asexual reproduction, pockets of the sphere depress inward and pinch off, making a new sphere within the parent. Some cells of the colony are specialized for sexual reproduction. Female cells lose their flagella and remain in the colony and become the eggs. Male cells are able to swim and thus can fertilize eggs from their own or from other colonies. Each fertilized egg can give rise to an entirely new colony. These daughter colonies are often visible inside the mother colony. Both asexually and sexually-produced daughter colonies are released through a hole in the wall of the mother Volvox.

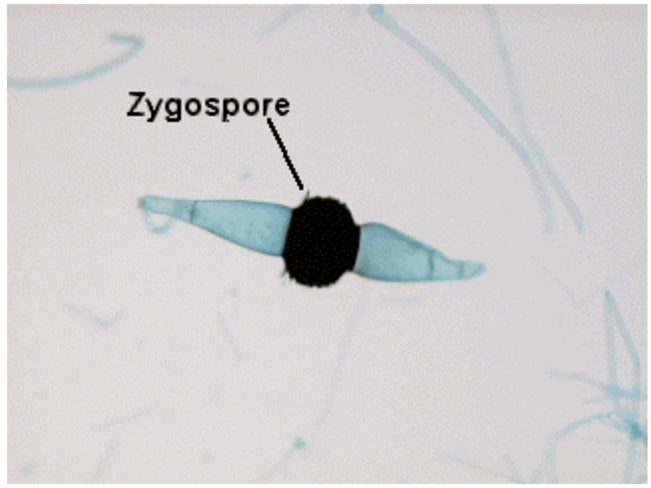


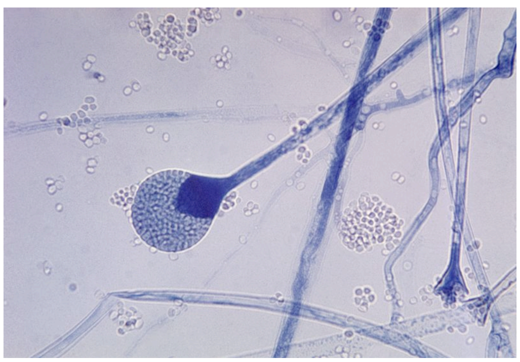
Place several drops of Volvox culture on a clean slide. Cover with a cover slip. View prepared slide if necessary. Determine if your Volvox has daughter colonies within the mother structure, as is shown in the image below.

III. Observation of Rhizopus (Bread Mold)

1. In Rhizopus, the body of the fungus is called the MYCELIUM (myketos is Greek for fungus) and consists of non-sexually reproducing cells in strands called HYPHAE. Specialized areas of hyphae invade starchy foods such as bread and secrete digestive enzymes. These hyphae are called RHIZOIDS. The end products of digestion are then absorbed by the mold. The black dots are called SPORANGIA and contain spores. Observe the sporangia on one of your prepared slide mounts (They are structures which contain spores); draw what you see at low power, 100x. The spores are formed by mitosis during asexual reproduction. Sporangia are held aloft by stalks called SPORANGIOPHORES, groups of which are connected by lengths of hyphae called STOLONS.

2. REPRODUCTION IN RHIZOPUS
Rhizopus can reproduce by fragmentation just as Spirogyra does. It undergoes conjugation also, but there are some differences. When two hyphae from molds of two different mating types fuse, GAMETANGIA are formed. Their 1N nuclei fuse and form a 2N zygote or ZYGOSPORE. The zygospore (as shown at right) undergoes meiosis, but only one nucleus survives to emerge in the next generation of hyphae. Observe a prepared slide of Rhizopus conjugating. Locate the hyphae, a sporangium, a sporangiophore, a stolon, some spores, gametangia, developing zygospores, and mature zygospores.





http://en.wikipedia.org/wiki/File:Mature_sporangium_of_a_Mucor_sp._fungus.jpg
Pictured above is a sporangium from a species (Mucor) related to Rhizopus.

IV. Examination of a Scallion Leaf

1. Place 2 drops of water onto your slide.
2. Scallions are a common vegetable that are similar to onions. The leaves that grow above the ground are wrapped around a central stem. Using your forceps, carefully remove a section of one leaf. Using the scalpel, gently scrape the tissue of the outer epidermis. Place the epidermis in the water on your slide. Cover it with a coverslip.
3. Observe the leaf under low power. Note the arrangement of the cells. Look for the kidney bean shaped guard cells. Switch to high power and observe.
4. In order to make the cells appear more distinct, you may use Lugol's Solution to stain the leaf. Place two drops of Lugol's Solution next to the coverslip. Place a small piece of paper towel next to the opposite side and draw the stain under the coverslip. Observe again under low, then high power.
5. **DRAW** and **LABEL** a **high-power** sketch of the leaf cells of the scallion. Indicate the magnification used. Please do all drawings in pencil.

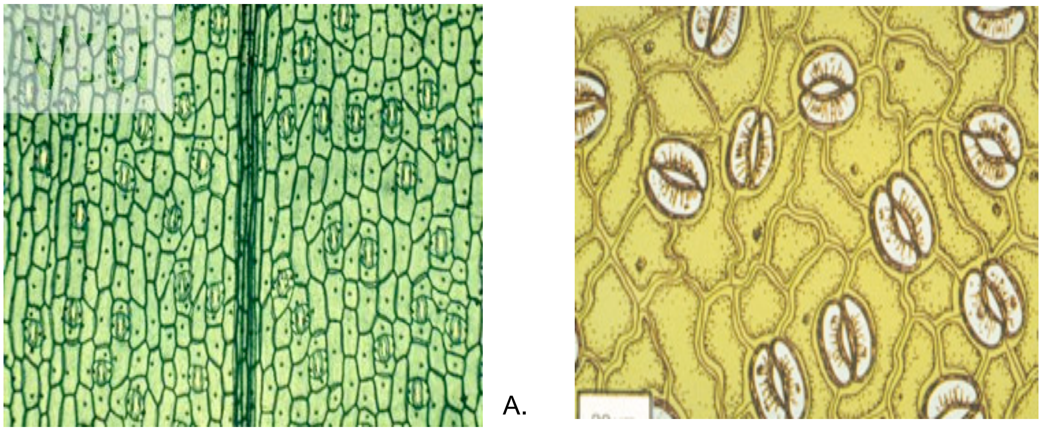


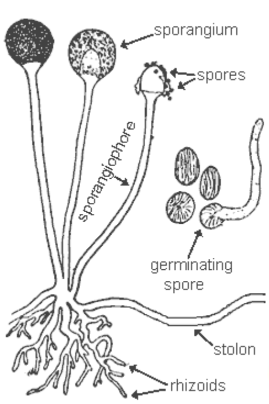
Fig. 18.2 Lower epidermis of a plant leaf with stomates. A. Low power, B. high power

V. Examination of a Lettuce Leaf

Pick up and observe the whole lettuce leaf, if available. There is a distinct top and bottom. Note the differences between the two sides. The top is shinier and greener while the bottom is paler. The main rib of the leaf bulges out on the lower side. You will observe the lower epidermis of the leaf and of the rib itself.

1. Place two drops of Lugol's Solution on your slide.
2. Hold the lettuce leaf in your hand, bottom side up.
3. Break a side vein so that part of the lower epidermis is separated from the rest of the leaf. Use your forceps to pull the tissue **AWAY** from the main rib. **MAKE SURE NO GREEN MESOPHYLL IS CONNECTED TO THE LOWER EPIDERMIS.** Pull off an 0.5 cm piece and place it in the Lugol's Solution.
4. Cover the tissue with a coverslip.
5. Observe the leaf under low power. Note the appearance of the epidermal cells.
6. Look for the darkly stained, bean-shaped guard cells. Switch to high power and observe.
7. **DRAW** and **LABEL** a **high-power** sketch of the cells and stomates of the lower epidermis of the lettuce leaf. Indicate the magnification used.

Embedded below is a series of images to assist for labeling.



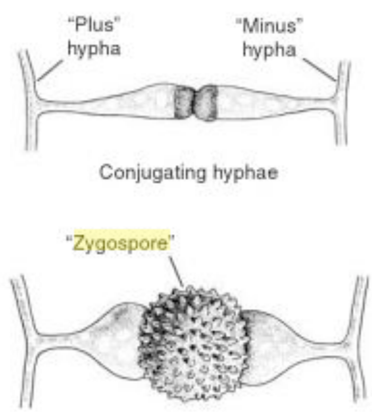
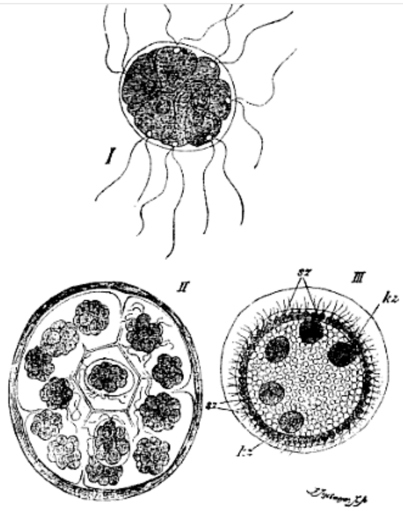
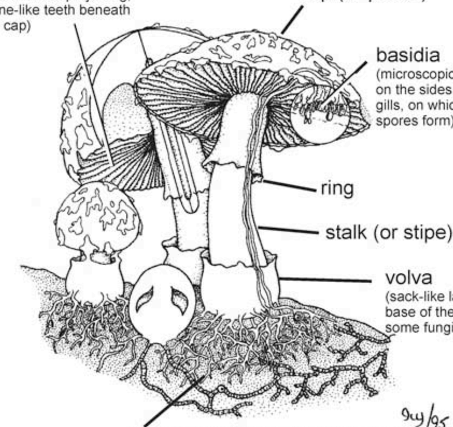


Figure E Conjugation in *Rhizopus*. [Drawing by R. Golder.]

Image at left: http://www.backyardnature.net/f/bredmold.htm



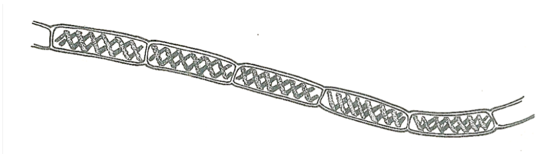
August Weismann's drawings of *Pandorina* and *Volvox* (from Weismann 1892b). His caption read: "I. *Pandorina morum*—A colony of swarming cells. II. A colony which has given rise to daughter colonies:—all the cells are similar to one another. (After Pringsheim.) III. A young individual of *Volvox minor*, still enclosed within the parent (after Stein): the cells are differentiated into somatic- and germ-cells."



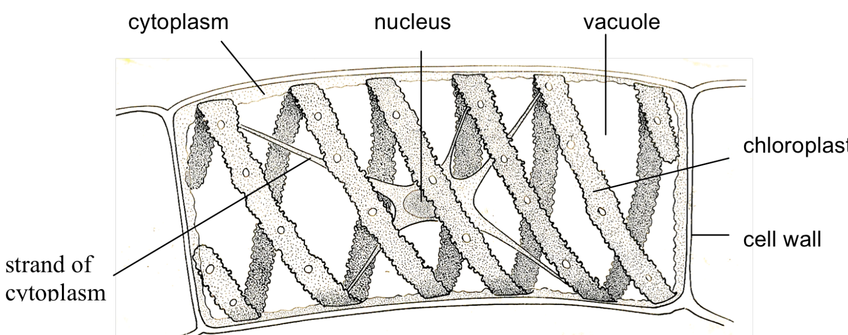
mushroom included for reference only
in the context of fungal reproduction. Drawing optional.

Spirogyra

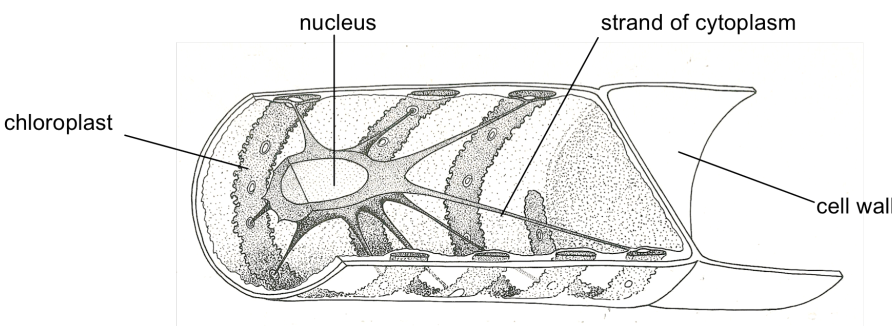
Spirogyra is a member of the Algae. These are simple plants ranging from single-celled organisms (*Chlamydomonas, Euglena*) to complex seaweeds. They contain chlorophyll and make their food by photosynthesis. Spirogyra is a filamentous alga. Its cells form long, thin strands that, in vast numbers, contribute to the familiar green, slimy ‘blanket weed’ in ponds. Seen under the microscope, each filament consists of an extensive chain of identical cells.



Each cell contains a helical chloroplast, a nucleus, cytoplasm and a vacuole enclosed in a cellulose cell wall.



Single cell of spirogyra



3D representation of spirogyra cell structure

Growth.

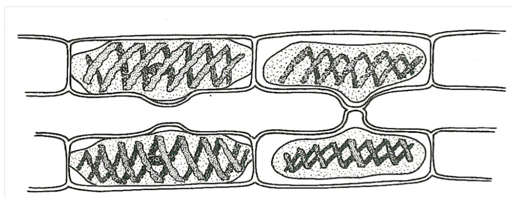
Each cell can divide transversely and grow to full size thus increasing the length of the filament. When the filament breaks, this is a form of asexual reproduction but there is a sexual process called *conjugation.*

© D.G. Mackean

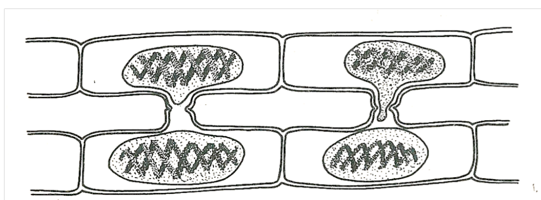
Spirogyra 2

Conjugation

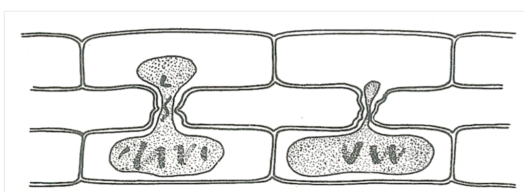
At certain times of the year, tubular structures grow out from each cell of a pair of filaments lying parallel to each other. The tubes join up to make a passage between each cell and its partner.
The chloroplasts and other structures become less distinct and the cytoplasm pulls free from the cell wall to form a rounded structure. The cytoplasmic contents of the cells of one filament then pass through the tube (conjugation tube) and fuse with the cytoplasm of the cells of the adjacent filament.



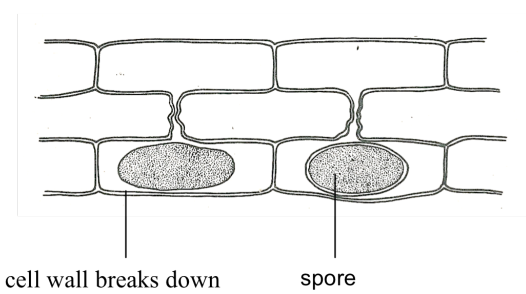
Cells of each filament put out a conjugation tube



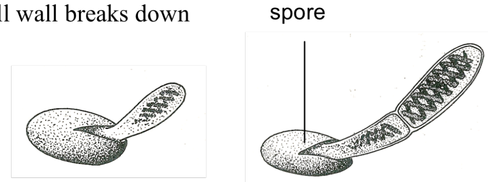
The conjugation tubes meet and join up. The chloroplast starts to break down. The cell contents become a gamete.



The cell contents of one filament pass through the conjugation tube and the nuclei fuse.
Although there is no visible difference between the filaments, one is clearly acting as the producer of 'male' gametes.



The zygote forms a wall round itself and becomes a spore which can resist adverse conditions, e.g. cold and lack of light.



When conditions improve, the spore germinates to produce a new filament

© D.G. Mackean