

Description of events

Mating

Mix one ml of CC620+ gametes and 1 ml of 621- gametes in a test tube. Note the time(*). Immediately place two drops on a slide and add a coverslip. Examine at 10x, 20x and 40x with and without phase contrast. Initially you should see pairs and clumps of cells aggregating by their flagella. The adhesion interferes with swimming, leading to the characteristic jerky appearance of mating cells. Continue to watch, focusing up and down.

Loss of cell wall:

After anywhere from a few seconds to a couple of minutes you should see the cell walls pop off. Careful examination under 40x may show that the cell wall is in two parts, one large part and a smaller fragment from around the flagella. You may even be able to see the two holes in the cell wall where the flagella emerged. Soon after this the clumps should break up and pairs of cells (+/-) should become apparent.

Cell fusion:

Once the wall is gone, the cells are free to fuse. This occurs where the two are held together by the flagella. Initially a dumbbell shape forms and then the two fuse completely into a spherical cell with four functional flagella. This quadriflagellate is motile, and lasts for several minutes up to an hour. The two nuclei are still separate, and this is termed the dikaryon stage

Zygote formation:

The flagella resorb and a heavy cell wall starts to form around the cell. Internally the nuclei and chloroplasts from the two parents are

fusing.

* Kinetics of the events:

Every 15 minutes, take two drops and mix with two drops Lugol's iodine in a fresh test tube. Put a drop under the microscope and estimate the percentage of cells unmated, clumped, paired off, lost cell wall, fused, and as zygotes at each time point.

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MATING

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A Series of Experiments on Mating

Focused Reading: p 538-541 "Chlorophyta"

p 540 Figure 26.24 26.15

Overview:

Over the next three weeks you will become comfortable with a fundamental tool in biology - the compound microscope. In order to familiarize yourself with the microscope, we will conduct a series of experiments on a unicellular green alga, *Chlamydomonas reinhardtii*, or Chlamy for short. Chlamy is a biflagellated green plant that reproduces asexually (by mitosis) and sexually (via meiosis, mating, and zygote formation). Each week, you will want to bring your calculators to lab. A brief description of these three weeks of experiments are outlined below:

1) The first week's experiment will be to observe the mating of Chlamy and to measure the efficiency of this mating. Our results will come in the form of % mating as a function of time.

2) The second week's experiment will examine the rate at which Chlamy can regenerate its two flagella once they have been amputated.

3) The exact nature of the third week's Chlamy experiment will depend on your interests. You will ask a specific question related to flagella regeneration and gene expression, design an experiment and a protocol with good controls, perform your experiment, collect the data, and present your results to the rest of the class.

The laboratory is a place where scientists (that includes you) come together to work as teams and talk about methods, results, and conclusions. It is also a place to assert yourself, take responsibility for your own education, and trust your common sense. For example, you will measure the length of flagella which requires you to kill the cells by chemically **cross-linking** the all proteins, or euphemistically called **fixing** the cells. The other three people in your group suggests that you pour the Lugol's fixative solution into your one and only supply of cells even though you also are supposed to mate LIVING cells later in the lab. Unfortunately, you have been cursed with short-sighted lab mates. To you, it seems obvious that you cannot kill all the cells in step 3 if you need live ones for step 7. So, you assert yourself and persuade your lab mates that they have made a miscalculation (this works better than calling them idiots, even if they are). This is not confrontation but cooperative learning. Each of you can be both student and teacher if you **think** while you are in lab; don't just hurry through in order to finish. What you learn from each other in the lab is just as important as what you learn in the class (that's why lab material is tested in the "lecture" exams).

How efficient is Chlamy sex?

There are several reasons why *Chlamydomonas* is such a useful model organism. It is a haploid organism which means there is only one copy of each chromosome. Therefore, the genotype is always

expressed in the phenotype (unlike diploids that may have a recessive mutation that is not revealed in the phenotype). It has a generation time of 2 weeks (from mating of one generation to when the next generation can mate). Finally, there are hundreds of mutant strains (stored at Duke University) that have been generated over the years and can be used for research. For example: *ac-17* cannot fix carbon during photosynthesis, *arg-7* requires the amino acid arginine to be added to the medium since it cannot synthesize its own; *act-1* is resistant to the translational inhibiting drug cycloheximide; and *pfl4* has straight and paralyzed flagella so it cannot swim.

Why would anyone want to know how efficient Chlamy sex is? Well, if you are trying to study the process of gametogenesis at the molecular level, you would need to be able to compare wild-type mating to abnormal, or mutant, mating. To compare these two, you might use mating efficiency as an indicator of the ability of a gamete to mate. Since human subjects are reluctant to submit to experimentation, especially experiments on mating efficiency, you would be forced to find an alternative organism to study and one that has a short generation time. For example, you are trying to learn how gametes fuse and you decide to generate a mutant strain of Chlamy that cannot fuse (actually, this strain was isolated in the '70s). Once the mutant is generated, you can try to clone the gene that has been altered and this will allow you to identify the gene that encodes the "fusing gene". Maybe a new contraceptive will result from your research.

Chlamy cells come in 2 sexes called **mating-type plus (mt+)** and **mating-type minus (mt-)**. When a mitotically dividing cell is deprived of nitrogen, it differentiates into gametes; mt- cells differentiate into *minus* gametes and mt+ cells differentiate into *plus* gametes. These two opposite sexes will fuse to form a diploid zygote that becomes a metabolically inactive spore. When conditions are favorable for mitotic growth (i.e. there is enough nitrogen), the zygote spore undergoes meiosis and germination to produce a tetrad of four haploid progeny: two mt+ cells and two mt- cells. We will be working with plus and *minus* gametes in today's lab.

Below is a set of directions that each person should follow. You should collect your own data, record your own observations, and answer the questions as you go along. Make sure you complete each step in the protocol before moving on to the next. Some steps will

require a group effort, but those come later in the protocol.

Protocol

A) Place 25 μl of *minus* gametes on a clean (use a kimwipe) glass microscope slide and cover this with a coverslip. Do not press down on the coverslip or else you will crush the cells. Place the slide on the **stage** of the microscope and use the 10X **objective lens** to observe the cells swimming around.

1. Can you see the flagella?
2. What can you do to see them better?
3. What is the total magnification you are using with a 10X objective lens and the **10X oculars**?

B) Increase the magnification by using the 40X objective lens.

1. What is your total magnification now?
2. Can you see the flagella? What can you do to improve your ability to see the flagella?
3. Can you see any other organelles in these cells?
4. Do you see any other colors besides green? If so, where and what organelle could this be? (Hint: "The better to see you with, my dear.")

C) On the same slide but separate from the previous sample, place 12.5 μl of mt- cells into an 12.5 μl Lugol's fixative (this is a dye that stains the sugars which are covalently bound to the proteins (sugar coated proteins are called **glycoproteins**) on the surface of the flagella. Examine this preparation of stained cells under the microscope.

1. What structure(s) can you see better now than when you observed live, unstained cells? Give 2 reasons why.
2. Two microscopes in each group have oculars that contain a **micrometer** (very small ruler). Use this to measure the length of the cell bodies and the flagella - using the 40X objective, every hash mark is 1 micrometer or **micron** ($1 \mu\text{m} = 10^{-6}$ meters). Measure 10 cells and average your results with those of the rest of your group.
3. How long is an average flagellum? How long is the average cell

body?

D) In a microfuge tube, mix 100 μ l of *plus* and 100 μ l *minus* gametes and record the time (e.g. 4:25 am).

1. Each person should take out a 25 μ l subset, or **aliquot**, of the mating cells shortly after you have mixed them together and observe these mating cells (still alive) under the microscope using the 10X and 40X objectives.

2. What is going on? Describe how Chlamy cells mate?

3. After the cells have mated for at least 15 minutes, take an aliquot of mating cells and fix them in Lugol's stain (record the time; e.g. 4:45 am). Observe these stained cells using the 40X objective lens.

4. Do you see any cells that look like diploid **zygotes** instead of haploid gametes?

5. What 2 or 3 features are noticeably different in zygotes? (Look carefully for a cell that is different from the haploids you have looked at until now.)

6. Each person should count the first 25 cells with flagella that you see and "score" them as either gametes or zygotes. Record the number of each type of cell. Try to be random in your selection of cells to count; do not hunt for one kind of cell over the other.

7. Compare your data with the data from the other 3 people in your group. Did you all get the same numbers?

8. Average your group's numbers and determine the % mating efficiency using the formula below. Write your results on the board.

% mating efficiency = $2(\text{no. of zygotes}) \div [2(\text{no. of zygotes}) + (\text{no. of gametes})]$

= no. gametes fused \div [total of all gametes seen]

9. Do your numbers agree well with your colleagues in the other groups?

10. Can you imagine any reason why your results would vary significantly from another person's?

11. What modification to your technique would you make to avoid this problem next time (like next week!)?

Before you leave the lab, you should know the following:

- How can you see flagella better without staining them?
- What colors do you see in a Chlamy cell and what structures are responsible for the colors?
- How do you calculate the total magnification you are using on a microscope?
- How long is a Chlamy flagellum and a cell body, on average?
- With what appendage(s) do Chlamy cells mate? and how efficient was this mating process?
- How can you standardize your methodology so that 2 people can get similar results when counting cells?

7) Before next week, your lab group should meet so that you can be prepared for next week's lab. Timing will be very important - you snooze, you lose. You can meet at the end of lab today or later.

Lugol's Fixative (protect from light)

1 g iodine

2 g K⁺ Iodide

12 ml H₂O

dissolve the KI first, then add the iodine.