كلية الآداب والعلوم
College of Arts and Sciences
QATAR UNIVERSITY جامعة قطر

College of Arts and Sciences
Department of Biological and Environmental Sciences

Laboratory Name: Genetics **Course Number: BIOL 212** **Semester: Spring 2016**	**Title: Lab manual**	
	Date: 31/03/16	**Pages: 103**
Laboratory Address: C01-C122 **Telephone Number:**		
Name of Lab Instructor: R. Stefan Rusyniak & Huda Al-Muraikhi **Name of Lab Technician: Mr. Abdel Matheen & Ms. Hind**		

Genetics Lab Manual

BIOL 212

Prepared by: R. Stefan Rusyniak

Qatar University

Table of Contents

General Information

Dear Student,

Welcome to the Genetics Laboratory.

The course you're about to take is a work in progress – I started writing this manual two years ago and am still working on the different sections and trying to come up with new exercises. This lab is also very dependent on the resources that are available to us from week to week, and as such, it may deviate a little from the schedule listed in this manual and your syllabus.

This manual and any handouts that might be posted online for you will form the laboratory manual for the course. They will contain the protocols for your exercises for each lab as well as any relevant theoretical information and definitions that you will need to perform your lab work effectively. Please be sure to bring this manual and any posted handouts to every lab.

It is important that you read through each laboratory exercise and theory before coming to the lab. This will help you understand why you will be doing certain things and will make it more likely that you will successfully complete the exercises in the allotted time, it also helps to ensure that the labs are conducted safely. Prior preparation and understanding are important and will be periodically tested using quizzes (these are likely to occur near the start of the lab).

The lab component of this course will involve a prolonged experiment, which will span a majority of the semester. In it, you and your lab partners will determine the inheritance pattern of specific physical traits of *Drosophila melanogaster* (fruit flies). This is an experiment I have done successfully for many years with my students in Canada and thus the "*Drosophila* portion" of this lab manual is heavily influenced by the laboratory manual written by Dr. Clare Hasenkampf for her Transmission Genetics course at the University of Toronto at Scarborough.

It is important to note that due to the nature of the experiment, there will be times (especially early in the semester) when you will need to perform work in the laboratory outside of the scheduled lab time.

I hope that you enjoy the labs and I would appreciate any comments or suggestions that you have for future improvements (although, please keep in mind that I have limited resources and time).

I wish you much success in this semester,

R. Stefan Rusyniak

Tentative Schedule of Work in the *Drosophila* Experiment

	***Drosophila* work to be completed**
1st week:	▪ Intro week – no fly work
2nd week:	▪ Familiarize yourself with the wildtype phenotype ▪ Learn to determine the sex of the flies ▪ Perform a self-cross
3rd week:	▪ Discard parental flies from the wildtype self-cross ▪ Familiarize yourself with some mutant phenotypic traits ▪ Obtain stock cultures for your experiment ▪ Perform self-crosses of all stocks assigned to your group ▪ Begin making reciprocal crosses (this will be done outside of scheduled lab time)
4th week:	▪ Observe mutant self-crosses and discard parental flies if you see larvae in the media. ▪ Perform assigned reciprocal crosses (this will be done outside of scheduled lab time)
5th week:	▪ Observe mutant reciprocal crosses and discard parental flies if you see larvae in the media (usually 10 days after making the cross). ▪ Perform assigned reciprocal crosses if needed (this will be done outside of scheduled lab time)
6th week:	▪ Observe mutant reciprocal crosses and discard parental flies if you see larvae in the media. ▪ Observe any emerging F_1 flies and make note of the phenotypes of the flies from each reciprocal cross. ▪ Perform F_1 self-crosses (this can be done outside of scheduled lab time)
7th week:	▪ Remove the F_1 flies from F_1 self-crosses if they're 10 days old ▪ Observe any emerging F_1 flies and make note of the phenotypes of the flies from each reciprocal cross. ▪ Perform F_1 self-crosses (this can be done outside of scheduled lab time)
8th week:	▪ Remove the F_1 flies from F_1 self-crosses if they're 10 days old ▪ Perform F_1 self-crosses if needed (performed outside the lab time) ▪ Observe any F_2 flies and record their phenotypes
9th week:	▪ Record the phenotypes of F_2 flies
10th week:	▪ Record the phenotypes of F_2 flies
11th week:	▪ Data analysis / meeting with TA
12th week:	▪ Oral Presentation of Data
13th week:	▪ Lab Reports Due
14th week:	▪ Review week – no fly work
15th week:	▪ **Final exam**

Please note that during the first few weeks of the experiment you **will need at least one member of your group to do some work on your project every morning and every afternoon**. Your TA will give you more details on this.

Lab 1

In this lab, we will be discussing some of the administrative details relevant to the lab part of the course. Please be sure you've looked at the syllabus and read through all of "Lab 1" in this manual **before** start of the lab.

For the main part of the lab, you will be dealing with Mitosis. You will spend some time looking at prepared slides, and will also make some of your own. Lastly, you will work in groups to model and explain the whole process to each other and to your TA.

Learning Objectives:

Students will:

- Demonstrate their knowledge of Lab Safety Rules by coming in dressed appropriately.
- Identify all pieces of safety equipment in their lab.
- Identify stages of Mitosis on a microscope slide.
- Describe the main events occurring at each stage of mitosis.
- Demonstrate good technique in the preparation of temporary slides.
- Describe the stages of the cell cycle, with a focus on what happens to the chromosomes at each stage.

Pre-Lab Questions:

Use your textbook and any online resources you think will be helpful to answer the following:

What is the purpose of mitosis?

List the stages of mitosis and describe the main events of each.

What is a centromere? How is it used in mitosis?

What is a meristem?

Why do we want to specifically look at the root apical meristem in the lab today?

Lab Safety

Your concern for safety should begin even before starting your lab activity. Please make sure you **always read your lab manual / handouts**, and think about each laboratory assignment **before starting**. Doing so, will alert you to any chemicals and other potential hazards that you might encounter in the lab that day. It will also ensure that you've had a chance to think about how you will perform certain activities and why they must be performed in certain locations or in a certain order.

Always remember that you are not the only person working in a lab and your actions and knowledge (*or lack of knowledge*) can affect the safety of others. For this reason please familiarize yourself with the locations of safety equipment (safety shower, eye wash station, first-aid kit, fire extinguisher, and blanket) in the lab, as well as the location of the medical clinic office in case medical assistance is needed. Also, please make sure your books, bags and clothing are placed out of the way where they are unlikely to cause problems.

In order to protect yourself and others follow basic safety procedures in the labs. Some of our classes involve the use of chemicals so please know and follow the following rules:

1. You **must wear a laboratory coat** for all laboratory work. You will not be allowed to work in the lab without one (and will be marked as absent).
2. **Eating, drinking, and chewing gum are prohibited** in the laboratory at all times.
3. Bulky clothes, abaya, long scarf, dangling jewelry and short skirts can be a hazard in lab. Please do not wear them to the lab or make sure they are secure and unlikely to cause problems.
4. **Confine long hair and scarf** when in the laboratory so that it will not catch on fire or come into contact with chemicals.
5. Wear shoes rather than sandals at all times in the laboratory. **Wearing open-toed shoes is hazardous** in a lab environment (you will be asked to leave the lab and marked as absent).
6. Special care is needed if you wear contact lenses since chemicals splashed in the eye may get under the lens and therefore be difficult to rinse.
7. Mascara has harmful effect on ocular lens (eyepiece) of microscope, and therefore should be avoided.
8. Do not use flammable liquids near open flames. Most organic liquids are flammable. Diethyl ether is especially dangerous.
9. Smell chemicals carefully and only when instructed to do so. Waft odors towards your nose rather than sniffing directly.
10. **Wash hands thoroughly** with soap and water immediately at the end of each lab secessions and before leaving the lab.
11. Broken glass should be removed from a work area and placed in the glass receptacle.
12. Biological waste should be placed in the appropriate waste container.
13. Notify your lab instructor immediately if you are injured in any way.

Notebooks

A laboratory notebook contains an accurate and detailed account of experimental procedures and results. It needs to be neat, well-organized and as complete as possible. The level of detail should allow another researcher to repeat your experiment and replicate your results.

It is important to keep an accurate and detailed laboratory notebook. Sometimes, a small detail may not seem important when you first see it, but it might be useful when you're trying to analyze the results – especially when they are not what you expected. Being able to go back through your procedures and observations in your notes can help you explain why your results are different from what you would expect. It might turn out that you skipped a step in the protocol, or that you made a mistake somewhere along the way (sometimes mistakes are good things – they lead to new discoveries).

The lab notebook is the property of the lab and not of the researcher who is writing in it. In a research setting, a laboratory notebook never leaves the lab. This is important, because many researchers working in that lab may need to refer to that notebook in order to perform their work.

For example, you might be a graduate student who is studying a particular gene. When you finish your project, you will need to be able to refer to your notebook to write your thesis. However, others may need to be able to use your notebook as well.

- Perhaps you made some modifications to a procedure that gave improved results – other people in your lab would benefit from having access to your notes on this procedure.
- Maybe one of your friends in the lab asked you to complete some part of an experiment for them, you would put your notes and observations in the notebook and your friend would later need to access them.
- Also, after you have completed your studies and left the lab, other graduate students might be asked to continue your research to find out more – these new grad students will benefit from having access to your notes (so they don't have to start from the beginning).

Advice on keeping a “proper laboratory notebook” varies depending on who you ask in a teaching lab, but there are a few things that are commonly done. Because some research can lead to financially rewarding discoveries and a laboratory notebook can be used as evidence of prior discovery in cases of disputes over intellectual property / patents, there are a few commonly agreed guidelines for keeping a notebook. Much of it has to do with ensuring that the contents of the book cannot be easily altered:

1. Notebooks should be permanently bound. This means that it should not be possible to remove (or add) pages from them without it being easily noticed.
2. All pages should be numbered. Again this will make it easier to notice if a page has been removed.
3. You should have a date on every page. This allows the identification of when an experiment was first performed or when a discovery was made.
4. You should write in pen and never use white-out or corrective tape. If you need to make corrections then just neatly cross out the incorrect material. A notebook that shows evidence of erasing or removal of data (use of corrective tape, etc.) is not trusted.
5. A laboratory notebook should have your name in it, and a contact number in case the book is found by someone who wishes to return it.

Because a laboratory notebook tends to contain the details of many experiments, it is also a good lab practice to include a Table of Contents on the first 1-2 pages. In the Table of Contents you should list the important experiments and items, and indicate on which pages those items can be found.

For the purposes of this course, you will also be maintaining a group notebook (you will be working in a group of 2-4 students). This notebook should have the names and contact info for you and your lab partners as well as your TA's name. It will be the responsibility of all group members to maintain this notebook and to ensure that it contains a complete record of your experiments. For example:

- It should contain detailed descriptions of the phenotypes of each *Drosophila* stock you have
- It should include the dates of any controlled matings and the dates on which the parental flies should be removed (also an indication that this was done)
- It should include the data, and dates on which it was collected
- It should include procedures as well as any changes you made to the protocols (or mistakes)
- It should include any observations you make about your flies. (for example if you observe mould growing in your culture, or a new phenotype, etc.)

You should write these things as you do them or very shortly after you've done them – it should be completed before you leave the lab each day. It is much better to have an accurate but somewhat messy description of what you did and your observations, than it is to have a less messy and less accurate description, that was written based on your memory, a day or two after the lab was completed.

Groups

In this course, you will work in groups of 4-5 students. Working in a group is not always easy but it is often a necessity in a research setting (or any work setting), so besides research skills, this course will also allow you to work on your teamwork and leadership skills. An important aspect of this type of work is that you all make an effort to divide up the work equally, that everyone contributes equally and that everyone feels like their contributions are valued by the other members. Your TA will not be able to monitor or enforce this, so it will be up to each group to ensure that the work is fairly distributed (you will also be able to evaluate your own performance and each of the other group members at the end of the course).

The Cell Cycle and Mitosis

Living organisms are made up of cells, and the number of these cells can be increased through a process called **Mitosis**. During mitosis, a **cell divides it's genetic material equally** and generates two new and identical daughter cells. In a multicellular organism, this process is responsible for growth in the size of the organism, while in unicellular organisms it results in the increase in the number of organisms.

A typical eukaryotic cell is comprised of a plasma membrane, cytoplasm and all the organelles and nucleus within it. The nucleus controls all the cell's biological functions and contains the genetic material in the form of chromatin which is condensed to chromosomes during cell division. The cells of different organisms contain specific numbers of these chromosomes, for example human somatic cells contain 46 chromosomes whereas onion cells have 16 chromosomes.

In their normal state, chromosomes exist as mostly decondensed chromatin (1 chromosome = 1 long DNA molecule and its associated proteins). They could be represented as seen in Fig.1a and b.

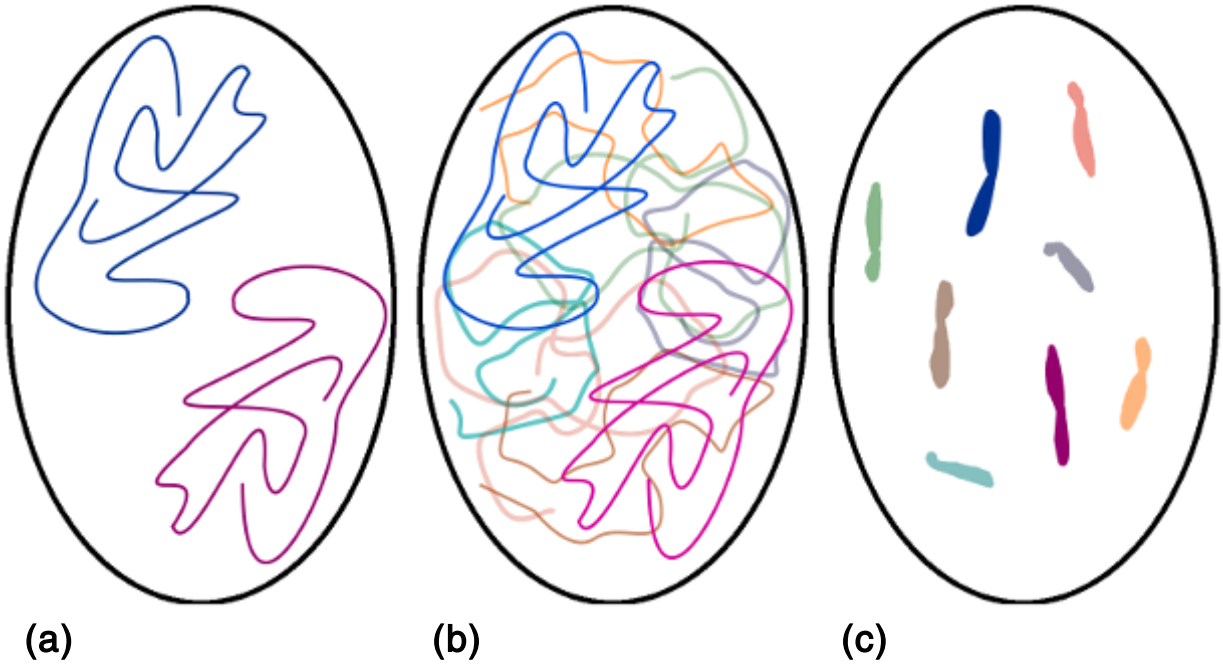


Fig. 1. Representations of chromosomes in a nucleus at the end of mitosis. Two homologous chromosomes are shown in (a), notice that they are in different areas of the nucleus. A total of eight chromosomes - 4 pairs - are shown in (b). Imagine how full this nucleus would look with 46 chromosomes. This is usually simplified for textbook figures to look like (c), but this is only done to make it easier to see what's going on.

These **chromosomes need to be replicated** before they can be divided in mitosis. If this didn't happen then the new cells would have have fewer chromosomes than the original cell. This replication happens before mitosis begins, during **interphase**. Interphase is the part of the cell cycle when the cell prepares for cell division. One of the main tasks that needs to be accomplished is the synthesis of new DNA from the old DNA. This results in the doubling of the amount of total DNA in the cell, but does not increase the number of chromosomes – instead, each chromosome is now composed of 2 identical sister chromatids.

Figure 2 shows how the above nuclei might look after the DNA synthesis phase of interphase. Most textbooks will show something similar to Fig. 2c because it's easier to see the difference (compared to Fig.1c), but in reality, the nucleus at the end of S-phase is probably a little more similar to Fig.2b in appearance.

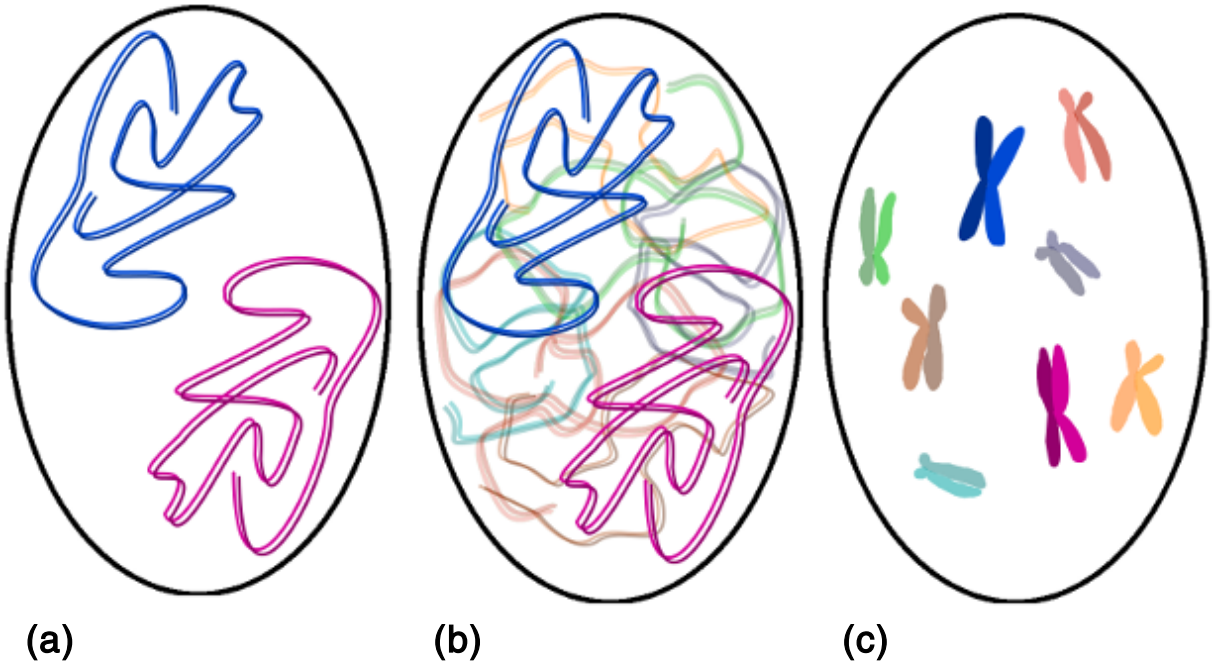


Fig. 2. Representations of chromosomes in a nucleus at the end of DNA synthesis phase. Two homologous chromosomes are shown in (a), notice that each has an identical sister chromatid now (shown in a slightly lighter colour). A total of eight chromosomes – 16 chromatids - are shown in (b). A typical textbook representation of this is shown in (c).

Please note that **the number of chromosomes has not changed**. This can be a tricky concept to understand – the amount of DNA has doubled but the number of chromosomes is still the same as before S-phase.

It may be helpful to think of it as follows:

- Each chromosome in a nucleus is unique. Even homologous chromosomes are different from each other. For example, while they might both have the gene for eye colour, the one you got from your mom might have the blue eye colour gene while the one from your dad has the brown eye colour gene***. Thus the two **homologous chromosomes are not identical in sequence**.
- Sister chromatids are identical to each other, they are the result of replication of DNA to make an **exact copy** of the template. Since their DNA sequences are exactly the same, the new copies are not called chromosomes, but chromatids.

Thus a diploid cell does not become tetraploid after S-phase – the amount of DNA has doubled but the chromosome number has not.

*** Please note that eye colour is a complex trait – it is not determined by just a single gene. It is being used here as an example of a gene that might have two different versions on homologous chromosomes and is easy to visualize and understand.

Once the cell has completed the DNA replication and has completed the remainder of its tasks in interphase, it enters M-phase (usually Mitosis). Mitosis involves the equal **division of the genetic material** and the cytoplasm. It is a very complex and continuous process, but we usually simplify it for ourselves by dividing it up into easily identifiable stages.

The first stage is **prophase**, here the chromosomes start to condense and therefore can be seen clearly under the microscope. Each mitotic chromosome is comprised of two chromatids attached together at a specific point called the **centromere**. The second stage is called **metaphase**, this is where chromosomes become denser and line up at the middle of the cell at a region called the metaphase plate. Then the nuclear membrane disappears and spindle fibers appear. The spindle fibers will attach to the chromosome at the centromere and then pull sister chromatids toward different cell poles at the **anaphase** stage.

Once separated to opposite poles, the chromatids start to decondense and a nuclear membrane reforms around each group at the final stage - **telophase**. The chromatids are now referred to as chromosomes. After nuclear division, the cytoplasm is divided to from two separate cells.

Exercises: Cell Cycle & Mitosis

Today, you will be studying slides of mitosis in plant cells, and also producing temporary slides of your own. Plant cell division is similar to animal cell division in the stages and chromosome shapes but it differs in two ways, one of them is the composition of spindle fibers. In animal cells the centriole is responsible for generating spindle fibers and cytoplasm division. On the other hand, the cell wall is responsible for completing the separation of the cells in plants.

Practical Aspects of Studying Mitosis in Plants

The parts of a plant that are active in cell division by mitosis are known as **meristems**. Meristems are regions of undifferentiated cells mostly found at the tip of the the stem [Shoot Apical Meristem – **SAM]** and at the tips of the roots [Root Apical Meristem – **RAM]** (Fig. 3).

In this lab, we will be looking at the roots of onions. In order to know where to look for evidence of mitosis, we should first understand a little about the structure of the root (Fig. 4).

The roots in plants must pass through soil as they grow. This means that they will be potentially exposed to abrasion, stress and damage. For this reason, the very tip of a root is covered by a **cap** [2] – the cap protects the **root apical meristem** region [1] from damage (Fig. 4).

Damage to the RAM of the main root will stop the elongation of that root tip, and will result in the generation of lateral roots, each with its own RAM (from secondary/lateral meristematic tissues).

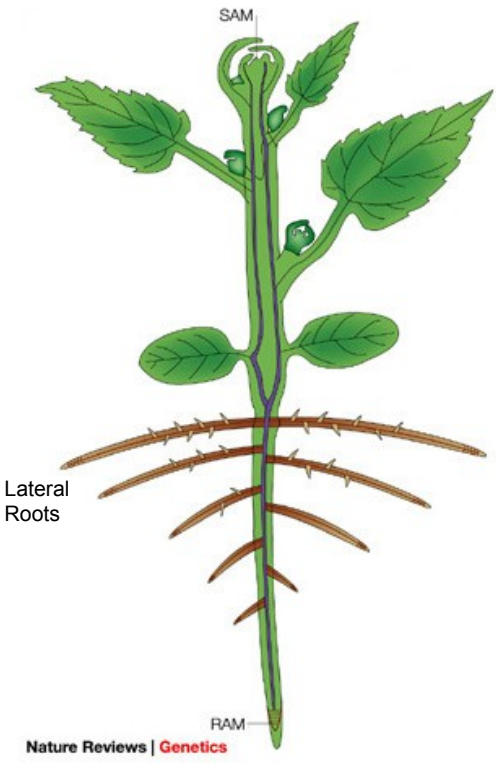


Fig. 3. Locations of meristem tissues in plants.

The root apical meristem is a region of rapidly dividing, **totipotent** cells. As new cells are produced at the RAM, the older cells (ones produced earlier) can start to differentiate into specific cell types of the **epidermis** [3], **cortex** [4], **endodermis** [5] and **stele** [6].

Thus, when looking for cells undergoing mitosis, we will only be interested in the very tip of the root (the first 2-3mm). Once you have isolated that part on your slides, you will want to search for mitotic cells near the middle of the plant tissue on your slide.

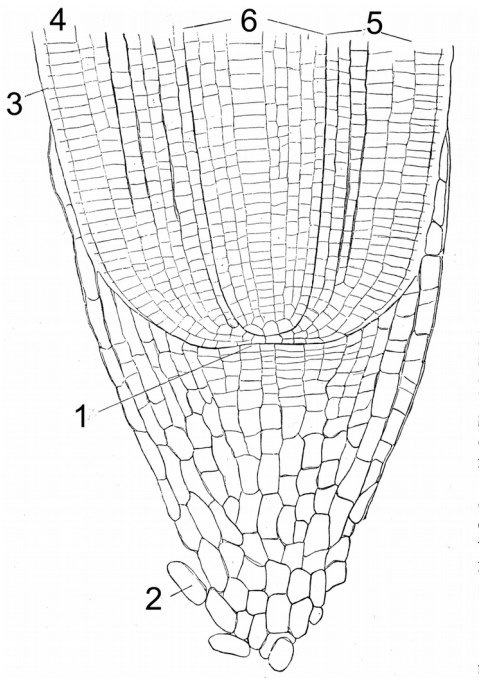


Fig. 4. Locations of various cell types in a typical plant root tip.

1. Study of Mitosis in Prepared Slides

Materials

- Compound microscope
- Prepared slides of onion root tips

Obtain a prepared slide of onion root tips and spend some time viewing them under a microscope.

1. Examine the slide containing the root apical meristem of the onion under the light microscope and check the following:
    - General morphology of the cell.
    - Location of cells undergoing mitosis.
    - Identify the stages (prophase, metaphase, anaphase and telophase)
    - The percentage of dividing cells in the different parts of the root.
    - The shape and arrangement of chromosomes at each division stage.

2. In your notes, draw the different stages of mitosis and label the important structures/features at each stage.

3. What differences would you see in animal cell mitosis?

2. Preparation of Temporary Onion Root Tip Squash Slides

Materials

- Compound and stereomicroscope
- Clean slides and coverslips
- Root tips in 70% Ethanol (previously fixed in a 3 ethanol: 1 glacial acetic acid solution)
- Aceto-orcein stain
- Small Kimwipes
- Pencils with eraser-ends
- Scalpels and forceps
- Water bath at 60°C with a beaker with 1N HCl and floating eppitube holders
- Eppendorf tubes
- Beaker of water

Procedure

1. Obtain 2-3 root tips and place them into an eppitube.
2. Add 1ml of warm 1N HCl into your tube and place it into the water bath for ~5min. The acid will help to dissolve the cell wall and make it easier for the stain to penetrate into the cells.
3. Carefully remove the HCl, add 1ml of water into the tube and mix.
4. Discard the water and again add 1ml of water. Repeat this step one more time. In this way you have washed the tips in water 3 times and have removed most of the acid.
5. Use forceps to gently take out a root tip and place it on a clean slide.
6. Use the stereomicroscope to help you find the root cap and the tip of the root.
7. Use a scalpel to cut the root about 2mm from the tip and remove the remainder of the root. You want to only have the very tip of the root (the area with the meristem).
8. Put one drop of the stain onto the slide and keep your specimen in the stain for about 5min. You can use your scalpel to squash and cut up the root tip while it's staining, this will help the stain penetrate more easily.
9. Cover your specimen with a cover slip and dry out extra stain using a Kimwipe.
10. Squash the specimen further by pressing down gently on the cover slip. Do not let the coverslip move as you do this. You can use the eraser-end of a pencil to help to distribute the cells more under the coverslip. You are trying to generate a monolayer of cells.
11. Examine your specimen under the low and high magnification of the compound microscope and try to find mitotic cells. Most of the cells will be at interphase, so use the low magnification objective lens to find areas with some mitosis and then use high magnification to look at details.

You have just prepared a temporary slide.

3. Modeling the Cell Cycle and Stages of Mitosis

Materials:

- Foam “chromosomes”
- Hair clips to act as “genes”

For this exercise, you will work as a team to help ensure that all members of the team understand the key events of mitosis and can explain and demonstrate them. You will use a set of foam “chromosomes” to demonstrate what happens to them at each stage.

You will receive a set of blue and a set of pink “chromosomes”. The pink set can represent the chromosomes that were inherited from the individual's female parent and the blue ones can represent the chromosomes inherited form the male parent. You will use these models to help you describe the cell cycle and mitotic stages to the other members of your group (feel free to use your notes here).

1. Interphase - Describe what is happening to the chromosomes at the different stages.

2. Prophase - Point out what a chromosome and a chromatid.
3. Metaphase - Line up the chromosomes on the metaphase plate. Are homologous chromosomes associated?
4. Anaphase - Show chromosomes moving to opposite poles. How many chromatids are there per chromosome?
5. Telophase - Describe the changes that occur with the chromosomes.

Repeat the exercise but this time, let's put some genes on the chromoromes. Obtain 4 hair clips – 2 of one colour and 2 of another – these will represent a specific gene (maybe the "eye-colour" gene). You will be modeling mitosis in a diploid heterozygous individual - one that has two different versions of the "eye-colour" gene. Use the hair clips to represent the two different eye colours – be sure to apply them to the right chromosomes.

Once everyone in your group has has demonstrated the process, you will explain this process to your TA. Any member of your team may be asked to demonstrate and explain, so make sure everyone in your group is capable of doing it correctly. Additionally, your TA may choose to ask questions during or after your demonstration.

Post-Lab Questions:

Please think about these and answer them in your notebook:

What is the purpose of mitosis?

Why do cells condense their chromosomes during prophase?

In your own words, explain the difference between a chromosome and a chromatid.

Assignment: Sketches of Mitotic Cells

Draw two sample stages of Mitosis (your TA will tell you which ones to draw), and submit your drawings to the TA at the end of the lab.

Name: _____ Student ID: _____

Mitotic Staging of Microscopic Specimens

Name of Slide:	Slide #:
Title:	Stage:
	Objective Lens Magnification:
	Ocular Lens Magnification:
	This drawing is larger than what I see by a factor of:
	Total Magnification:
Explanation:	

Name of Slide:	Slide #:
Title:	Stage:
	Objective Lens Magnification:
	Ocular Lens Magnification:
	This drawing is larger than what I see by a factor of:
	Total Magnification:
Explanation:	

Lab 2

Today's lab introduces you to the model organism we will be using for the remainder of the semester. This lab will focus on observing wildtype flies, and differentiating between male and female flies. In the process, you will be learning skills that you will need for your major project this semester. Make sure you take some time to practice them.

You will also prepare some mitosis slides for use in next week's lab. Because you will need these slides to still look good in a week, you will be adding some extra steps the the protocol, that you didn't do last week. These steps will help to preserve the slides, without them the slides would be dried out and unusable in the next lab.

Learning Objectives:

Students will:

- Describe the life cycle of *Drosophila*.
- Differentiate between male and female flies.
- Describe the wildtype phenotype in *Drosophila*.
- Demonstrate appropriate technique in etherizing flies and performing a fly cross.
- Identify stages of Mitosis on a microscope slide.
- Demonstrate good technique in the preparation of permanent slides.

Pre-Lab Questions:

- Look up the MSDS-s for Ether and Xylene. List the potential hazards from these chemicals in your notes. In each case, read through the section on first aid.

- Go to this website: http://www.yourgenome.org/topic/animals-and-plants

  Read the page about Model Organisms.

  What is a model organism and why do we use them?

  Read the page about Fruit Flies in the Laboratory.

  Why was the discovery of the white eyed flies important?

  How many chromosomes do *Drosophila* have?

  Read the page about Why Use the Fly in Research.

  What percentage of disease-causing human genes are also found in *Drosophila*?

Introduction to *Drosophila melanogaster*

Fruit flies (*Drosophila melanogaster*) are small insects, which have been used in the study of Genetics since 1910. This is partially because they're easy to maintain and do not require many resources, but also because many of their phenotypic traits are easy to observe. Many of the traits that geneticists have used to study inheritance in *Drosophila* have to do with their eye colour, body colour and wing shape and size. These traits may not seem like they are particularly useful to study, but it was the study of such traits that allowed geneticists to learn enough about genetics to discover gene linkage, sex linkage and chromosome mapping techniques.

The fruit fly is still frequently used in genetic studies. While it may not seem very similar to us, it continues to yield genetic information that is relevant to human health. Genes involved in human diseases like Leukemia, Cystic Fibrosis, Hereditary Deafness and several forms of Cancer can all be studied in *Drosophila*.

Life Cycle of *Drosophila*

The *Drosophila* life cycle consists of 4 main stages: egg, larva, pupa and adult (Fig. 1). Fertilized eggs are usually deposited within suitable media and hatch within 15hrs (at room temperature). The *Drosophila* initially emerge as white worm-like larvae that burrow into the medium.

Since the insect cuticle does not stretch, a larvae must shed its 'skin' twice as it grows – this is called molting. At the first instar stage, the larvae has not molted yet. After it molts for the first time, it is known as second instar larvae, and third instar larvae after it has molted a second time.

Throughout this period, the larva feed on the media until they are ready to pupate. Once they reach this stage, they crawl out of the media to a dry place on the side of the media container. Here, they stop moving and begin the next stage of their development.

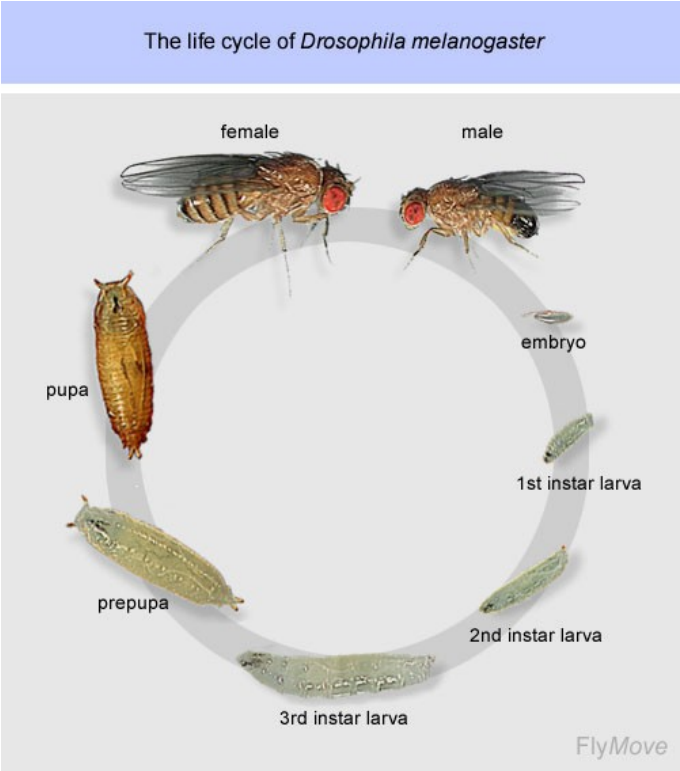


Fig. 1. The life cycle of Drosophila melanogaster.
Source: flymove.uni-muenster.de

After 4 days of development as a pupa, adults emerge from the pupal cases. Newly hatched females become sexually active 8-12 hours after hatching, and can begin to deposit eggs around their third day of adult life, and thus the cycle can continue.

In total, it takes about 10-14 days from the time that eggs are laid until the emergence of adult lies. The actual time depends on the temperature – usually, the warmer the temperature, the faster the development. If the cultures are kept between 21 and 25 C a new generation will be produced in about two weeks from the date that adult flies are put together in culture medium. Adults can survive and stay fertile for about one month if kept under good conditions.

Maintaining and Manipulating *Drosophila*

Flies are typically grown in vials containing a medium which consists of a mixture of cornmeal, corn syrup and agar. They are handled using clean/sterile tools to avoid contaminating the flies or vials with molds or mites that can kill the flies.

In order to determine the sex and assess the phenotypes of the flies, they are usually immobilized using either cold-anesthetization or a brief exposure to ether. Once the flies have been anesthetized, they are individually examined under a dissecting microscope.

Sexual Dimorphism in *Drosophila*

Sexual dimorphism refers to the difference in appearance between males and females of the same species. This includes differences in shape, size, ornamentation and behavior. This allows scientists to easily distinguish male from female flies and carry out mating experiments correctly.

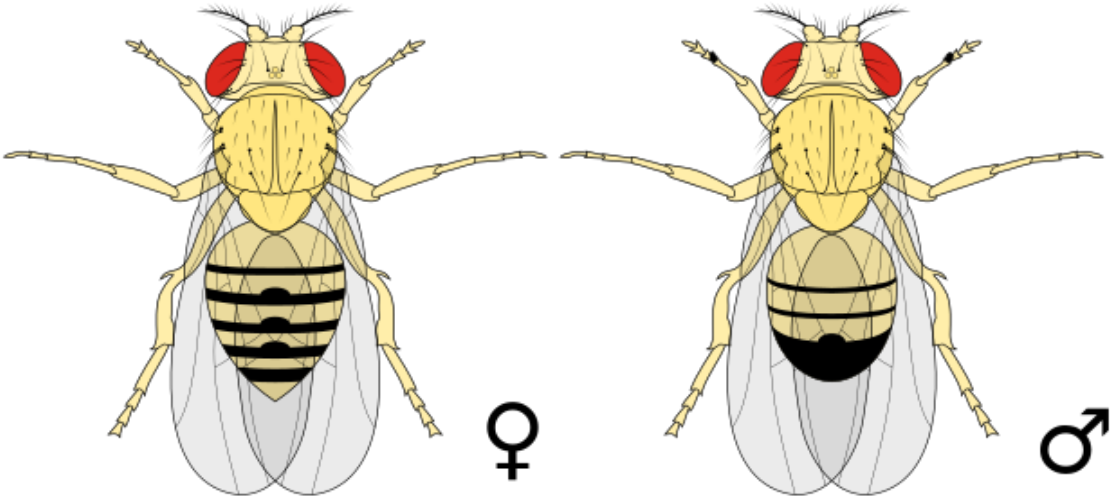


Fig. 2. Schematic diagram of a female and male fruit fly, showing some of the morphological differences between them.

Source: commons.wikimedia.org

Several criteria may be used to distinguish male from female *Drosophila*:

1. Females are usually somewhat larger than males
2. The female abdomen is more pointed, while the male abdomen is rounder and shorter
3. The pattern of stripes on the abdomen is different in males and females – the tip of the male abdomen is heavily pigmented. This is probably the most easily identifiable difference (except in newly emerged flies which are still not fully pigmented)
4. Males have a tiny tuft of bristles/hairs (sex combs) on their front legs

Exercises: Examination and Handling of *Drosophila*

In this exercise, you will familiarize yourself with the appearance of **wildtype** *Drosophila*. You will also learn to distinguish between males and females – this skill will be very important in the coming weeks.

Working with *Drosophila* can be challenging because the flies are small and very quick, thus in order to view and manipulate them, you need to have a reversible way of immobilizing the flies. This is done through anesthetization using a short exposure to ether. Only use a small amount of ether and work in the fume hood in order to minimize the fumes in the lab room. Your TA will demonstrate the process for you.

Once the flies have been anaesthetized (etherized), transfer them onto a clean, white index card and view them under a dissecting microscope. Use a clean paint brush to manipulate the flies so that you can see the necessary details. The brushes can be sterilized by dipping them in 70% ethanol, but be sure that they are dry before using them afterwards.

As you look at the anaesthetized flies, you may notice some that appear quite large and very pale (gray); the wings of these flies look very small. These are not mutant flies – they are flies that have recently emerged from the pupal casings. If you were to give these flies a few hours to mature, they will become darker and extend their wings to their normal adult size. When looking at your flies, try to only examine the adult flies and not these newly-emerged flies – the phenotypes will be easier to identify in adults.

Materials

- Wild type flies
- paint brush
- etherizer
- diethyl ether
- 70% ethanol squirt bottle for sterilizing brushes
- fly morgue with 70% (for disposing of *Drosophila*)

1. Sexing *Drosophila*

1. Obtain a bottle of wild type *Drosophila* and anaesthetize them. Do this as a group – each bottle contains many flies and there aren't enough bottles to give one to each student. Leave the flies exposed to the ether until there aren't any flies moving around for at least 30sec. (you may see their legs twitching – that's okay).
2. Transfer some flies onto a clean index card and place them under your stereomicroscope (also known as a dissecting microscope).
3. Use a pen or pencil to draw two circles on your index card and label them as “males” and “females” or “♂” and “♀” (the symbols for male and female). You will move your flies into those circles as you identify their sex.
4. Use the following criteria to help you identify the sexes of your flies:
    - Females are generally larger than males. This can be difficult to determine if you have little experience with *Drosophila*.
    - The female abdomen is more pointy, while the male abdomen is more rounded and shorter.

- The pattern of stripes on the abdomen is different in males and females. Females have stripes going all the way to the tip, while **males have a heavily pigmented tip** ("black bum"). This is one of the easiest characteristics to identify.
- Males have **sex combs** on their front legs. This is a small, dark tuft of hairs at one of the joints of both of their front legs. These are very dark even in newly emerged flies, and can be used to confirm the sex of the flies.

5. Try to separate all your flies into their assigned areas on your index card.

As you're performing this exercise, you may notice that some of your flies are starting to move. You will initially see their legs twitching and maybe you'll see their mouth-parts moving – this is normal and you shouldn't worry about the flies flying away at this point. At some point, however, the flies will start to get up onto their legs and slowly walk around (they're still waking up). If you notice this, then you need to finish up your work quickly or get the flies re-etherized – they will escape if you don't.

You will need to learn to work (sex and identify phenotypes) quickly because you should avoid re-etherizing *Drosophila* – re-etherizing has a tendency to sterilize the flies which means that they won't be able to reproduce.

6. At any point during this exercise, ask your TA to check to make sure you're identifying your flies correctly.
7. Draw a sketch of a male and a female fly on the provided handout. Try to make your drawing look as much as what you're seeing under the microscope as possible. You can do this at the end of the lab when you're doing the 3rd exercise.

2. Self-cross of wildtype *Drosophila*

In this part of the lab, you will be performing a self-cross. This means that you will be placing male and female flies from the same bottle in a fresh bottle to allow them to mate. You only need to produce one such bottle per group, so discuss this part with your lab-mates to see who will do this part.

1. Obtain a bottle of fresh media and label it with your group name and the strain of flies you're putting in (in this case "wildtype" or "wt").
2. Unplug the bottle and place it on its side.
3. Take 4-5 male flies from the circle on your index card (from the previous exercise) and use your brush to gently get them into the bottle.
4. Take 8-10 female flies from the other circle on the index card and get them into the bottle.

As you're adding the flies, make sure the bottle is on its slide. If you keep the bottle standing up, you will be dropping the flies into the media, where they will get stuck and will not be able to move. Such flies will not be able to mate and produce progeny. If you keep your bottle on its side then the flies will only be in contact with the glass while they're knocked out. Once you see your flies start to walk around the inside of the bottle, you can go ahead and stand it up in its normal orientation (with the media at the bottom). At this point the flies will be able to walk around on the media without getting stuck.

5. Plug the bottle as soon as all the flies are in.
6. Wait for all the flies to “wake up” and start walking around. Once you see this, it is safe to put the bottle away in your designated storage area.

3. Observation of *Drosophila*

In this year’s lab, you will be dealing with wildtype and non-wildtype (mutant) flies. The mutant flies could have mutant **phenotypes** that affect eye color, eye shape, body color, wing shape, wing size, wing venation, and head & thorax bristle shape and number. It is therefore important for you to become familiar with the wildtype phenotype – this way you can tell when something looks different (a mutant phenotype).

As you learn to recognize the wild type phenotypes, put this information in the table below. Be sure to discuss these with your group members – you all need to be able to agree on how you describe wildtype characteristics, this way you will all know when one of your colleagues is describing a mutant phenotype. Before you leave the lab, please make sure you show the table to your TA.

1. Observe any remaining wildtype flies that you have after the last exercise. Etherize them again if necessary – you won't be breeding these flies, so you don't have to worry about making them **sterile**(first definition).
2. Observe the eyes of the flies. Wild type eyes are red in color (they are darker if a fly is dead) and have an oval shape. Draw a fly head and sketch the shape of wild type eyes in the table. Try to draw them to scale.
3. Observe the body (thorax and abdomen). Wild type body color is mixture of beige and gray. The males may appear darker than the females, but that's mostly because of the dark pigmentation of their abdomen. Be sure you look at more than just one or two flies so that you learn to recognize the wild type body color in both females and males. Draw the abdomen of a male or female (indicate which one in the table) and add color.
4. Observe the bristles (hairs) on the thorax of the flies. Wild type bristles are numerous, fairly long, straight and smooth. Draw a few of the bristles, keeping shape and relative size correct.
5. Observe the wings of a few flies. Always make sure you look at more than one fly for this – sometimes a few flies may have damaged wings (sections of a wing might be torn or bent), these are not a mutant phenotype.
    - Wild type wings are large (the wings extend significantly past the abdomen), have smooth edges, and have veins which are inflated to extend the wings after a fly emerges from the pupal casing. It is important to observe the pattern of the veins closely because there exist a few mutations that affect the venation pattern (especially the short veins which connect the longer veins). Draw the abdomen and wings of a wild type fly. Be sure to keep the relative proportions correct.
6. Fill in the table below with as much detail as possible. Feel free to add labels and any extra useful descriptions to the drawings.

Strain:	Wildtype	
Eyes		
Shape		
Colour		
Size		
Body		
Colour		
Thorax stripe pattern		
Bristles		
Length		
Shape		
Colour		
Wings		
Length		
Shape		
Veins		

Exercises: Preparation of Permanent Slides

In this exercise, you will prepare some slides for viewing next week. Last week's slides used a quicker protocol that would normally be used to allow you to view your slides for diagnostic purposes (for example: is there any evidence of mitosis in this tissue?) before performing an experiment on the rest of the tissue. Because these slides are only used for diagnostic purposes, we make no effort to preserve them and and they are discarded (the cells would have dried out by now).

If you are interested in preparing slides that will be viewable for a longer period of time (months or years), you would want to follow a slightly different protocol. Last week we only stained our slides; this week you will be adding dehydration, clearing and mounting to the protocol.

These extra steps are there in order to remove water from the cells and replace it with the mounting medium. While it might seem like the procedure is unnecessarily long, every step is needed in order to preserve the tissues and make the slide permanent.

The ethanol is used to dehydrate the cells – this means that water is removed and replaced by ethanol. We place our samples into several different concentrations of ethanol in order to remove water gradually and minimize tissue damage and shrinkage. We have to remove the water because it does not mix with an organic compound like Canada Balsam.

Unfortunately, ethanol is also not miscible with Canada Balsam, but it is able to mix with an organic solvent like Xylene. So the next step is the replacement of ethanol with Xylene – this is called “clearing”. Once the ethanol is completely removed (after an incubation in 100% Xylene), Canada Balsam can be used to mount the coverslip onto the slide. Xylene can mix easily with the mounting medium, so the Canada Balsam will be able to get into the cells on the slide and stick to them.

Materials

- Compound microscope
- Clean slides and coverslips
- Root tips in 70% Ethanol (previously fixed in a 3 ethanol: 1 glacial acetic acid solution)
- Feulgen stain
- Ethanol series (70%, 80%, 95%, 100%)
- 50% Ethanol : 50% Xylene
- 100% Xylene
- Canada Balsam
- Small Kimwipes
- Pencils with eraser-ends
- Scalpels and forceps
- Water bath at 60°C with a beaker with 1N HCl and floating eppitube holders
- Eppendorf tubes
- Beaker of water
- Slide warmer

4. Preparation of Permanent Onion Root Tip Squash

Procedure

1. Obtain 2-3 root tips and place them into an eppitube.
2. Add 1ml of warm 1N HCl into your tube and place it into the water bath for 12min. The acid will help to dissolve the cell wall and make it easier for the stain to penetrate into the cells.
3. Remove the acid and rinse out the root tips with water 3x
4. Remove water, add 0.5ml of the stain and incubate for 10min.
5. Remove the stain and wash the tips in water 3x
6. Put a drop of 45% acetic acid on a slide
7. Transfer a root tip to the slide and remove the unstained part of the tip.
8. Squash/cut up the root tip with your scalpel.
9. Cover your specimen with a cover slip and squash the specimen further by pressing down gently on the cover slip. Do not let the coverslip move as you do this. You can use the eraser-end of a pencil to help to distribute the cells more under the coverslip. You are trying to generate a monolayer of cells.
10. Prepare a second slide with one of your other root tips.
11. Examine your specimen under the low and high magnification of the compound microscope and try to find mitotic cells.

    You have just prepared a temporary slide. To make the feulgen-stained slide permanent, perform the following steps:

12. Place the above slide on ice for two minutes. This will help to release the coverslip.
13. Carefully use a razor blade or the edge of a scalpel to lift the edge of the coverslip and remove it from the specimen.
14. Pass the slide through a series of alcohol preparations 70%, 80%, 95%, 100%, 50% alcohol + 50% xylene and pure xylene for 2-3minutes in each preparation. Use your forceps to transfer the slides to each solution.

    The treatment with increasing concentrations of ethanol is known as Dehydration (it removes water from cells and replaces it with ethanol). The dehydration needs to be done gradually to minimize shrinkage of the cells. The treatment with xylene replaces the ethanol with xylene – this is the Clearing step.

15. Add one drop of Canada balsam to your slide and slowly put a new coverslip back on the specimen. Avoid trapping air bubbles and do not touch the Canada Balsam that spills around the edges of the coverslip.

    The addition of Canada balsam is the Mounting step. Do not get the Canada balsam on your hands – it is not water-soluble and will not wash off easily

16. Put, your slides on a slide warmer plate at 50°C for two days to dry and harden.

You have now prepared a permanent slide.

Post-Lab Questions:

Please think about these and answer them in your notebook:

When setting up crosses of fruit flies, you should never make a cross where you only have one male fly, or only one female fly with flies of the opposite sex. Why?

Why is it important to prepare slides in a way that produces a single layer of cells?

Lab 3

Today's lab introduces you to the fly strains you will be using for your project this semester. This lab will focus on observing these flies and identifying their mutant phenotypes. This lab will also allow you to practice some of the skills you learned last week and which you will need for your major project this semester. Make sure you take the time to practice them.

You will also be looking at last week's mitosis slides to determine how active the meristematic tissues are. You will also determine a base phase rate – an indication of how common the different stages of mitosis are in a normally cycling cell population. This will become important in later labs when we talk about problems with mitosis.

Learning Objectives:

Students will:

- Identify the mutations in their *Drosophila* stocks.
- Be able to appropriately etherize flies and perform a fly cross.
- Identify stages of Mitosis on a microscope slide.
- Determine the mitotic index and phase rates in normal onion root tips.
- Explain why knowing the phase rate and mitotic index might be useful when studying the effect of various chemical on cell reproduction.

Pre-Lab Questions:

You are sitting at an airport watching a group of people as they get off their airplane. As the people walk past you, you notice that 7% of them are carrying umbrellas and 2% of the people in this group have sunglasses.

You don't know which country these people came from, but if you had to guess, does this country have more sunny days or more rainy days?Explain.

Are most days in this country sunny, rainy or cloudy? Explain.

Start of *Drosophila* Project

This week marks the starting point of your *Drosophila* project. In this project, you will be cross-breeding (crossing) laboratory **strains**(first definition) of flies in order to determine the inheritance pattern of a variety of mutations. From your experiments, you will be able to find out if the mutant traits you're following are controlled by genes on **autosomal** or on sex chromosomes. In some cases, you will be able to determine the distance between these genes on a chromosome.

In order to do this, we will be setting up a set of controlled crosses with **true-breeding** strains of flies, this means that all the flies in one strain have the same genotype and phenotype (they have been bred for many generations to ensure that they have identical genes). Here is a general overview of the experimental design:

- You will be assigned a set of fly strains, they will be labeled with a number. Arrange the numbers of your strains from lowest to highest. For the purposes of the lab manual, we will relabel your strains with letters: A, B, C, etc. This means that if you receive strain 1, 3, 4 and 9, then strain 1 will be referred to as “A”, strain 3 will be “B”, strain 4 will be “C”, etc.
    - We do this because different groups will get different combinations of strains – you might get 1, 3, 4 and 9, but another group might get 3, 5, 7 and 8. Using the new labels allows us to more easily give everyone instructions.
- The first set of crosses will be **reciprocal crosses**. You will take male flies from strain A and cross them with females from strain B. You will also take strain A females and cross them with strain B males. This will generate the F_1 generation (first **filial generation**).
- You will collect the F_1 flies and observe their phenotypes – they might give you some clues about their **mode of inheritance**. You will then self-cross the F_1 flies to generate the F_2 generation.
- You will collect the F_2 flies and catalogue their phenotypes. You will need to keep track of the numbers of flies with each phenotype, so developing a good, well-organized system for doing this will be important.
- You will then compare the experimentally-determined phenotypic ratios to expected phenotypic ratios based on your knowledge of Mendelian laws.

At the end, you (as a group) will present your results to your peers in the class. You will then (individually) produce a written lab report that describes this experiment and your results.

Some of this work will have to be done outside of regular laboratory hours, especially at the start or the experiment. You will need to make sure you all work well as a group and that you're all well prepared and knowledgeable about what you're supposed to be doing.

Exercises: Work with Mutant *Drosophila* Stocks

Today you will receive the mutant stocks that you will be using for your experiment this semester. Each of these fly strains can have from zero to three mutant traits (depending on the strains you receive). It is important that you correctly identify all of the mutant traits present in your flies – this will be the focus of today's lab.

It is also important that all the members of your group can identify/agree on what the mutant traits are. If someone in your group can't accurately identify a mutant trait then she will not be able to provide accurate data for your experiment, so please make sure that you work as a group and ensure that everyone can differentiate between a mutant trait and a wildtype trait.

Once you have identified the mutant phenotypes in your fly strains, check your observations with your TA. Your TA will either confirm that you're correct or suggest that you look more closely. It may be helpful to have a few wildtype flies from last week for comparison (you know that those flies have a wildtype phenotype for all traits). Since they've had a week to mate and lay eggs, you don't need them in that bottle anymore and can etherize them for use in today's lab.

Use the table on the next page to help you describe your experimental strains.

Materials

- Mutant fly stocks
- paint brush
- etherizer
- diethyl ether
- 70% ethanol (for sterilizing brushes)
- fly morgue (for disposing of Drosophila flies)

1. Self-crosses of Mutant *Drosophila* Strains

Procedure

Repeat this procedure for each strain you receive (you can assign one strain to each member of your group to speed things up). In each case, perform the self-cross with a few of your flies and then use the rest for the next exercise.

1. Obtain a stock bottle of your assigned *Drosophila* and make note of the label on the bottle. Anaesthetize the flies using the method you used last week. The flies in each bottle are “true-breeding”. Leave the flies exposed to the ether until there aren't any flies moving around for at least 30sec.
2. Transfer some flies onto a clean index card and place them under your stereomicroscope.
3. Identify and separate a few males and females. Use these to make self-crosses using 4-5 males and 8-10 females using the same technique you used last week. Be sure to label the new bottles appropriately.

2. Identification of Mutant Traits in Assigned Drosophila Strains

1. Using the remaining flies from the previous exercise, observe the phenotypes of the flies from each strain and make note of them in the provided table.
2. Once you think you have identified all the mutant traits, check with your TA.

Strain:		Strain A	Strain B
Eyes			
	Shape		
	Colour		
	Size		
Body			
	Colour		
	Thorax stripe pattern		
Bristles			
	Length		
	Shape		
	Colour		
Wings			
	Length		
	Shape		
	Veins		

Exercises: Determination of the Mitotic Index and Phase Rate

Mitosis results in increasing cell numbers thus contribute in growth and replacement of the cells that die during individual growth. Mitosis often occurs in specific regions of the organism such as meristem tissues in plants.

In order to determine the rate of cell division in the active tissues such as meristematic cells in the plant's apical regions, we need to measure the mitotic index. Mitotic index can be defined as the ratio of the dividing cells to the total number of cells in the tissue expressed as a percentage. This number gives an indication of how active a tissue is. If the number is high, that means there is more mitosis, it it's low then there is less mitosis. In this way we can compare different tissues – a root tip might have a higher mitotic index than a mature leaf – or to compare the same tissues under different experimental conditions.

We can also calculate the phase rate, which is the ratio of the number of cells in a specific phase/stage to the total number of dividing cells. This gives us an indication of the percentage of cells at each stage and can be an indication of the length of each stage – the more cells we see at a particular stage, the more likely it is that this particular stage is takes a long time to complete. Similarly to the mitotic index, it is also possible to find out the effect of different external factors on the different mitotic stages by calculating the phase rate.

Materials:

- Your slides of onion root tips
- Compound microscope

2. The Mitotic Index and the Phase Rate in Onion Root tips

The mitotic index may be calculated as follows:

1. View the slide under high magnification.
2. Choose one field on the slide which contains different division stages and calculate the number of undivided cells.
3. Identify and count all the dividing cells at each division stage (prophase, metaphase, anaphase and telophase)
4. Move the slide and choose another field and repeat what you did in step 2.
5. Repeat this 2 more times.

Field	Prophase	Metaphase	Anaphase	Telophase	Interphase
1					
2					
3					
4					
Total					

6. Write your totals on the board to share them with your classmates.

Class Data for Onion Root Tips					
	Prophase	Metaphase	Anaphase	Telophase	Interphase
Total					

7. Add up the class data and enter the totals in the table above. Add up the totals for all the mitotic stages to give you the “Total number of cells”, and calculate the mitotic index. Use this equation:

$$\text{Mitotic index} = \frac{\text{number of dividing cells}}{\text{Total number of cells}} \times 100$$

8. From the tables, calculate the sum of all the cells in mitosis (dividing cells) and calculate the phase rate as follows:

$$\text{Phase rate} = \frac{\text{number of cells at a particular stage}}{\text{number of dividing cells}} \times 100$$

		Onion Root Tips
Mitotic Index		
Phase Rate	Prophase	
	Metaphase	
	Anaphase	
	Telophase	

Post-Lab Questions:

Please think about these and answer them in your notebook:

You're looking at two different samples of cells. Sample #1 has a mitotic index of 5.6%, while sample #2 has a mitotic index of 7.8%. What can you conclude about the cell samples based on these results? Explain.

What would it mean if you found a sample that had a mitotic index of 0%? Do you think that's even possible? Explain.

The work described below will be completed over the next 2.5 - 3 weeks and need to be mostly done outside of regular lab hours. The reasons for that will be explained in class and can be found a little further below.

You will be making reciprocal crosses between your fly stocks. You will probably (depending on number of stocks available at the start of the semester) be making two sets of reciprocal crosses. In most cases it will be between stocks A and B and between B and C, but if you were given 4 different fly stocks then do the second set of crosses between C and D (instead of B and C).

As indicated earlier, for the purposes of this lab manual, the two fly stocks with the two lowest numbers are stock A and stock B. You should try to generate at least 3 bottles of Stock A females mated with Stock B males. You should try to do the same for the reciprocal – aim for at least 3 bottles of Stock B females mated with stock A males.

- For each bottle there should be at least three females and two males. Larger numbers would be preferred, and having more females than males is also better.
- You should end up with at least 6 bottles completed by the end of the 3 weeks.

For this set of reciprocal crosses Stock A and Stock B are the Parental generation, and their offspring will be the F_1 generation of that reciprocal cross.

For the second set of reciprocal crosses, you will be working with either stocks B and C (if you were given 3 stocks) or C and D (if you were given 4 stocks). Set up these crosses in the same way as already described for stocks A and B.

Important Information for All Crosses

The time it takes for an adult *Drosophila* to develop from an egg is about 10-12 days at a typical room temperature (approximately 20°C), and females typically lay eggs about 2 days after mating. This means that once you've made a cross (put some males and females together in a bottle), you can expect to see their offspring emerge about 2 weeks later. When they do, you do not want the parental flies mating with the new generation, so you need to make sure you remove and discard the parental flies before the new generation hatches.

Tip #1:

- For each cross you make, write down the date on which it was made on the bottle. Based on this date, determine when you need to remove the parental flies (about 7-10 days after making the cross) and make sure that this is done for every cross.

When making crosses, you will be manipulating etherized flies. Make sure that you keep the bottles on their sides until you see that the flies you have combined actually wake up and crawl around the vial. Dead flies do not mate.

After the new generation emerges in your bottle, the new flies will start mating with one another and laying eggs. This means that the next generation (ie. F_2) will start to hatch about two weeks after that. At that point it will be nearly impossible to identify which flies belong to which generation and using these flies could negatively affect your results.

Tip #2:

- Write down the date on which you first noticed the new offspring (ie. F_1) emerging (if you had cleared out the parental flies, this will be easy). If you first noticed the new flies on a Sunday then assume that they may have emerged on a Friday. Once you have figured out this date, calculate the date when you should expect the next generation (F_2) to start emerging in that bottle and write that down as your "expiry date" or "best before date". Once you've passed this date, do not use the flies from that bottle any more.

Important Information for Reciprocal Crosses

In order to make sure that you're **making controlled crosses** in your experiment, you will need to make sure that the females you use only mate with the males you provide. One of the difficulties with this is that ***Drosophila*** females often mate with more than one male before laying eggs. They actually **store the sperm** in specialized organs and can fertilize their eggs several hours or days after mating. Thus the only way of ensuring that female *Drosophila* will **only** mate with the males that you selected is to only use virgin female flies.

Drosophila females become sexually active 8-12 hours after they emerge from the pupae. Thus in order to obtain virgin females someone from your group must separate the females from their male siblings within 8 hours of the females hatching so that the females can not mate with their male siblings.

This is why you will need to be available outside of regular lab times very frequently for the next few weeks – this will only be necessary for the reciprocal crosses, the F_1 self-cross will not require virgin females.

Someone from your group will need to be able to come to the lab early every morning and clear the stock bottles (all flies should be removed). It is absolutely critical that no living flies are left in the bottles.

Someone from your group will then have to come into the lab every afternoon – less than 8 hours after the morning clearing. She will etherize any newly-emerged flies and collect any females. Since these females could have only hatched during the last several hours, it can be safely assumed that they have not copulated with any males yet and can be used in further experiments.

Collecting flies in this way may not seem like something that will take 3 weeks, but it is not uncommon to come in the afternoon and find that only 1 fly had hatched since that morning and it was male. Collecting enough females for all the crosses may take some time, so be patient.

Tip #3:

- Arrange a schedule for this with your lab mates, and try to make sure at least two of you can show up in the mornings. Life happens, and sometimes one student may not be able to make it in to the lab. There should always be someone she can ask to take over.

Tip #4:

- Make sure you use your notebook to communicate what you've completed. If you cleared out all the vials in the morning, then write it down in the group's notebook so that the student who comes in the afternoon knows that she can safely collect and use any females she finds. Conversely, if there is nothing written in the notebook from that morning, you should assume that noone came in the morning and that you can't collect any flies that afternoon.

Culture bottles should continue to be checked in the mornings and afternoons until both sets of reciprocal crosses have been generated (remember you want 3 bottles of each side of the cross). If you need males for a cross, you can collect them in the mornings (they don't need to be virgin). In the afternoon, as long as vials were cleared that morning, all females and male flies can be collected for use in making crosses.

Tip #5:

- Don't wait too long to make the crosses – you don't need to use large numbers of females (like in the self-crosses in the last two labs). You can easily get the number of flies you need in this experiment if you start with 3-4 females and 2-3 males per bottle. Remember that each *Drosophila* female is capable of laying 300-400 eggs in her lifetime, so even one female could generate lots of F_1 flies for you.

Tip #6:

- Don't make crosses where you only have one female with a few males, one male with a few females. If that one fly dies or is sterile then you will have wasted your time and resources.

Lab 4

In today's lab, you will be dealing with Meiosis. We will spend some time discussing meiosis and its stages in detail. You will then be looking at prepared slides of meiotic tissues from lilies, and will also make some of your own slides as well.

In the second part of the lab, you will work in groups to model and explain the whole process of meiosis to each other and to your TA. We will be focusing on linking what is happening during meiosis to Mendel's Laws.

Learning Objectives:

Students will:

- Visually identify stages of Meiosis
- List ways in which Meiosis differs from Mitosis and explain the advantages of those differences
- Describe events occurring at each stage
- Explain how Mendel's Laws are related to the movement of chromosomes during Meiosis
- Link the event of Meiosis to the generation of allele combinations in gametes

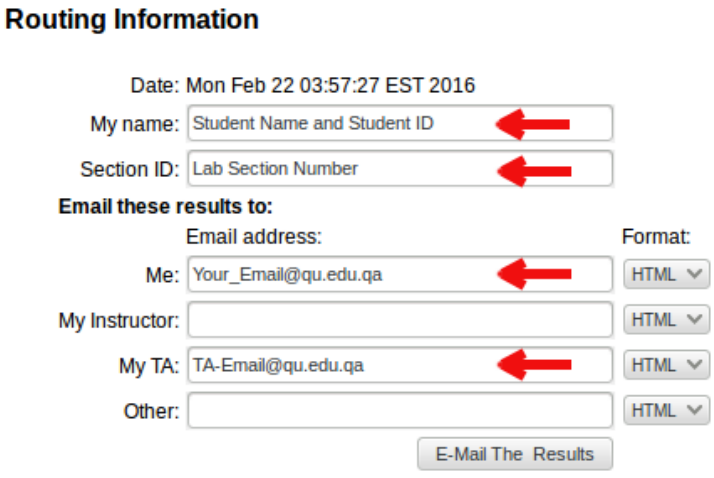
---

Pre-Lab Questions:

View the animation at the site listed below and complete the quiz under the video.

http://goo.gl/lf3K6D

Once you have submitted the quiz, you will see a form like the one below:



Please fill in the indicated fields with the appropriate information (be sure to include your student ID, and to use the correct email addresses), and send this quiz result to your TA to show that you've completed the exercise before the lab starts.

Meiosis

During sexual reproduction, at fertilization, an egg nucleus from one individual is combined with a sperm nucleus from another individual. This input of genetic material by two individuals generates new gene combinations. This does however, generate a potential problem. If the egg and sperm are like any other cell, then they would have the same chromosome number as any other cell of each of the donating individuals. Thus the fusion of the two nuclei would lead to a doubling of the total number of chromosomes in the progeny (ie. If the parents were diploid then the offspring would be tetraploid). Meiosis allows cells to avoid this problem.

Meiosis is a special type of cell division which leads to the production of gamete cells with a reduced chromosome number. This is done in anticipation of fertilization, where two such gametes will fuse and restore the normal chromosome number, and thus the chromosome number of individuals in a species is kept constant.

The process of gamete formation is generally called gametogenesis. In animals, gametogenesis in males is known as spermatogenesis while in females it is called oogenesis. In higher plants the equivalent terms are microsporogenesis and macrosporogenesis respectively. Meiosis is simply a part of the process of gametogenesis.

Stages of meiotic division:

Meiosis consists of two successive divisions, the first one is known as Meiosis I and the other one is termed Meiosis II. The result of the two divisions is the formation of four cells each containing half the chromosome number of the original mother cell.

The first meiotic division:

Meiosis I has been divided into the same main stages as mitosis, namely prophase, metaphase, anaphase and telophase, however the aim of meiosis differs significantly from mitosis. The aim of mitosis is to produce two genetically identical cells, while the aim of meiosis is to do the opposite – to produce genetically different cells.

In fact, the products of meiosis are meant to be different from each other and from the cell that produced them, and to have half the number of chromosomes of the original cell. The reduction in chromosome number is accomplished by having a single DNA replication followed by two divisions of the genetic material. The introduction of variation is accomplished by reciprocal genetic exchange (“crossing-over”)

This requirement for RGE (reciprocal genetic exchange) introduces a lot of complexity into the process and as a result, the first stage of meiosis is very prolonged and subdivided into substages.

Prophase I

1. **Leptotene**

   The chromosomes appear, thin, single stranded and have bead-like structures known as chromomeres. The chromosomes are quite long but are starting to condense. Each chromosome is made up of two sister chromatids (DNA was replicated in S-phase). At this stage the chromosomes are finding their homologues (this is called pairing).

2. **Zygotene**

   The two members of each homologous pair have located each other and are starting to align. A process known as synapsis begins. Chromosome condensation continues.

3. **Pachytene**

   Synapsis of chromosomes is finished through the completion of the synaptonemal complex, this will allow recombinant genetic exchange to occur safely. In the meantime, chromosomes are still becoming shorter and more tightly condensed. The paired homologous chromosomes are now in the form of tetrads (because they're composed of 4 chromatids) and are sometimes visible as two closely apposed “threads” under the microscope.

   During this stage an important genetic event takes place, namely crossing over. This process involves exchange of segments between two non sister chromatids out of the two homologous pairs of chromosomes (at least one cross-over must occur in every tetrad). This is the step responsible for generating genetic variability in a species.

4. **Diplotene**

   Chromosomes continue to become more shortened and thickened. The synaptonemal complex holding the homologous chromosomes together starts to break down and homologues start to separate away from each other. This occurs along most of the tetrad, except at certain points where two out of four of the chromatids form a crossover. Each of these points is known as a chiasma (plural is chiasmata). Chiasma formation is a cytological expression of the process of crossing over.

5. **Diakinesis**

   The chromosomes become more shorter and thicker. The number of chiasmata have become reduced as the chromatids that were involved in crossing over are starting to separate from one another (and integrate the newly exchanged genetic material into their structure). This separation occurs because the chiasma move toward the ends of the tetrad; this process is known as terminalization.

   The bivalents are scattered amongst the spindle fibres and their centromeres start to attach to them.

Metaphase I

This stage includes the arrangement of chromosomes along the equatorial plate of the cell. The spindle fibers arrange the chromosomes such that each homologous chromosome faces the opposite pole of the cell.

Anaphase I

Each homologous chromosome with its two chromatids and undivided centromere moves towards one of the opposite poles of the cell. Thus, two groups of chromosomes are produced each representing the half (reduced) number of that of the original mother cell.

Telophase I

The nuclear membrane is formed around each of the two groups of chromosomes. Then the chromosomes become uncoiled and the nucleoli make their appearance in the nuclei.

The second meiotic division:

This process is mainly similar to mitosis in its stages. Each of the daughter nuclei produced from the first division undergoes the second meiotic division which includes the following phases:

Prophase II

The chromosomes condense. This is usually a very short stage – it's rare to see it on slides.

Metaphase II

The spindles are formed, one for each nucleus. The chromosomes are arranged at the equatorial planes of the cells.

Anaphase II

The centromere of each chromosome divides and thus the two daughter chromatids of each chromosome spearate away from each other. Each chromatid travels towards the corresponding pole of the cell.

Telophase II

Chromosomes lose their state of condensation and the nuclear membranes of the two daughter nuclei are reconstructed.

At the end of meiosis, four cells are produced from the original mother cell, each containing the haploid number of chromosomes.

Exercises: Microscopic Examination of Meiotic Cells

Today, you will spend some time looking at mitotic and meiotic cells in order to become more familiar with the appearance of the different stages of each type of cell division.

Materials:

- Compound light microscope
- Slides of meiosis in lily anthers

1. Observation of Meiosis in Plant Anthers

In this exercise, you will be viewing some prepared slides of cross sections of lily anthers to observe plant microsporocytes at various stages of meiosis. Microsporocytes are diploid stem cells which produce pollen cells (the male gametes) in plants. These cells are found in the anthers of flower buds (Fig. 1.) and can be easily obtained from a variety of flowering plants. Try to find as many stages as possible.

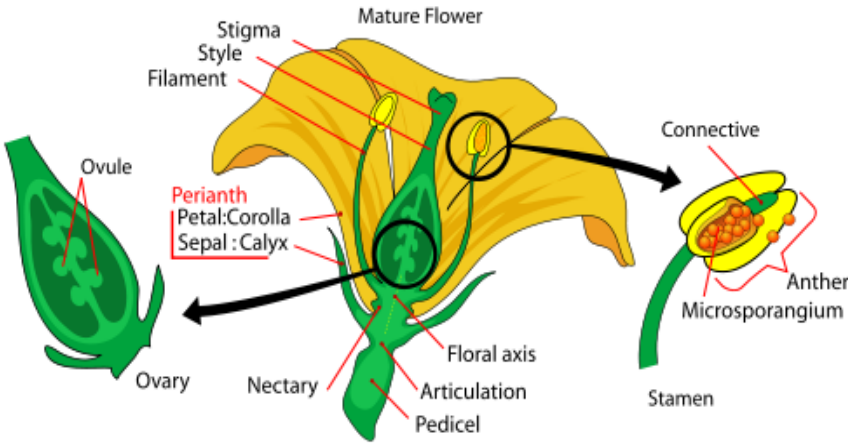


Fig. 1. Anatomy of a mature flower. The central portion is the ovary, which contains the female gametes. The ovary is surrounded by several stamens which end in anthers. The anther produces pollen grains – the male gametes. It is these pollen grains that are picked up by insects, such as bees, and used to "pollinate flowers". These structures exist, on a smaller scale, in an unopened flower bud.

Source: wikimedia.org

When looking at the slides today, it will be helpful to orient yourself on the slide first – look at the specimen at low magnification and find a cross section of the whole anther (Fig. 2A) and then use a higher magnification to view the anther sacs (Fig. 2B) to observe the meiotic cells.

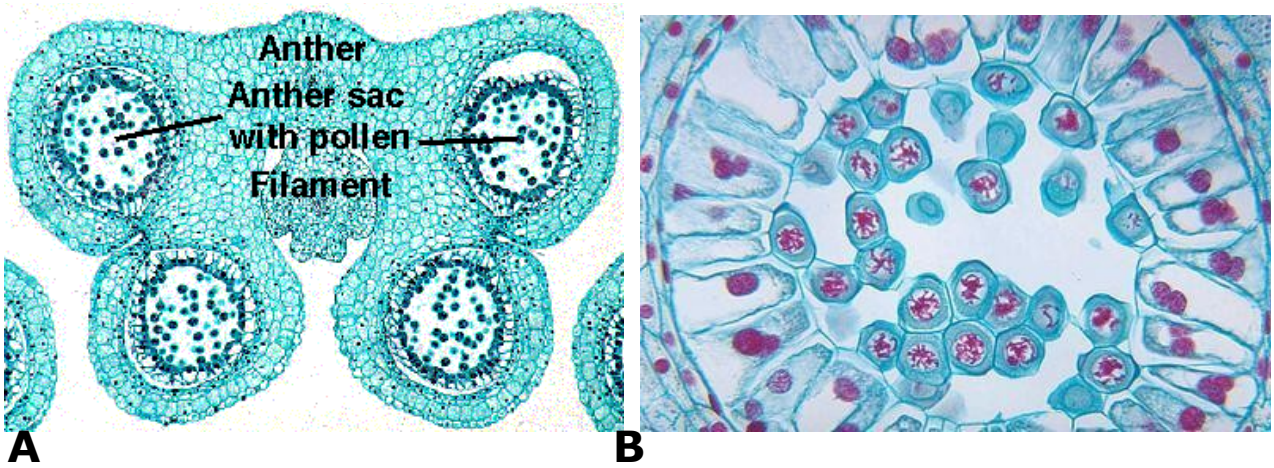


Fig. 2. Cross section through a lily anther. A low power micrograph of the whole structure (A). Most of the cells in this slide are not meiotic. You should focus on the open circular structures (the anther sacs) with the individual cells (B).

2. Preparation of Squashes of Meiotic Cells

Materials

- Compound microscope
- Clean slides and coverslips
- Lily Anthers in 70% Ethanol (previously fixed in a 3 ethanol: 1 glacial acetic acid solution)
- Aceto-orcein stain
- Small Kimwipes
- Pencils with eraser-ends
- Scalpels and forceps

Procedure

1. Obtain 2-3 anthers and place them onto a clean slide.
2. Use a scalpel to cut off one end of the anther and discard it. You want to only cut off the very tip of the anther.
3. Use your finger or the flat surface of the scalpel to gently press on the anther to squeeze out the contents. You should see white material come out onto your slide. If the squeezed out tissue looks yellow then the cells are likely to be mature pollen grains and you will not see any meiosis in that sample. Start over with another anther.
4. Put one drop of the stain onto the slide and keep your specimen in the stain for about 5min.
5. Cover your specimen with a cover slip and dry out extra stain using a Kimwipe.
6. Press down gently on the cover slip, but do not let it move as you do this. You can use the eraser-end of a pencil to help to distribute the cells more under the coverslip. You are trying to generate a monolayer of cells.
7. Examine your specimen under the low and high magnification of the compound microscope. By squeezing out the cells from the anthers, you emptied the anther sacs and collected most of the microsporocytes onto your slide, therefore most of the cells should be in the process of going through meiosis.

Exercises: Meiosis as the Basis for Mendelian Laws

The exercises below are modified from those developed for BIO C15 Transmission Genetics Course by Claire Hasenkampf at the University of Toronto at Scarborough.

Materials:

- Foam “chromosomes”
- Hair clips to act as alleles

3. Modeling the Stages of Meiosis

For this exercise, you will work as a team to help ensure that all members of the team understand the key events of meiosis and can explain and demonstrate them. You will be given a set of “chromosomes” and your job will be to demonstrate what happens to these chromosomes at each stage of meiosis.

Feel free to use your notes and the textbook to help you describe the stages initially. Once everyone in your group has demonstrated the process, demonstrate this process to your TA. Any member of your team may be asked to demonstrate and explain, so make sure everyone in your group is capable of doing it correctly. Additionally, your TA may choose to ask questions during or after your demonstration.

1. Prophase I - Point out what a chromosome is and what a chromatid is. Show chromosome pairing, reciprocal genetic exchange and a chiasmata.
2. Metaphase I - Line up the chromosomes on the metaphase plate. Are homologous chromosomes associated?
3. Anaphase I - Show chromosomes moving to opposite poles. How many chromatids are there per chromosome?

4. Telophase I - Describe the changes that occur with the chromosomes
5. Prophase II - Point out what a chromosome is and what a chromatid is.
6. Metaphase II - Line up the chromosomes on the metaphase plate. Are homologous chromosomes associated? Are sister chromatids associated?
7. Anaphase II - Show chromosomes moving to opposite poles. How many chromatids are there per chromosome?
8. Telophase II - Describe the changes that occur with the chromosomes.

4. The Chromosomal Basis of Mendel's Laws

Let's re-use the chromosome models to understand how genes are segregated during meiosis. Think about two sets of alleles. We will call the first set of alleles A and a, the second pair of alleles B and b. We will be using coloured hair clips to symbolize the alleles – you may wish to use a more intense/dark colour to symbolize the dominant version and a lighter colour for the recessive allele (ie. A = red, a = pink, B = dark blue, b = light blue).

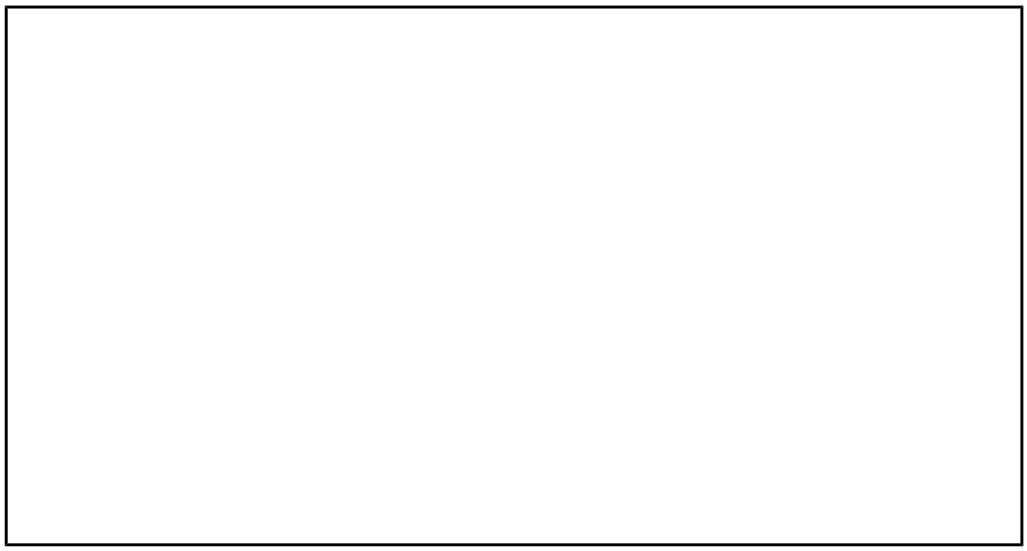
1. Go back to your chromosome models. To one chromosome clip an 'A' onto each of the two sister chromatids, and clip an 'a' onto each of the two sister chromatids of the homologous chromosome.
2. Now quickly take these homologous chromosomes through anaphase I, and then take sister chromatids through anaphase II. What happens to the alleles A and a?

You should find that alleles 'A' and 'a' started out together in the same diploid nucleus, but by the time meiosis is completed the two alleles have segregated from each other. The haploid meiotic products have either A or a, but not both. This simple and rather obvious fact (once you understand meiosis) is **Mendel's Law of Segregation of Alleles**.

3. Now let's add another set of chromosomes and alleles into the picture - 'B' and 'b'. Clip the alleles onto the other pair of homologous chromosomes. (keep the A and a alleles clipped to the original chromosomes).
4. Now take the chromosomes through meiosis again. If you did it correctly then alleles 'B" and 'b' also segregated from each other, each haploid nucleus has only 'B' or 'b', but not both.

    - What would be the genotyes of the gametes you've just generated?
    - Are these the only possible genotype? (feel free to do this exercise again to check)
    - Write the all the possible allele combinations in the box below:

    - If this organism was self-crossed, what would the Punnett square look like?



Let's look at how the 'A and a' alleles segregate relative to the 'B and b' alleles.

Homologous chromosomes act dependently when they orient on the metaphase I plate (they are tied together by the chiasmata), orienting to opposite poles. But one pair of homologous chromosomes (like the ones carrying 'A' and 'a'), acts independently of all other pairs of homologous chromosomes (like the ones carrying 'B' and 'b') when orienting on the metaphase I plate.

Thus if we survey each of our four groups in the class we should see that some groups generated haploid nuclei with 'A' and 'B', and 'a' and 'b', but others generated haploid nuclei with 'A' and 'b', and 'a' and 'B'. This phenomenon you've just re-enacted with the chromosome models is Mendel's second law known as the **Law of Independent Assortment**.

As you've just seen independent assortment is a consequence of chromosome behavior during meiosis, and it is a great source of new gene combinations.

Post-Lab Questions:

Please think about these and answer them in your notebook:

A more complicated version of the above exercise would involve crossing over. In this case, you would place the 'A' and the 'B' alleles on one chromosome and the 'a' and 'b' alleles on it's homologue. If you do this, then the only way get an combination of 'Ab' and 'aB' would be if crossing over occurs somewhere between these two alleles.

If you tried to do this exercise twice – once when 'A' and 'B' were very far (~5cm) away from each other on the same chromosome, the second time when 'A' and 'B' were very close together (~1cm) – would you be able to get all possible allele combinations at the end of meiosis in both examples? How likely is it?

Assignment: Sketches of Meiotic Cells

Draw two sample stages of Meiosis (your TA will tell you which one to draw), and submit them to your TA for grading.

Name: _____ Student ID: _____

Meiotic Staging of Microscopic Specimens

Name of Slide:	Slide #:
	Stage:
	Objective Lens Magnification:
	Ocular Lens Magnification:
	This drawing is larger than what I see by a factor of:
	Total Magnification:
Explanation:	

Name of Slide:	Slide #:
	Stage:
	Objective Lens Magnification:
	Ocular Lens Magnification:
	This drawing is larger than what I see by a factor of:
	Total Magnification:
Explanation:	

Lab 5

In today's lab, we will be discussing polymorphisms, transmission of alleles to offspring, as well as pedigree charts – a form of family tree used in Genetics. You will also be given a part of the lab to work on your first Problem Set.

Learning Objectives:

Students will:

- Explain the difference between dominant and recessive phenotypes
- Define the terms: homozygous and heterozygous
- Distinguish between the terms “polymorphism” and “mutation”
- Determine the mode of inheritance of a trait based on the information in a pedigree chart
- Construct a pedigree based on a polymorphism found in their family

---

Pre-Lab Questions:

- Please see this online presentation to see the proper format for a pedigree.
- Please see this online presentation to see pedigree charts “in action”.

Based on the above presentations, how common is the Hemophilia phenotype?

What are some of the differences between the inheritance patterns in the hemophilia and eye colour examples?

Human Genetics

Children tend to look like their parents because they inherit genes from their parents, and it is these genes that influence characteristics such as skin, hair and eye color. A gene is a relatively short region of DNA on a chromosome which influences how a cell works, and in some cases it can directly influence our physical characteristics (our phenotype).

The DNA sequence of a gene provides instructions that a cell uses to make proteins and enzymes. As an example, one such gene codes for an enzyme called Tyrosinase. Under normal circumstances, Tyrosinase catalyzes the first reaction in a pathway that converts the amino acid Tyrosine into melanin, which is the pigment molecule responsible for your skin, hair and eye colour. A change to the DNA sequence of the Tyrosinase gene could result in the production of a non-functional version of the enzyme, which would prevent the production of any pigment. Thus, inheriting a **mutation** in the DNA sequence could directly affect phenotype (Fig. 1).

Fig. 1. Group of babies of African descent (in a nursery in Malawi). The baby in the middle is unable to produce a functional copy of the Tyrosinase enzyme and therefore can't produce any melanin pigment. This is a condition called Albinism.

Source: https://simple.wikipedia.org/

Inheriting a mutated copy of a gene from one of your parents, however, doesn't always result in an alteration of phenotype. This is because each cell in your body has two copies of each gene – you inherited one copy from your mother and one from your father – and these are often different from each other. These different versions of a gene are known in Genetics as **alleles** and, in a diploid individual, it is often the presence of two alleles that determines the phenotype. If both copies of the gene have the same sequence (same allele), the individual is **homozygous**. Having two different versions of a gene, makes an individual heterozygous for that trait, and the phenotype of that person or cell would depend in which of the alleles is **dominant** (ie. will determine the phenotype) and which is **recessive** (ie. will not affect the phenotype).

Mutations versus Polymorphisms

It's important to note that not all mutations cause disease, and not all are rare. Some DNA changes have absolutely no effect on the proteins and enzymes produced by the cells, some changes have no detectable effect on the phenotype, and some changes do produce a phenotypic change but that change has very little or no effect on the organism's ability to live and reproduce – no effect on fitness. Mutations that have very little or no effect on reproductive fitness will likely remain in the population and will result in phenotypic variation in that species. This is the case with many of the mutations that you are studying in Drosophila this term. This is also the case with many human traits, like eye colour, height, hair length, etc.

As a generally accepted rule in Genetics, changes to a DNA sequence that are found in 1% or more of the population are referred to as polymorphisms, while changes that are considered rare – less than 1% of the population – are referred to as mutations (some people prefer to use the term: "rare variant").

Exercise: Punnett Squares and Human Genetics

In this exercise, you will be modeling the inheritance of a mutation in the TYR gene – the gene that encodes Tyrosinase. For the purpose of this exercise, we will assume that the mutation causes the production of a non-functional enzyme, which would result in Albinism – this would be the observable phenotype (as seen in Fig. 1.).

Materials

- Coin
- Pedigree in Fig. 2.

1. Randomness in the transmission of alleles to offspring

In our exercise, we will be studying the inheritance of the faulty TYR gene in a family where both parents are heterozygous for the mutation.

In last week's lab, we modeled the movement of chromosomes and alleles during meiosis. At that time you learned that alleles assorted independently (ie. randomly). In this exercise, we will be looking at how the products of meiosis – gametes – are combined at fertilization. It's also random.

To help us model this randomness, we will be using a coin to help us decide which allele will be inherited by the children of the heterozygous parents. You will assign one side of the coin ("heads") as the dominant allele, and the other side of the coin ("tails") as the recessive allele.

Procedure

1. The first thing you will want to determine is if the mutant allele is likely to be dominant or recessive. Based on the fact that, in some parts of the world, the albino phenotype occurs in 1 out of 5000 people, discuss in your group which you think is more likely.
    - Discuss your conclusions with your TA.
2. Use the pedigree chart in Fig. 2. to help you.
3. Once you have discussed the dominance/recessiveness of the TYR mutation, draw a Punnett square to symbolize a mating between two heterozygous individuals and indicate the expected phenotypic ratios.

		Female Gamete	
Male Gamete			

The two parents in the Punnett square above, want to have 4 children. Use the table below to fill in the genotypes of the 4 children using data generated by coin tosses. Since each parent is heterozygous, Each of them could donate a dominant allele or the recessive allele – we will represent that as either one side of the coin (heads) or the other side (tails).

4. For each child, use two coin tosses to help you determine the alleles donated by the mother and the father. Fill in the genotypes below:

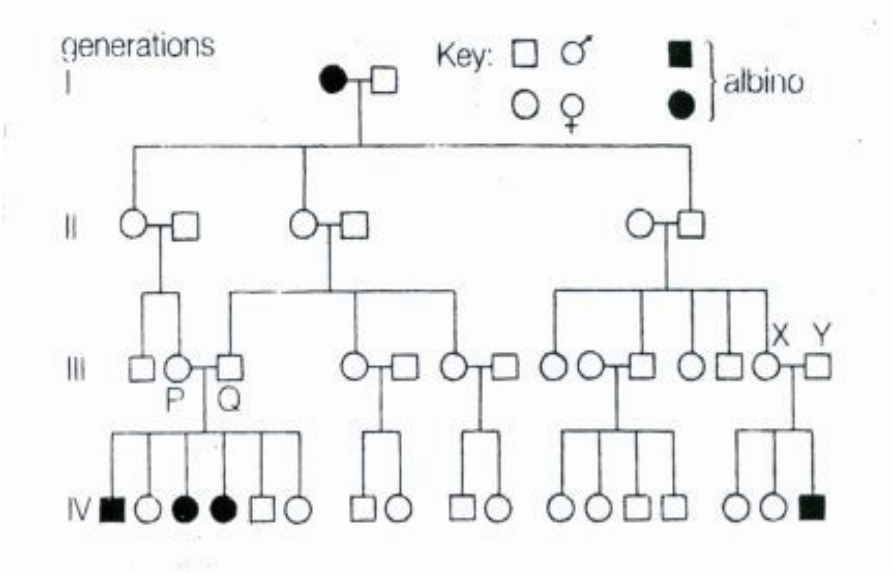
	Child #1	Child #2	Child #3	Child #4	Ratio
Genotype of each child					
Phenotype of each child					

5. Put up your data on the board for the class to see.
6. Look at the class totals. Are the phenotypic ratios in your own data matching the expected ratios (based on your Punnett Square above)? How does the class data compare to the expected ratio?

Pedigrees.

Fruit flies allow for ease of study of the transmission of their traits because they produce hundreds of offspring in each generation and they can be used in controlled matings. The same cannot be said of humans. Studying human traits, therefore, requires a different approach.

A pedigree chart allows the analysis of the inheritance pattern of specific human traits by collecting and arranging the information about a family's genetic history in a way that makes tracking the appearance of such traits easier. The scrutiny of pedigree charts from many different families can be useful in determining the mode of inheritance of a particular trait. This will tell the researchers whether an allele is dominant or recessive, and whether the trait is autosomal or sex linked.

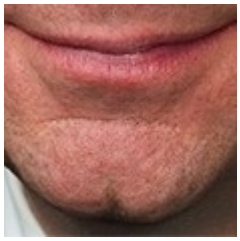


`Fig. 2. Pedigree of a family showing the inheritance pattern of albinism. Based on the information presented here, what are the genotypes of the individuals labeled with"P", "Q", "X" and "Y"?

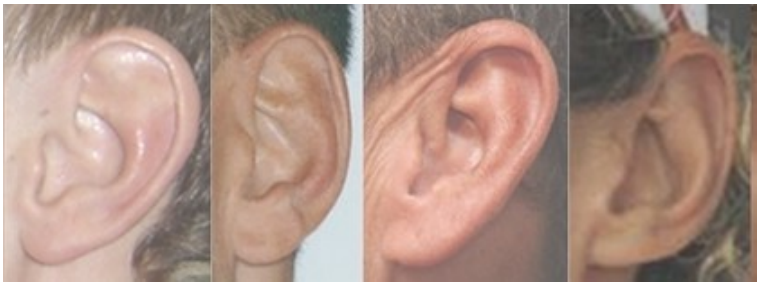
Using this information, a geneticist can make predictions about the probability of that trait occurring in future generations in that family. In most of the pedigree examples frequently described for humans, one allele is associated with a normal phenotype and the other allele is associated with a 'disease' phenotype. However there are some non-disease, morphological variations in humans that are associated with different alleles. Here, all of the different phenotypes are normal; an example is hair colouring, or eye colour. .

Exercise: Human polymorphisms

The following traits have been used for many decades, as examples of polymorphisms that follow simple Mendelian genetics.

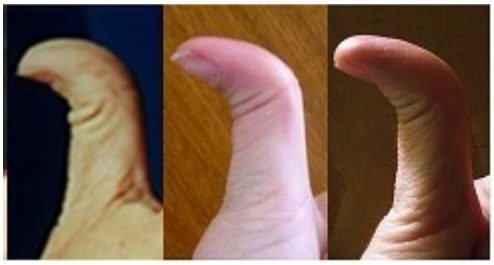


- **Dimpled chin** – Some people's chins have an indentation in the middle; this is the dimple. This trait is more pronounced in some people than in others. The cleft is a dominant trait, symbolized D. The allele for undimpled chin is d.
- **Free earlobe** - Earlobes hang free or are attached directly to the head. The trait is clearly more complex than just those two options, but let's just classify people into two catogories. We will free hanging earlobes as a dominant trait symbolized by E. The allele for attached ear lobes is symbolized by e.



- **Widow's peak** – You have a widow's peak if your hairline forms a distinct peak in the center of your forehead. Again, this trait is more obvious in some people than others. The distinct point is caused by a dominant allele symbolized by W. The allele for a smooth hairline is recessive and symbolized by w.

- **Hitch-hiker's thumb** – Assess whether or not you can bend the top joint of your thumb back towards the knuckle. The allele for an unbent top joint is considered dominant and is symbolized H, the allele for the bent joint is recessive and symbolized by h.



- **Tongue-rolling** – This is a fun one to ask your family members, though it has not genetic basis at all. Assess whether you can turn the two sides of your tongue inward so that the two sides nearly touch at the tip. The ability to roll the tongue is often said to be due to a dominant allele symbolized by T. The non-rolling allele is symbolized by t.

For many of the above, if not all, there is limited or no scientific evidence that they follow simple Mendelian inheritance patterns, but we will use them anyway because in most cases the pedigrees do show the "expected" inheritance patterns. They also allow students to gain some experience in constructing simple pedigree charts, and can be fun one to research in your family. How else are you going to convince your grandmother to stick out her tongue at you?.

Materials

- none

2. Determining prevalence of polymorphisms in the class

Procedure

1. You and a partner should assess yourselves for the human polymorphisms listed above.
2. Fill in the table below with the data for the whole class.

	Dimpled Chin	Free Earlobe	Widow's Peak	Hitch-hiker's Thumb	Tongue-rolling
Affected students					
Unaffected students					

3. Look at the class tally sheet to see the number of students with each version of the polymorphism, to see how frequent the polymorphisms are. Are the phenotypes associated with the dominant allele always common, and the recessive ones rare?

4. Try to determine your “genotype” based on the above phenotypes.

Assignment: Human Polymorphisms and Pedigree Analysis

In order to get practice with pedigree analysis (and learn learn something new about your family members) each student should construct a pedigree for at least three generations, for one of the traits above. Collect information about grandparents, parents, siblings (and spouses) and children of siblings and self.

Generate a pedigree chart for your family and turn it in to your TA at the start of the lab in **two weeks**.

1. The pedigree should be based on a trait that you, or someone in your family actually has (ie. Do not hand in a chart that says that no one in your family has that trait).
2. Your pedigree should show at least 3 generations.
3. Please be sure to indicate the generations and genotypes (or possible genotypes) for each individual. Please see your textbook for examples of the type of information included in a typical pedigree chart.
4. Write a brief explanation of your reasoning behind the genotypes you assigned to each individual (ie. "I think II-2 is heterozygous because he has the dominant phenotype but his mother does not – she has the recessive phenotype").
5. Indicate which of the individuals on the chart is you.

Lab 6

Hopefully, you have completed most of your reciprocal crosses by now and have some F_1 flies to work with. If you don't have any F_1 flies by the time you do this lab, then you will need to do the listed exercises on your own time during the week. Once you have completed your initial F_1 analysis, please talk to your TA to check if your results look okay and to make sure you understand what to do next.

In this week's lab, we will be focusing on reading scientific literature. For this reason, you are asked to read the posted article on your own time **before coming to class**. We will be discussing the contents of the article as a class (you're not expected to understand everything that's in there, but you should try). Besides talking about the article contents, we will be discussing the structure of a typical research article and its similarities to a formal lab report.

Learning Objectives:

Students will:

- Explain the difference between Co-dominance and Incomplete dominance
- Describe the structure of a research article and explain how it's similar to a lab report
- Differentiate between a Procedure and a Materials & Methods section
- Write a Materials & Methods section based on a written procedure

---

Pre-Lab Questions:

Work through the following exercises dealing with Incomplete Dominance and Co-dominance:

http://goo.gl/QChHbw

http://goo.gl/Yus8z5

In your own words, and using a different set of examples, explain the differences between the two types of allele relationships.

A Review of Genetic Terminology

Before you begin, it may be useful to review some basic genetic terminology. Most genetic experiments are done on organisms that are like us in that they have two sets of chromosomes (**diploid**). This is not the only chromosomal combination seen in nature however, there are some eukaryotes which spend at least part of their life cycle with only one set of chromosomes (**haploid**). Such organisms can still reproduce sexually by becoming diploid briefly during their reproductive stage. There also organisms that are **triploid** (three sets of chromosomes) or **tetrapoid** (four sets of chromosomes). Tetraploid plants are more common because they can undergo normal meiosis, while triploid plants are often generated by us through selective breeding in order to produce desirable characteristics like a lack of seeds. This works because triploid cells can't undergo proper meiosis and thus can't produce gametes – this is how we get seedless watermelon.

When you started this experiment, you made some reciprocal crosses between flies from the **parental generation (P)**, these individuals displayed **phenotypic traits** (mutations) which are encoded by a particular **gene**. This gene is a DNA sequence at a particular location on a chromosome (**locus**), and it's expression by the cell results in a phenotype. There can be different versions of this gene (ie. a wildtype version and a mutant version), known as **alleles**, which can either always dictate the phenotype whenever it is present (**dominant allele**), or can only affect the phenotype if it is the only version of the gene in an individual (**recessive allele**).

In genetics, we generally name **gene loci** (plural of locus) after the mutant form of the allele and we try to be descriptive. Thus, if you have a fruit fly without any wings (there is an actual mutation that can cause this), then the locus would be named: "*wingless*" (please note that mutations are usually **italicized**) and would be given a two letter symbol like " *wl* " (the wildtype would be symbolized as " *wl+* ").

If the mutant allele is recessive then a fly with the mutant phenotype would be described as having two mutant alleles at the *wingless* locus and would be **homozygous** for that locus. If a fly has two different versions of the allele, one dominant and one recessive, then it would be described as being **heterozygous** at the *wingless* locus.

In order to determine if an allele is dominant or recessive, you perform controlled matings – **crosses**. A cross between two pure-breeding (homozygous) lines of organisms that have different versions of the allele of interest would produce a **First Filial Generation (F_1)** composed of heterozygous individuals. The phenotypes of these individuals will show the dominant trait. Thus the trait that is not seen in the F_1 generation is recessive. There are some exceptions to this rule. Sometimes, a trait does not follow simple recessive/dominant relationship – some alleles are incompletely dominant and some are co-dominant.

When the expression of both alleles in a heterozygote results in a blended phenotype (ie. Parental phenotypes are white and red, but F_1 phenotype is pink), then the relationship is described as one of **incomplete dominance** – in this case the wildtype phenotype does not completely mask the mutant phenotype. **Co-dominance** is seen when both the traits are seen in an individual at the same time. The human blood groups A and B are a good example of this, but it is easier to describe in terms of colours of individuals – an individual that displays both of the parental colours at the same time (patches of different colours – this is common in animals) is co-dominant.

A  B 

Fig. 1. Co-dominance and Incomplete Dominance. The mating of a white and a red flowered individual in different plant species has resulted in co-dominance (A), where the red and white alleles are both expressed in the same plant equally, or in incomplete dominance (B) where the dominant trait does not completely mask the recessive trait.

Sources: (A) wikimedia.org , (B) media.web.britannica.com

The F_1 generation can reveal more about the mode of inheritance than just a dominant/recessive relationship. Normally, a dominant allele will mask the presence of a recessive allele in a heterozygous individual, however a recessive trait can be seen when it is present in a **hemizygous** state (when it is the only copy of the allele in that individual). This happens in cases of the sex chromosomes – a female individual has two X chromosomes, but a male has only one, therefore any alleles found on the X chromosome are present in only one copy in a male individual.

This is useful because F_1 males will inherit the X chromosome from their female parent, and if that female has any X-linked recessive alleles, they will be present as the only copy in that F_1 male. Thus the F_1 male will show all the phenotypes of any recessive alleles that are X linked.

This is also the reason that we started our experiment with reciprocal crosses. If you cross a wildtype female with a mutant male then all the F_1 males will show the wildtype phenotype. If the mutant trait is recessive, then all the F_1s will have the wildtype phenotype and you will assume they're autosomal. If you look at the F_1 results from the reciprocal of that cross, however, you will see that all the F_1 males have the mutant phenotype for any alleles with a locus on the X chromosome.

Drosophila Project – F_1 Self-crosses and Preliminary Data Analysis

This week you will likely be seeing some F_1 flies emerging from your reciprocal crosses and should use them to make self-crosses and also to do some simple analysis. It is possible however that you may not have enough data to analyze today. You may have data from one side of the reciprocal cross and not the other – do not make any final conclusions about the mutant traits in your flies until you've looked at all your crosses and discussed your conclusions with the other members of your group.

Since different groups will be getting their F_1 results at different times and may not have enough data, you may have to do the analysis on your own whenever the data is available. Regardless of when you do it, please use the information outlined above to help you.

Exercises: Examination of F_1 Progeny

In this portion of your *Drosophila* project, you have two tasks. The first task is to make self crosses between the flies from the F_1 generation for each side of the reciprocal cross. Be very careful as you make the crosses and look for unusual results. If a mistake was made in one of the initial reciprocal crosses, this could show up here, and if you notice it you will have a chance to make sure it doesn't have a negative impact on your final results.

The second task is to observe this generation of flies very carefully and make note of the phenotypes of all the flies, keeping in mind that it may be possible for the males and females to differ in their phenotypic appearance. This part of the experiment is very important because it will allow you to make some very important conclusions about your flies.

The results of the reciprocal crosses will help you determine the dominance/ recessiveness of the traits you're following, they will also allow you to determine if the traits are x-linked or autosomal. If the traits are autosomal, then all the F1 flies will have the same phenotypes, but if you notice that all the males look different from the females in the F1 generation and that they look like their female parent, then the traits are found on the sex chromosome.

Materials

- F_1 flies
- tubes of fresh media
- paint brushes
- etherizer
- ether
- 70% ethanol (for sterilizing brushes)
- fly morgue (for disposing of Drosophila flies)

1. Self-crosses of F_1 *Drosophila*

Once you've obtained some flies from the F1 generation. Make self-crosses and produce the F2 generation. It is a good idea not to mix the flies from different bottles / tubes.

Repeat the following procedure for each tube/bottle of reciprocal cross you have. In each case, perform the self-cross with as many of the flies as you can, but leave yourself a few to use the rest for the next exercise. Alternatively, you could do both of these exercises at the same time.

1. Obtain your reciprocal cross tubes/bottles and make note of the label on the bottle. Anaesthetize the flies.
2. Transfer the flies onto a clean index card and place them under your stereomicroscope.
3. Identify and separate a few males and females. Use these to make self-crosses using 4-5 males and 8-10 females (the more flies you use per cross the more F_2 offspring you will get). Be sure to label the new bottles appropriately.

2. Phenotypic Analysis of F_1 *Drosophila*

1. Separate the males from the females.
2. Observe the phenotypes of the flies from each of the reciprocal crosses separately and determine if any of them display any of the mutant traits you observed in the Parental generation.
3. Fill in your observations in the table below (do in your group notebook as well).

Cross	F_1 ♂ Phenotype	F_1 ♀ Phenotype
A♂ x B♀		
B♂ x A♀		
C♂ x D♀		
D♂ x C♀		

4. Answer the following questions:
    1. Are the mutant traits you're studying dominant or recessive?
    2. Is there a difference between females and males in any of your F1 populations? What do the F1 males look like?
    3. If there is a difference, what could account for the difference?
        1. Is the trait autosomal or sex-linked?
5. Based on your answers to the above questions, assign a genetic notation to each mutant trait.

If you don't have any F_1 flies yet, please complete the above table when you do have some flies to analyze.

Then, discuss your results with your TA.

Scientific Communication

Scientists do research in order to seek out new knowledge and to test existing theories. Once they have found our something new, they share their findings with the rest of the scientific community in the form of journal articles and scientific presentations. The results presented in these articles and presentations are evaluated by the scientific community, and if they can stand up to close scrutiny and criticism, they become part of the general body of knowledge that will be drawn upon by other scientists to support their future hypotheses and their experiments.

Thus, the dissemination of experimental results is an important skill for a scientist, and it is a skill that you will need to learn as an undergraduate student. This is a skill that you will develop and improve over several years, so you are not expected to master it by the end of this lab.

There are three main types of articles that are commonly published in scientific journals, these are: letters or short communications, research articles and review articles. Today, we will be reading and discussing a research article, because the structure of this type of article is very similar to a formal lab report. We will also try to apply what we learn about research articles to a writing exercise.

A typical research article follows a format very similar to you might think of as a “lab report”, and that is what it is. The main difference between a research article and a lab report that you're used to writing, is that a research article is often used to summarize many months' worth of research activities, while a typical undergraduate lab report generally summarizes one or two labs. The basic structure, however, is the same - a typical scientific article usually consists of the following:

1. Title
2. Abstract
3. Introduction
4. Materials and Methods
5. Results
6. Discussion
7. References

Although some journals differ in how these sections are presented - sometimes in the Materials & Methods section is presented at the end, sometimes the Results and Discussion sections are combined – the content of each section is fairly specific. Here is a brief summary:

Title

The title is generally fairly descriptive of the main finding and can sometimes look quite long. Titles of research articles are not designed to catch attention, but rather to summarize the main result. Since there are thousands of articles published every month, scientists use the contents of the title and abstract to help them decide which ones to read. A good title is therefore informative, and uses keywords that your peers and fellow researchers will likely be searching for.

Abstract

Like the title, the abstract is there to help your fellow scientists decide whether your research is relevant to what they want to learn about, and whether they should read the entire report. A good abstract is a short and accurate summary of the whole report. It is generally less than 250 words and summarizes a little bit of each of the sections that will follow. It will summarize the introduction by outlining the purpose of the report, it will summarize the data and give an

overview of some of the main methods used to gather that data, and it will give a brief summary of the author's main conclusions (from the discussion).

Introduction

The introduction of a research report tends to focus on outlining what is already known about the subject being studied. This tends to start out as a general overview and becomes more focused – this style is sometimes referred to as an “inverted pyramid”. The introduction of a typical research article tends to end in a brief overview of the gaps in the current knowledge about the subject, and indicates how the research which is being presented aims to fill those gaps in our collective knowledge.

In most cases, the introduction tends to be very brief and limited only to materials that are relevant to the experiments that will be presented in the paper. These articles tend to be aimed at an audience of fellow researchers in the same field. As a result, it can be difficult for a newcomer to the field – like an undergraduate student – to gain enough background knowledge from such an introduction to be able to fully appreciate the contents of the article. This is why it can be so challenging to read scientific literature for undergraduate students.

Materials and Methods

This section is generally very brief and only gives a summary of the methods used in the experiment(s). The biggest challenge in writing this section is knowing how much detail to include. The Materials & Methods section is meant to answer the question: “How?” and differs from a procedure because it does not list all the steps. The M&M section is not a set of instructions or explanations, but rather is a brief overview of the parameters used in the collection and analysis of the data – ie. things that could affect the results. For example, it probably doesn't matter that you used a “test tube” or that you labeled it “A”, but it does matter that you incubated the samples at a particular temperature for a specific amount of time.

In a research paper, the aim of this section is to convince the readers that you used the appropriate methods to generate the data and that you controlled for any confounding variables. In some cases, authors don't even describe a particular method, but instead they simply cite the article where that method had been initially described.

Results

This section is usually presented separately from the Discussion in an attempt to allow the reader to analyze the data without the writer's own interpretations biasing their thinking. It therefore summarizes the data from the experiments but does not provide any interpretation – that is left for the discussion.

This section tends to include tables, graphs and figures but it's important to understand that those should not be the only components of the Results section. The Results section is where you “tell the story of what you did” and provide a textual summary of the main findings, trends and differences between different groups. The tables and figures are then used to support the text and provide a more complete picture.

All figures and tables should have descriptive captions and titles, should be numbered, and should be referred to in the text. The captions of the figures and titles of the tables should provide enough information to allow the reader to understand them without referring to the text.

Discussion

This is where the data from the Results section is analyzed and interpreted. This is where authors look at their data and present their interpretation in a logical fashion, often presenting several pieces of evidence and linking them together to tell a full story. It should not just be a

restatement of the results, but should draw some meaning out of them and connect them to what is already known. This is where the author can connect the experimental results to the introduction, and show how they fill in the gaps in our knowledge identified earlier.

References

This section lists all articles or books cited in the report. It does not list references that were useful but not actually cited. The sources are listed in alphabetical order by the last name of the lead author. You may notice that different biological journals use different citation formats for citing literature (unlike Psychology where APA is used), however we will be asking you to use the CSE format (http://writing.wisc.edu/Handbook/DocCSE_NameYear.html).

Exercise: Writing Exercise

While the order of the sections presented above is how a report is submitted, it's not actually the order in which it is usually written. Most people tend to start by writing the parts that are easiest because they don't require much prior literature research – this is the Materials & Methods section and the Results section. In this exercise, you will practice writing a Materials & Methods section by taking a procedure and working with your group to convert it into the proper format.

Materials

- none

1. Writing a Materials & Methods section

1. Obtain your copy of the procedure.
2. Identify the components of the procedure that are likely to affect the results.
3. Write a few sentences to accurately represent the important parts of that procedure.

Assignment: Writing a Materials & Methods section for the report

In order to get practice writing and to get you started on your final lab report, you will be starting to work on a Materials & Methods section for your *Drosophila* lab report. In this assignment, you are asked to write about the project work that you have performed up to this point. This should assignment should be fairly short, if you're doing it right, it probably will not be longer than about 1-1.5 pages.

This assignment should be done individually. It will be submitted online (through Blackboard) and checked for evidence of plagiarism. Please upload your work in the “Assignments” section on the course website.

Lab 7

Today's lab deals with the cell cycle and some of the chemicals or treatments that could affect it. More specifically, you will be preparing more mitotic slides – some will be of root tips grown under normal conditions, others will use root tips grown under conditions which might affect various parts of the cell cycle. At the end of the lab, you will be submitting your two best slides for grading by your TA.

Learning Objectives:

Students will:

- Explain why it's important that cells with damaged DNA do not enter mitosis
- Describe some of the precautions taken by cells to ensure that serious DNA damage is not passed on to the next generation
- Identify some of the checkpoints in the cell cycle
- Describe the effect of colchicine on mitosis and what is likely to be seen on slides
- Describe the effect of various antimitotic drugs on the mitotic index and phase rate

---

Pre-Lab Questions:

Please read the linked page (feel free to view the video on cancer as well) and answer the following questions:

- https://goo.gl/CIv4Rr

At which point does the cell commit to cell division? How can you tell?

Why is it so important for the cell to keep checking it's DNA integrity?

What could happen if the cell doesn't check for tension at the spindle checkpoint?

The Cell Cycle

Dividing cells progress through a series of stages as they exit mitosis and prepare to complete the next division. Students often simply memorize the names and order of the stages and often don't give much thought to what is actually happening at each of those points, or why those stages exist. While the M-phase appears to be the point where all the “action” happens, it is important to realize that this stage is actually very complex and all the other parts of the cell cycle serve to prepare the cell to successfully complete it.

The cell cycle starts with the formation of a new cell (at the end of mitosis). This cell enters what is known as the G1 phase. It is diploid and starts accumulating the necessary “machinery” to synthesize DNA in the next stage. Accurate DNA replication is absolutely crucial; for this reason, the cell scans it's DNA for mutations and DNA damage before it can enter S-phase. If any damage is detected at this point, the cell will become arrested (stuck) in G1 phase until the mutations in the genome are repaired. This prevents the copying of damaged DNA and spreading the mutations to the new cells that would be formed in this process.

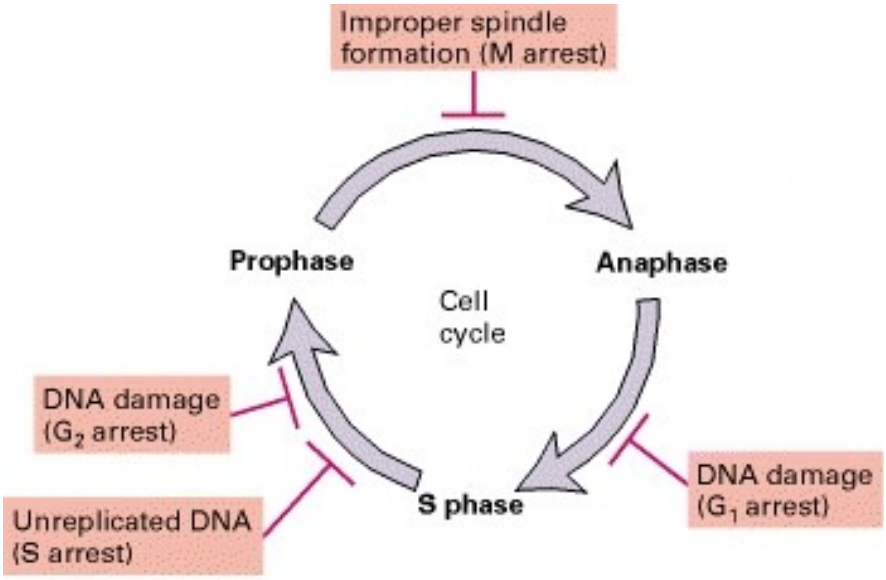


Fig. 1. Stages at which checkpoint controls can arrest passage through the cell cycle. DNA damage due to irradiation or chemical modification prevents G_1 cells from entering the S phase and G_2 cells from entering mitosis. Unreplicated DNA prevents entry into mitosis. Defects in assembly of the mitotic spindle or the attachment of kinetochores to spindle microtubules prevent progression into anaphase. Cells do not enter anaphase until all kinetochores are bound to spindle microtubules. [Adapted from A. Murray and T. Hunt, 1993, The Cell Cycle: An Introduction,W. H. Freeman and Company.]

Image modified from one obtained through the NCBI Bookshelf.

If the cell successfully passes this checkpoint, it will begin to replicate its DNA. It cannot continue to mitosis until all of the DNA has been replicated, so there is a mechanism that keeps the cell arrested until that happens. The DNA will also be checked again for any damage before the cell is allowed to move forward in the cycle – this is the G2 checkpoint. This checkpoint ensures that a cell will not be able to enter M-phase with DNA that is damaged/mutated. At this stage, the cell also accumulates the necessary proteins and structures that will be used in the next part of the cycle. Once the cell is ready, it enters M-phase.

The Spindle Assembly Checkpoint at the end of metaphase controls whether the cell is able to continue through M-phase (mitosis or meiosis), and is based on the ability of the kinetochores to attach to the spindle. This checkpoint ensures that the chromosomes do not get pulled to the

poles of the cell until all of them are properly attached and capable of being evenly separated. In theory, the cell should not be able to proceed unless this happens.

Activation of the checkpoints

There are many ways to cause an arrest at one of the checkpoints. One of the simplest is to introduce a DNA mutation or physical DNA damage by using radiation. When such damage occurs, the typical cell is able to detect it and stops cycling (ie. does not continue with mitosis.

We can also use various and drugs to interfere with the cell cycle leading to many abnormalities. Some chemicals inhibit DNA synthesis while others stop division at a specific stage, or affect the spindle fibers, or lead to breaks within the DNA molecule. Some of these chemicals can be quite useful in stopping the uncontrolled proliferation of cells in the body and are used as therapeutic drugs. Drugs that interfere with the cell cycle act are called cytostatic drugs.

Some cytostatic drugs act on the S-phase and inhibit DNA synthesis:

- Methotrexate
- Fluorouracil
- Mercaptopurine

Some cytostatic drugs cause cells to accumulate in G_2:

- Mitomycin C
- Adriomycin
- Cyclophosphamide

Some drugs inhibit the formation of the mitotic spindle and cause cells to stop in metaphase:

- Colchicine

There are many reasons why we might want to do this. Some of these drugs can be used in chemotherapy to stop proliferation of cancer cells, but we can also use them to help us understand what happens at various stages of the cell cycle and how different molecules might interact.

Exercise: Effect of Chemicals on the Cell Cycle

As indicated earlier, there are many ways to stop the cells at various times during the cell cycle. One of the ways to stop the cell cycle is by exposing cells to a drug like Colchicine.

Colchicines are drugs that prevent formation of spindle fibers; therefore chromosomes will not easily separate and move towards the cell pole. Chromosomes will appear scattered irregularly in the cytoplasm leading to a stage known as C-metaphase where the chromosomes become shorter and thicker and each chromosome appears with both chromatids joined together by the Centromere.

Later, the centromere divides and chromatids move from one another but not towards the cell pole, this is C- anaphase. At last, a nuclear membrane appears inclosing all chromosomes (double in number) resulting in a polyploid cell (ie. the final result is one cell with an increased number of chromosomes) and if cells are exposed to colchicine for more time, the same process

will repeat. The effect of colchicines depends on many factors, like concentration, duration of treatment and the way the treatment is performed.

Depending on which other options are available to us this semester, we may also provide you with root tips treated in other ways for use in your slides. In any case, your task in this lab will be to prepare a slide of untreated and treated root tips. You will view them to observe the differences (recall the mitotic index exercise – this could be useful here), and then you will take your best slides and make them permanent.

Materials

- Compound and stereomicroscope
- Clean slides and coverslips
- Root tips in 70% Ethanol (previously fixed in a 3 ethanol: 1 glacial acetic acid solution)
- Aceto-orcein stain
- Small Kimwipes
- Pencils with eraser-ends
- Scalpels and forceps
- Water bath at 60°C with a beaker with 1N HCl and floating eppitube holders
- Eppendorf tubes
- Beaker of water

1. Permanent slide preparation

Procedure

1. Obtain 2-3 untreated root tips and place them into an eppitube. Do the same with treated root tips.
2. Add 1ml of warm 1N HCl into your tubes and place them into the water bath for ~5min. The acid will help to dissolve the cell wall and make it easier for the stain to penetrate into the cells.
3. Carefully remove the HCl, add 1ml of water into the tube and mix.
4. Discard the water and again add 1ml of water. Repeat this step one more time. In this way you have washed the tips in water 3 times and have removed most of the acid.
5. Use forceps to gently take out a root tip and place it on a clean slide.
6. Use the stereomicroscope to help you find the root cap and the tip of the root.
7. Use a scalpel to cut the root about 2mm from the tip and remove the remainder of the root. You want to only have the very tip of the root (the area with the meristem).
8. Put one drop of the stain onto the slide and keep your specimen in the stain for about 5min. You can use your scalpel to squash and cut up the root tip while it's staining, this will help the stain penetrate more easily.
9. Cover your specimen with a cover slip and dry out extra stain using a Kimwipe.
10. Squash the specimen further by pressing down gently on the cover slip. Do not let the coverslip move as you do this. You can use the eraser-end of a pencil to help to distribute the cells more under the coverslip. You are trying to generate a monolayer of cells.
11. Examine your specimen under the low and high magnification of the compound microscope and try to find mitotic cells. Most of the cells will be at interphase, so use the

low magnification objective lens to find areas with some mitosis and then use high magnification to look at details.

You have just prepared a temporary slide. If you like the results, then you can proceed to the next part, which is making the stained slide permanent. If you're not quite happy with the result, try to fix it (perhaps you need to squash it a little more to get a nice mono-layer of cells) or make another slide. Examine this slide and determine if there is any evidence of mitosis. Can you identify any specific stages?

- Take a picture of a region of your slide that contains some mitotic cells and identify the stage of mitosis – you will email that picture to your TA and indicate which stage you identified on your slide.

Once you have a slide you wish to preserve (and present to your TA for marks – see next page), perform the following steps to make it permanent:

12. Use a **pencil** to label your slide on the frosted portion of the slide.
    - The following should be on the label:
        - Your first name
        - An ID code composed of your initials, the number of the slide, and an indication if this is normal (N) or Treated (T).
            - for example: RSR2T
                - "RSR" are my initials
                - "2" means it's my second slide / attempt
                - "T" means it shows a treated root tip
    - This gives your slide a unique identifier and makes it easier to refer to it in anything you might write,

13. Place the above slide on ice for two minutes. This will help to release the coverslip.
14. Carefully use a razor blade or the edge of a scalpel to lift the edge of the coverslip and remove it from the specimen.
15. Pass the slide through a series of alcohol preparations 70%, 80%, 95%, 100%, 50% alcohol + 50% xylene and pure xylene for 2-3minutes in each preparation. Use your forceps to transfer the slides to each solution.
16. Add one drop of Canada balsam to your slide and slowly put a new coverslip back on the specimen. Avoid trapping air bubbles.
17. Put, your slides on a slide warmer plate at 50°C for two days to dry and harden.

Assignment: Preparation of Feulgen-stained Squash Slides

You have had some practice producing squash slides and looking at mitosis. In this assignment, we will be assessing your skill at producing a good squash of plant tissue and at identifying a mitotic stage.

By the end of this lab you will prepare one slide of a normal root tip and one slide of a treated root tip. You will submit these to your TA for evaluation. You will do this by providing your TA with the ID code of the two slides you prepared for this purpose.

When you get home, you will email the picture of your slide to your TA. In your email, you should identify the stage of mitosis that is visible in the photo (identify a few stages if you see more than one in the picture). **Be sure to send the e-mail to your TA by the end of this evening.**

Lab 8

In today's lab, you will be dealing with determining probabilities of genetic events. Most of the lab is dedicated to problem solving – you should use this as an opportunity to practice skills you will need to solve problems on your exams.

Learning Objectives:

Students will:

- Explain when to use the Product and Sum rules
- Use genotypic and phenotypic parental information to make predictions about offspring
- Apply appropriate combinations of Product and Sum rules in more complex problems
- Appropriately apply the Binomial Equation or Pascal's triangle to problems

---

Pre-Lab Questions:

Please view the following video for an overview of the basics of probability in genetics:

- https://youtu.be/Qcmdb25Rnyo

Identify what is wrong with the following statements:

- The probability of having a child with curly hair is 1½
- When rolling a six-sided die, it is more likely that you will roll a 6 and a 2, than a 1 or a 5.

Based on what you learned about colour-blindness in the video:

- What is the probability of a child being colour-blind if the parents do not show the colour-blind phenotype?
- What is the probability that these parents will have a colour-blind daughter?

Probability

At this point in the semester, you have learned about meiosis and have drawn some Punnett squares, so you know how traits can segregate during the production of gametes. You have also learned about phenotypes and genotypes, and you have produced a pedigree chart, so you know how you could go about collecting phenotypic data and using it to help you determine genotypes of individuals.

One of the ways that all of this knowledge of genetics and of the genetic makeup of individuals can be applied, is in the making of predictions. This is what Genetic Counseling is all about.

The reason this works is that the segregation of alleles in meiosis is a random event, and the fertilization of an egg with a specific genotype by a sperm with a specific genotype is also random. Thus, because we're dealing with random events in genetics, we can apply the rules of Probablity to make accurate predictions. All we need to know is the total number of possible outcomes, and the number of outcomes that matches our desired criteria.

As you might imagine, some predictions do not require any knowledge of probability, some can be made using some very simple probability rules, while others require rules that are more complex. The difference can be quite subtle, so you must learn to look at genetic questions/problems very carefully.

Let us begin with a very simple problem:

- ***A married couple is about to have a child. What is the probability that it will be a boy?***
    - This is a very simple question with a very simple answer. Since the only options are “Boy” or “Girl” , the probability of them having a boy is 50% (or ½)
    - This answer doesn't really require a genetic explanation, but you can easily do a Punnett square for this couple to determine the answer.

		Male Gamete	
		X	Y
Female Gamete	X	XX	XY
	X	XX	XY

    - As you can see, based on the distribution of the X and Y chromosomes to their progeny, there is an equal chance (2 out of 4, or ½) that the couple will have a boy or a girl.

A similar question could also be asked:

- ***If the couple already has 2 girls, what is the probability that this next child will be a boy?***

- It is still 50% (or ½). The previous children will have no impact on the combination of gametes produced by each parent. Each "event" (child) is **independent** of the others.

It is important to understand that each fertilization of an egg by a sperm is an independent event. It is not affected by previous events, and will not affect future events either. The above question can be asked in a different way however and that will make it a different type of problem.

- ***What is the probability of this couple having 2 girls first and then a boy?***
  - This question is asking you to determine the probability of three independent events occurring together (ie. for one couple).
  - So in reality, this question is asking:
    - ***What is the probability of having 1 girl and 1 girl and 1 boy?***
  - This will require the use of the **Product Rule**

Product Rule:

The Product Rule is used to determine the probability of a combination of two or more independent events happening together. When this is the case, you need to take the probability of each event and multiply it by the probability of the others

- Thus the answer to the above question is ½ x ½ x ½ = $^1/_8$

Another question that would fall into this category would be:

- ***What is the probability of a heterozygous brown-eyed (Bb) man and a heterozygous brown-eyed (Bb) woman having a blue-eyed girl?***
  - Here again we are dealing with the combined probability of two independent events:
    - the probability of having a girl (you already know that's ½)

      and

    - the probability of having a child with blue eyes

		Male Gamete	
		B	**b**
Female Gamete	**B**	**BB**	**Bb**
	b	**Bb**	**bb**

    - According to the above Punnett square, there is a ¼ chance of any of this couple's children having blue eyes.
  - Thus the answer to the above question is ½ x ¼ = $^1/_8$

Let's ask another question:

- ***What is the probability of this couple having two children – a girl and a boy (in any order)?***
    - This question may seem to be the same as one of the questions we asked above, but it isn't. This is where students often make their mistakes.
    - This question is really asking:
        - ***What is the probability of having:***
            - ***a girl and then a boy***

              or

            - ***a boy and then a girl?***
    - This will use the product rule for part of the answer, but you will also need to understand that you are now dealing with two **mutually exclusive** events. Having a girl and then a boy is different from having a boy and then a girl. You can't have both happen at the same time – you can have one **or** the other, but either one meets the criteria and is an acceptable way of reaching your goal (ie. having two kids, a girl and a boy).

Sum Rule:

The Sum Rule is used to determine the probability of the occurrence of one of two of more mutually exclusive events. When any of the different combinations of the events is acceptable, the probability of each event is added up to calculate the probability of the occurrence of any one of them.

- Let's return to our question above. The probability of having a girl and then a boy is ¼ (this is determined by product rule) – we can call this "event 1". The probability of having a boy and then a girl is also ¼ – let's call this "event 2".
- Now the probability of event 1 **or** event 2 happening is the sum of the two probabilities. ¼ + ¼ = **½**

Does this make sense?

Based on these rules, you are much more likely to have a boy and a girl in any order (P = 50%) than you are to have a girl first and a boy second (P = 25%).

The above rules work well for relatively simple problems. Once the problems become more complicated, you need to use a more complex formula to help you determine the probabilities.

- ***What is the probability of the above couple having 3 children, 2 girls and 1 boy (in any order)?***
    - This one can still be done using the methods we used above
    - This can be restated as:
        - What is the probability that the 1st child is a boy,

          or
        - What is the probability that the 2nd child is a boy,

          or
        - What is the probability that the 3rd child is a boy

          (always assuming that the remaining kids are girls).

But what is we ask this:

- ***What is the probability that the above couple will have 6 children, 2 girls and 4 boys (in any order)?***
    - This may not seem that much different from the above, but you need to understand that here you can have **many different combinations** of boys and girls.
    - In this case, you need to use the Binomial Theorem

Binomial Theorem:

Whenever you're dealing with some combination of two mutually exclusive events, and the order is not specified, you will want to use binomial theorem. There are two ways to solve such problems: using the binomial formula or the binomial expansion.

Binomial Formula

This is the binomial formula: $$P(\text{a or b}) = \frac{n!}{s!\,t!}\,p^s q^t$$

Where:

n = total number of events
s = number of type A events (ie. number of boys)
t = number of type B events (ie. number of girls)
p = probability of type A event
q = probability of type B event
Always true: s + t = n, p + q = 1

Thus, the answer to our sample problem is: $$P(\text{a or b}) = \frac{6!}{2!\,4!}\left(\frac{1}{2}\right)^2\left(\frac{1}{2}\right)^4$$

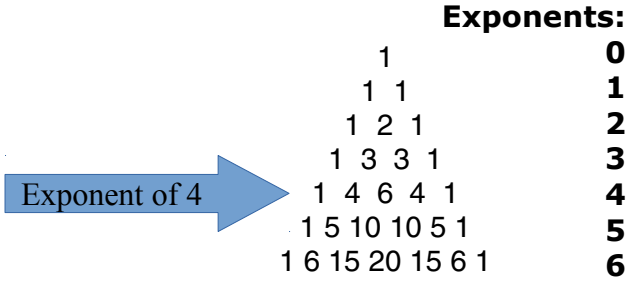
$P = 15(½)^2(½)^4 = \mathbf{0.234375}$

Binomial Expansion

$$(a+b)^n$$

This is used for sets of independent events where the order does not matter.

1. Create a series of "ab" terms. The number of terms should be 1 more than the exponent on the binomial.
    - ie: $(a+b)^4 = ab + ab + ab + ab + ab$
2. Add exponents to each of the "a"-s. Start with the exponent of the binomial and decrement them by one in each case.
    - ie: $(a+b)^4 = a^4b + a^3b + a^2b + a^1b + a^0b$
3. Add exponents to each of the "b"-s. Start with 0 (zero) and increase by 1 in each case.
    - ie: $(a+b)^4 = a^4b^0 + a^3b^1 + a^2b^2 + a^1b^3 + a^0b^4$
4. Use Pascal's triangle to find the leading coefficients for the appropriate exponent:



    - ie: $(a+b)^4 = 1a^4b^0 + 4a^3b^1 + 6a^2b^2 + 4a^1b^3 + 1a^0b^4$
5. Simplify
    1. anything to the power of 0 is equal to 1 ($a^0 = 1$)
    2. get rid of any "1"-s that are being multiplied by something ($1 \cdot a = a$)
        - ie: $(a+b)^4 = a^4 + 4a^3b^1 + 6a^2b^2 + 4a^1b^3 + b^4$
6. Figure out which one of the terms to use – you will not be using the whole equation. Look at the problem, how many individuals in each category? Each category is either an "a" or a "b", the number of individuals in each category is the exponent, the "a" and "b" are the probabilities of having each of these categories.

Thus, the answer to our sample problem is:

$$(a+b)^6 = 1a^6b^0 + 6a^5b^1 + 15a^4b^2 + 20a^3b^3 + 15a^2b^4 + 6a^1b^5 + 1a^0b^6$$

So, if girls are symbolized with an "a" and boys are symbolized with a "b", then we would use the following term:

Probability (of 2 girls and 4 boys) = $15(½)^2(½)^4$ = **0.234375**

Both methods work, just use whichever is easier for you to remember.

Lab 9

Today's lab deals with performing statistical tests on our data in order to determine whether alleles are linked. If linkage is indicated, we will be using mapping strategies to determine how close the alleles are to one another on a chromosome. This lab will also include the solving of a problem set to help you practice certain concepts in preparation for your exams.

Learning Objectives:

Students will:

- Identify categorical data in order to determine if a Chi-square test can be performed
- Use a table to Chi-square Critical Values to identify p-values for their statistical tests
- Describe the relationship between experimental and expected data using p-values
- Use statistical tests to determine whether to calculate map distances
- Calculate map distances between two or more allelic loci
- Define epistasis and describe how it might affect phenotypic ratios

---

Pre-Lab Questions:

What is the difference between continuous data and categorical data?

Please view the linked video dealing with the basics of the χ^2 test and answer the questions below:

- https://youtu.be/WXPBoFDqNVk

Based on what you saw, what is a Null Hypothesis (H_o)?

What does a p-value that is less than 0.05 mean?

The Chi-Square Test

The Chi-square test is performed on data that is organized in distinct categories (ie. non-continuous data), for example numbers of objects or individuals that belong to one category (number of yellow peas) or to another category (number of green peas).

It is a statistical measurement of goodness-of-fit between two data sets, in genetics this means actual data and expected results from a cross. The easiest way to think of it is that it's a way of determining if two sets of numbers are similar enough to be considered "the same".

The test uses the following formula:

$$\chi^2 = \sum \frac{(Observed - Expected)^2}{Expected}$$

You can use the following table to help you calculate the χ^2 value for each of your crosses.

Table.1. Calculation of the χ^2 value.

1. List your expected phenotypes:					
2. What is the expected phenotypic ratio?					
3. What is your total number of flies?					
4. What are the expected number of flies in each category? (#**2** x #**3**)/16					Exp.
5. List the actual numbers you obtained from your experiment:					Obs.
6. Subtract Exp. from Obs. for each column: (Observed – Expected)					
7. Square the number obtained in each column:					
8. Divide the above number by the expected value: (the numbers in row 4)					
9. Add up all the numbers from the above row:					χ^2

Once, you've calculated the Chi-square value, you will need to determine your "degrees of freedom", which is defined as the number of categories -1. So, with 4 categories in the F2 population of your dihybrid cross, the degrees of freedom is equal to 3.

Once you've determined both of the above numbers, you can use them to help you determine the goodness of fit of your data using the table below. The value you calculated for the degrees of freedom will help you decide which row of the table to use.

Table.2. Table of Chi Square Values.

Percentage Points of the Chi-Square Distribution

Degrees of Freedom	Probability of a larger value of x^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21
11	3.053	4.575	5.578	7.584	10.341	13.70	17.28	19.68	24.72

Source: http://whichbobareyou.com/uploads/2/9/4/6/2946053/9419235_orig.png

Across the top of the table are listed critical values. These values are the probabilities that the results you obtained (your observed) are different from the expected results purely by chance. Thus if your χ^2 value falls in the first column of numbers (the one under the "0.99", then there is a 99% chance that your results differ from the expected simply by random chance. If, on the other hand, your χ^2 value is larger than the values in the column under the "0.05", then there is only a 5% chance that your observed values are the same as the expected. It is at this point that scientists consider their results to be "**significantly**" different from the expected and reject the Null Hypothesis.

In Genetic terms, this means that a large Chi-Square value occurs because you have greater than expected numbers of flies with the parental phenotypes (ie. flies that look like the parental flies), and fewer flies with the recombinant phenotypes (ie. flies that look different from the parents). This generally means that the alleles did not assort independently and supports the hypothesis that the two alleles are linked.

Exercise: Determining Genetic Linkage

Once you have collected an adequate amount of F_2 data, you can begin your *Drosophila* experiment data analysis.

First, you need to decide whether the alleles are sorting independently, or are linked. Your Null Hypothesis (H_0) should be that the two alleles are not linked (i.e. they are sorting independently). If you're considering a dihybrid self-cross and the mutations are recessive to wildtype, then an expectation of independent assortment means that the expected F_2 phenotypic ratio is 9:3:3:1. In other cases, you can simply produce a Punnett square to determine your F_2 expected phenotypic ratios.

If your data does fit the expected ratio, then you can't reject the Null Hypothesis and you can conclude that the two genes are sorting independently. An exact fit of the 9:3:3:1 ratio, however, can be quite rare – natural variability and random chance can make your actual ratios somewhat different. Linkage between the two alleles can make the ratios very different from 9:3:3:1, but can also sometimes result in just a very slight difference.

So, when can you safely conclude that the two genes are linked? You can test your Null Hypothesis using a Chi-square test.

Materials

- StarGenetics Software (online: http://star.mit.edu/genetics/)
- Datafile (provided on Blackboard)

1. Linkage analysis

Procedure

Your group will be assigned two fly strains (ie. strain A and strain D). You will use these strains in the StarGenetics program to perform your experiment.

1. Identify the mutant traits from the descriptions provided by the software.
2. Make a set of reciprocal crosses between each strain and wildtype flies to generate F_1 data for each side of the cross.
    - Mate strain A females with wildtype males, and strain A males with wildtype females
    - Mate strain D females with wildtype males, and strain D males with wildtype females
3. Observe the phenotypes of the F_1 flies.
    - Do the mutant traits appear in the F_1 flies? Which traits are dominant and which are recessive?
    - Do all the flies look the same or are they different? Are your traits autosomal or X-linked?
    - Assign genotypes to the F_1 and the Parental flies (remember that the parental flies are true-breeding, so they're homozygous each mutant trait)
4. Make F_1 self-crosses
    - If the F_1 phenotypes are the same for each side of the reciprocal cross, then just make one self-cross (try to make enough matings to obtain close to 1000 flies in the F_2 generation
    - If the F_1 phenotypes are different for each side of the cross, then make a self cross from each side.
    - Make sure you generate a Punnett square to find out if the self-cross for that side of the reciprocal cross will actually generate useful data.
5. Observe the F_2 data
    - 1. What is your expected phenotypic ratio? (look at your punnett squares to determine that)
    - 2. Does the actual phenotypic ratio match the predicted/expected ratio

6. Do χ^2 analysis on the data

   - If χ^2 gives a P-value greater than 0.05 then you have to assume that there is no difference between your data and the expected results – thus no linkage
   - If χ^2 gives a P-value less then 0.05 then you know that your actual ratio is significantly different from expected and thus indicates linkage
   - this assumes that there are no gene interactions or epistasis

Epistasis

There is one complication of which you should be aware. There an assumption within your Null Hypothesis that all possible phenotypes are **equally viable** and that there is no **epistasis**.

However this is not always true. Some alleles, and some allele combinations are less viable (or the flies with those phenotypes are slower to emerge). Delayed birth, or reduced viability might make one or two of your categories have lower than expected values. Similarly if the two genes interact then the ratio could be altered due to a gene interaction such as epistasis. How can you distinguish failure to get a 9:3:3:1 ratio due to linkage from some other type of explanation?

If you do reject your Null hypothesis, think about whether it is because the two genes are indeed linked, or whether one or more of the genotypes is under-represented for reasons of viability or gene interaction. A careful inspection of your data can help you distinguish between these possibilities.

Epistasis occurs when the expression of the phenotype controlled by one allele is modified or suppressed (covered up) by the expression of another allele.

Consider this example:

You're studying two separate mutant alleles in mice. One codes for a brown fur pigment and the other produces hairless mice.

When expressed individually in separate mice, each is capable of producing a phenotype. But when both alleles are found in the same mouse, the brown fur allele will not be visible because the other allele will mask its presence. Together, these alleles will show the same phenotype as the hairless mutation, thus the hairless allele/gene is said to be epistatic to the brown allele/gene.

If you have rejected your Null hypothesis, look at the phenotypic ratios for each locus individually. For recessive mutations you should see a 3:1 ratio of dominant to recessive for each trait if there is no lethality or gene interactions. If you get the 3:1 ratio for each separate trait this suggests the deviations in the 9:3:3:1 ratio are not due to viability differences, developmental delay, or epistasis. Now look carefully at the four categories of flies, and see which categories are very different from the expected values, and in which direction there are differences.

Determining Map Distances

If you have rejected your Null Hypothesis that the two alleles are not linked, and if you observed an excess of parental-type phenotypes, then it is likely the two alleles are linked.

Once you've shown linkage between two alleles, you can calculate the map distance between them based on the frequency of recombination between the two alleles. This is based on the assumption that the closer the two linked alleles are located to each other, the lower the frequency of recombination between them.

To put it another way, the closer two alleles are to each other on a chromosome, the greater their chances of being inherited together. Thus, recombinant allele combinations are less likely to occur and the parental allele combinations should be found more frequently in the offspring. A crossover must occur between the loci of the two alleles to produce a recombinant-type offspring.

Crosses

Crosses can be set up in ***repulsion*** or in ***coupling*** with relation to the alleles.

When you set up a cross in ***coupling***, the parental flies either donate both of the dominant traits or both of the recessive traits. Thus, the parental gametes are AB and ab. Some authors call this ***cis***.

When setting up a cross in ***repulsion***, the parental flies are donating one dominant and one recessive allele from the two genes. Thus, the parental gametes are Ab and aB. Some authors call this ***trans***.

Furthermore, in order to do this mapping, you must understand that crossing over during meiosis does not occur in male *Drosophila*, thus if the two genes are linked, the linkage in males will be absolute. The F_1 males will only donate one of the parental- type of gametes to their F_2 offspring. In a cross set up in **coupling** this will mean that F_1 males donate both wild type alleles to 50% of their offspring, and both mutant alleles to the other 50% of the offspring. You must factor this in, when calculating map distances.

To determine the distance between two linked alleles, you need to determine the number of flies that obtained a recombinant-type chromosome from the female parent. Remember, that we only need to think about recombination in female flies – we're actually trying to find out what happened during meiosis in the F_1 females.

So the total number of progeny can be thought of as the total number of gametes produced by the female. If you think back to what you learned about meiosis and crossing over, you will recall that a crossover involves only two of the four chromatids of a synapsed pair of homologous chromosomes. Therefore, under “normal conditions” each crossover will produce two recombinants, and two parental-type gametes. In the case of linkage, the crossover still occurs, but it is less likely to occur between the linked allelic loci, thus generating more parental-type gametes.

In our case the parental-type genotypes might be something like AB or ab, and the recombinant-type gamete genotypes would be Ab and aB.

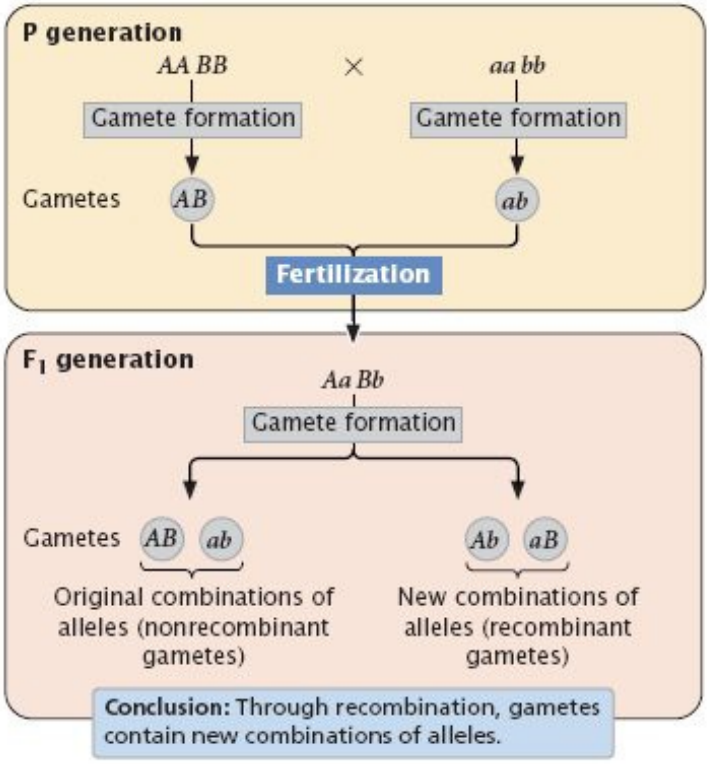


Fig. 1. Alleles and recombination. Parental-type gametes on the left and recombinant-type gametes on the right.

With this in mind, you will now need to look at your F_2 data and decide which of the offspring show evidence of recombinant-type allelic combinations. If they do, then it is evidence of recombination in the F_1 females (the males would have donated a chromosome containing all dominant or all recessive alleles).

Once you've identified the recombinant-types, you can determine the map distance using the following formula:

$$\text{Map Distance} = \frac{Number\ of\ F_2\ Recombinants}{Total\ F_2\ Offspring} \times 100$$

This is basically the recombination frequency expressed as a percentage and is given in units called Centimorgans (cM) in honour of Thomas Hunt Morgan.

One last thing to keep in mind when determining the number of recombinant type gametes produced by the female in your crosses is that the genotype of half of the female gametes will be masked by the dominant traits donated by the male parent (about ½ of the time, the male F_1 flies will donate the AB version of the chromosome). You will need to account for this in your calculations.

Discussion of Data

Some time in the 11th week, you will need to make an appointment with your TA to discuss your results. You will need to be prepared to present your findings (informally – no powerpoint is necessary at this time) and defend your conclusions.

At this point, you should have collected most of your data (but can continue counting flies if you need more) and you should do some preliminary analysis on it. Please make sure you've determined if you have linkage, and try to determine map distances in those cases. Having mapped your alleles, please take a look at the map of *Drosophila* chromosomes below and identify which genes you're most likely studying. At your meeting, your TA will let you know which ones you were actually given.

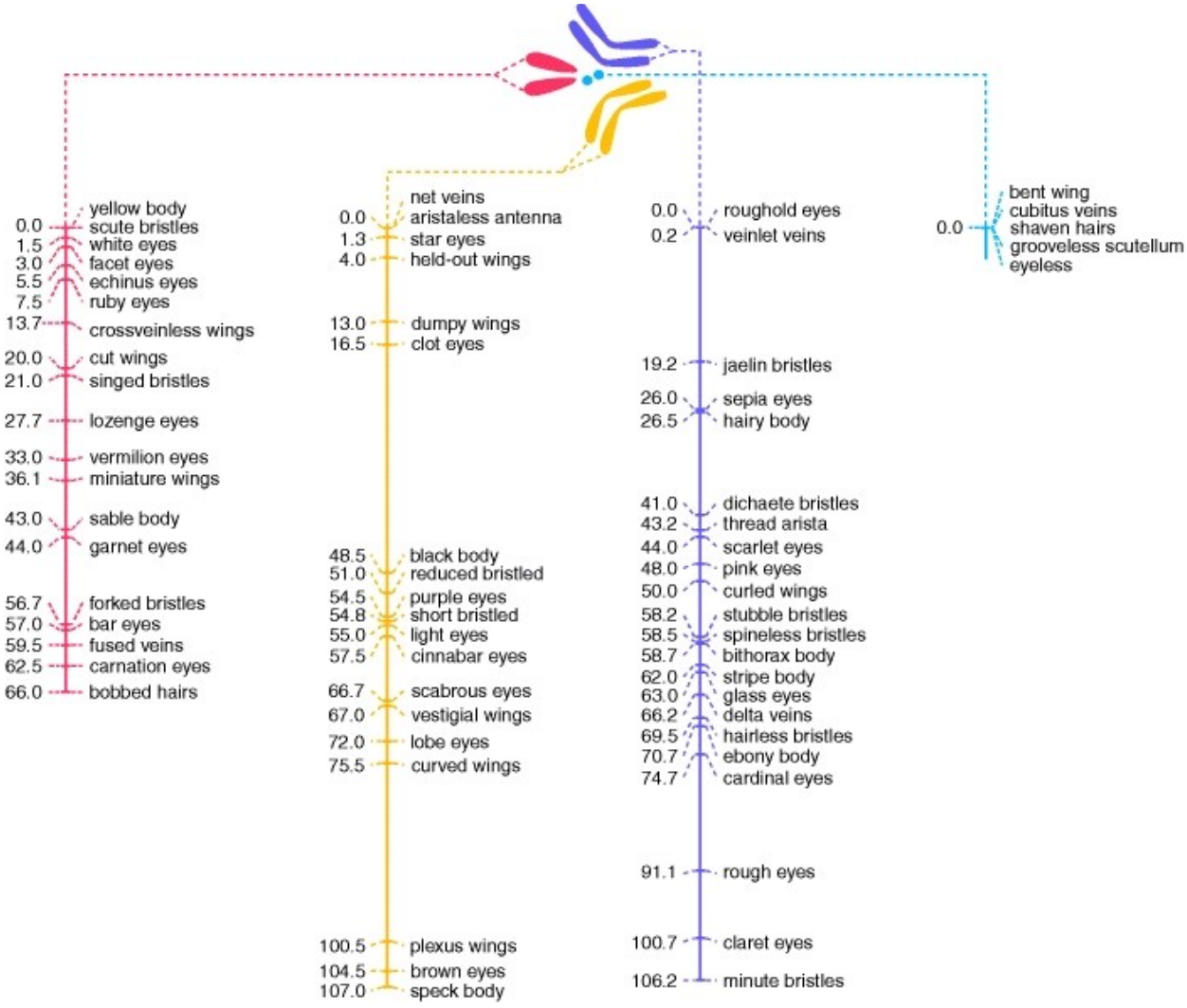


Fig. 1. The genetic map of the *Drosophila* genome. Values are given in map units measured from the gene closest to one end.

Source: Griffiths AJF, Gelbart WM, Miller JH, et al. Modern Genetic Analysis. New York: W. H. Freeman; 1999. Linkage Maps. Available from: www.ncbi.nlm.nih.gov/books/NBK21358/

Datasheet for TA

Group: _____

X-linked Trait Data			
Phenotypes	Genotypes	# of Flies	Comments
Total Number of Flies:			
Autosomal Trait Data			
Phenotypes	Genotypes	# of Flies	Comments
Total Number of Flies:			

Lab 10

Today's lab starts the molecular genetics portion of this course. Over the course of the next few labs, you will be extracting DNA and using it to perform PCR to determine the molecular genotype of the organism being tested. The DNA extraction protocol used today is a relatively short one, but it will still likely take up most of the time that we have in this class, for this reason it is important that you **come to class prepared** and have a fairly good idea of what you will be doing.

Learning Objectives:

Students will:

- Identify the main steps of a typical DNA extraction protocol
- Explain the purpose of the various reagents used in a DNA extraction protocol
- Perform a DNA extraction

Pre-Lab Questions:

View the video linked below and answer the following questions;

- https://youtu.be/90Gsxal57-g

What are some of the similarities and differences between the protocol in the video and the protocol we will be using in today's lab?

What does it mean when you're told to “balance the tubes” in a centrifuge?

View the video linked below to review the proper use of a micropipettor:

- https://youtu.be/uEy_NGDfo_8

Extraction of Genomic DNA for Genotyping

There are numerous methods for DNA extraction, but they all apply the same basic principles. The differences between the methods are mostly due to the unique characteristics of some organisms and the challenges they present to the scientist (another reason has to do with the availability of certain chemicals in the lab).

DNA extraction begins with cell lysis. The DNA is then separated from the contaminating (unwanted) macromolecules and debris. And lastly, it is precipitated so that it can be placed into an appropriate storage buffer.

Cell Lysis can be accomplished through physical, enzymatic or chemical methods. Essentially, the cell membranes and possibly cell walls (depending on the organism) need to be damaged enough to allow the release of DNA into solution. Some care must also be taken to ensure that this released DNA is not damaged.

Centrifugation is generally used for removing some of the larger cellular debris, while lipids and protein are generally separated away from DNA based on their preferential solubility in organic solvents. Enzymatic methods are also sometimes used to **remove macromolecules** like proteins and RNA.

Once relatively isolated, the **DNA is precipitated out of solution** by removing water from the DNA backbone. This is accomplished by increasing the ionic strength of a solution by the addition of a salt, and the addition of a high concentration of an alcohol (isopropanol or ethanol).

These two components will attract lots of water to themselves and thus pull much of it away from the DNA. Once the DNA backbone has had water removed from it, it loses solubility and starts to precipitate. Some researchers enhance this precipitation by performing it at very low temperatures (in a freezer) and for long periods of time.

This precipitated DNA can then be resuspended in any solution that the scientist might find appropriate for further experiments, but is usually placed in a buffered solution of Tris and EDTA.

Exercise: DNA Extraction

In this lab, you will be extracting DNA from one of your fruit flies in order to perform some PCR on it in the next lab for the purposes of genotyping it.

We will be following the CTAB protocol in this lab, but there are several different DNA extraction protocols. If you end up doing a research project in a lab where you are asked to do a DNA extraction, your supervisor will likely provide you with a protocol that they want you to use. It may not use CTAB – different scientists have preferences for different protocols. Part of that has to do with which chemicals they have available in their lab and part of it depends on which organism they work with – different protocols have been specialized to work best with different organisms. The reason we selected the CTAB method is that it is short enough for use in a teaching lab.

Regardless of which protocol you use in a research situation, they all have the same basic parts as described in the section above.

Materials

- 1ml of CTAB Buffer (2%CTAB, 100mM Tris, 20mM EDTA, 1.4M NaCl, pH 8)
- 24:1 Chloroform-Isoamyl alcohol solution in fumehood
- 1ml of 95% Ethanol
- 1ml of Isopropanol on ice
- 1ml 1xTE (10mM Tris-HCl, 1mM EDTA, pH 8)
- 65°C water bath or heating block
- Microfuge
- Eppendorf tubes
- P200, P1000 and tips

1. DNA Purification

Procedure

1. Working as a group, anaesthetize a few of the provided flies.
2. Transfer one *Drosophila* into an Eppendorf tube. Each group should prepare two tubes.
3. Add 25µl of CTAB buffer and grind *Drosophila* with a blue pestle or a yellow pipette tip for about 2min.
    - Do your best to crush the fly into the buffer to produce a fairly uniform mixture, but it is likely that you will still be able to see fly parts floating around in your mixture when you're finished.
4. Add 175µl of CTAB buffer and mix the contents of the tube by pipetting up and down gently a few times.
5. Incubate the tube at 65°C for 30 minutes, mixing again once after 15 minutes
6. In the fumehood, add 200µl of 24:1 Chloroform-Isoamyl Alcohol.
    - This solution may have some water on it's surface, so make sure you take the organic phase from the second layer.
7. Close the cap tightly and shake the tube vigorously to mix the contents.
8. Centrifuge the tubes for 10 minutes at maximum speed (13-15,000 rpm).
    - Be sure the centrifuge is in a "balanced configuration" before you start it. Ask your TA to explain or demonstrate if you're not sure what that means.

    Following centrifugation, you should have three layers:

    - top: aqueous phase (buffer, DNA and RNA, some proteins)
    - middle: debris and some proteins
    - bottom: organic phase (chloroform, cell membranes and some proteins)

    Proceed to the next step quickly so the phases do not remix

9. Set your micropipettor to 150µl and carefully pipette off the aqueous phase (top layer). Do not to suck up any of the middle or organic phases.
10. Place the aqueous phase into a new labeled Eppendorf tube and add 110µl of cold isopropanol and mix gently by inverting tube.
11. Place the samples in a freezer for 45 min to an hour (your TA will let you know based on the amount of time left in the lab).
12. Centrifuge your samples for 3 min at maximum speed.

13. Discard supernatant, being careful not to dislodge pellet.
14. Add 200 µl 95% EtOH, invert tubes 5 times.
15. Centrifuge for 1 minute at max speed .
16. Discard supernatant, being careful not to dislodge pellet. You may try gently tapping the inverted tube over a paper towel or kimwipe to remove an excess liquid.
17. Invert tubes on a clean kimwipe and allow to air-dry for 10 minutes on your bench.
    - Alternatively, you can place your Eppendorf tubes in a rack near the front of the fumehood with the sash pulled down as far as possible to have the air flow past the tubes at high speed.
18. Add 20ul of TE to the tubes to dissolve the pellets. Allow the pellets to resuspend overnight at room temperature.
19. Store the DNA in a refrigerator until the next lab.

Lab 11

In this lab, you will be using the DNA extracted in the previous lab to determine the genotype of the organism you were using. We will be amplifying a region of DNA that may or may not have a mutation that will be detectable by looking at the length of the DNA fragment generated. Please be sure to prepare for the lab by familiarizing yourself with the concept of PCR.

Once the PCR reaction is set up, you will discuss your *Drosophila* experiment with the TA.

Learning Objectives:

Students will:

- Explain the concept of PCR
- Describe how PCR primers can be used to distinguish between homozygotes and heterozygotes
- Set up a PCR reaction
- Explain their experimental data based on their understanding of Mendelian genetics

---

Pre-Lab Questions:

View the video explaining the concept of PCR (read about it if needed) and answer the question below:

- https://youtu.be/2KoLnIwoZKU

You're performing PCR on DNA from a heterozygous individual. If the two alleles are very similar in sequence but one of them is just a little longer (recessive allele) than the other (dominant allele), what kind of products would be generated in the PCR reaction?

Polymerase Chain Reaction

PCR, is a technique which allows the amplification of specific regions of DNA. It utilizes a cell's DNA replication mechanism, but does it outside the cell (in-vitro) and performs multiple replication cycles allowing for exponential increases in DNA product. PCR differs from normal DNA replication two very important ways:

1. Instead of using random hexamers as in typical DNA replication, PCR uses very specific primers to ensure that the DNA synthesis would start at a particular point along a single strand of DNA. This breakthrough concept allows scientists to target specific DNA regions for amplification by the DNA polymerase.

2. PCR bypasses the fairly complex process of unwinding the DNA and preventing it from reannealing that normally happens in a cell. Instead, it uses high heat (~95°C) for the efficient separation of the DNA strands to allow the DNA polymerase access to it's template. This is accomplished through the use of a thermostable DNA polymerase, which was isolated from a heat-loving bacterium – *Thermus aquaticus* (*Taq* Polymerase). Thus, multiple cycles of heating and cooling can be performed without the DNA polymerase enzme denaturing.

Once the DNA is denatured by high heat, the temperature is lowered somewhat to allow the specific PCR primers to bind to the template, and then the *Taq* polymerase synthesizes a new DNA strand starting at the primer. This synthesis happens at 72°C, its optimum temperature.

Exercise: Genotyping of *Drosophila*

Using the genomic DNA sample you extracted in the last lab, you will perform PCR amplification of a part of a DNA sequence.

Materials:

- 5X *Taq* polymerase buffer
- *Taq* DNA polymerase
- PCR Primers
- sterile water
- micropippettes and tips
- Sterile PCR tubes
- dNTP mix
- 25mM $MgCl_2$

1. PCR Reaction

Procedure:

1. Label the PCR tubes you need and place them on ice.

2. Set up the reactions as indicated and in that order, making sure to keep them cold.

DNA	2µl
5X Buffer	5µl
dNTP Mixture	10µl
25mM $MgCl_2$	2µl
Forward Primer	2µl
Reverse Primer	2µl
Taq Polymerase	2µl
Total Vol.	25µl

3. Place the PCR tubes inside open eppitubes and spin the contents down to the bottom.
4. Place the PCR tubes onto a thermocycler and start the prepared program

Oral reports

While we are waiting for the PCR reactions to complete, you will be presenting your experimental results from your project to your peers. Each group will be given a maximum of 15 minutes. It is expected that each student in your group will give a portion of the presentation. **All students are expected to attend all talks** and you are expected to ask questions.

Because of the time constraints, you should focus on your results. Remember that you will be speaking to peers who have done a similar experiment and have a fair amount of background knowledge. Do not spend too much time dealing with the basics of genetics in your introduction.

Then they should explain how their particular data set allowed them to determine the mode of inheritance of the genes in question, and to determine whether or not any of the genes were linked to each other. If the genes were linked, then the students should indicate the map distances between the loci, as well as the other relevant parts of their linkage analysis. As in the written reports, the students should focus on the key pieces of data that led them to each specific conclusion. If there were any particularly interesting (or anomalous) data they can comment on this.

Be sure to use correct nomenclature and the genetic notation that is appropriate for Drosophila. Each group will be expected to use their judgment on how to best use their time allotment.

*** Important ***

Your lab report is due at the start of thie next lab. Please make sure your TA receives it before the deadline. You should be sure that you submit a printed copy and also an **electronic version on the Blackboard system**. Lab reports that are not turned in online will not be graded.

In today's lab, you will finally get to see the results of your experiment. You will run your PCR reactions on an agarose gel and make some conclusions about genotypes based on the size and number of DNA fragments in each sample.

While you wait for your results, you and your group will present your *Drosophila* experiment results to the rest of the class. Everyone in your group should participate – you will be evaluated on this part as a group.

At the end, you will also clean up your project work (if you haven't done so already). Please dispose of any unneeded fly cultures appropriately – if in doubt, ask your TA.

Learning Objectives:

Students will:

- Explain the concept of separation of molecules by size using electrophoresis
- Interpret PCR results
- Identify genotypes based on the PCR results
- Explain how the DNA sequence is related to the concept of an allele
- Summarize their findings in a visual format
- Present their data in an oral presentation

Pre-Lab Questions:

View the linked video dealing with electrophoresis and answer the following questions:

- https://youtu.be/mN5IvS96wNk

What is a DNA ladder?

If you have two differently-sized DNA fragments in your sample, describe how they will be separated on an agarose gel.

Agarose Gel Electrophoresis

Agarose electrophoresis is generally used for visualizing DNA samples. At a molecular level, an agarose is composed of many cross-linked agarose fibers which form small openings (pores) between them. The size of these pores can be controlled by varying the concentration of agarose when the gel is cast, and will determine the size of DNA molecules that will migrate easily through this gel.

The DNA migration is in response to an electric current. Since DNA has a negatively charged sugar-phosphate backbone, it will be attracted to the cathode (positive electrode) once an electrical current is applied to the gel. Large DNA molecules will travel slowly in the gel because they are impeded by the gel matrix, while smaller DNA molecules are able to pass through the pores in the gel matrix more easily, and therefore travel more quickly.

Because the size of a DNA fragment determines how fast it travels through the gel matrix, the smaller the DNA molecule, the farther it will travel over a period of time. We can thus use this migration information to help us determine the sizes of the DNA molecules on the gel. We do this by comparing the migration of DNA in our samples to the migration distances of a sample of DNA fragments of known sizes – a DNA standard, also known as a Molecular Marker.

Visualizing DNA on a Gel

On its own, DNA has no colour and would be very difficult to detect, it is therefore necessary to attach it to molecules that can be visualized quite easily. There are several such molecules, but ethidium bromide is the most commonly used in the lab. Ethidium bromide (EtBr) is a small molecule that has the ability to bind to DNA, and is detected as an orange fluorescence upon excitation with ultraviolet (UV) light. It is usually added to the gel during gel casting and attaches to DNA molecules as they migrate in the electric current. DNA that passes through such a gel will show up as a bright band on a dark background due to the ethidium bromide that has been "picked up".

There are two potential safety issues associated with DNA electrophoresis:

1. Ethidium bromide is a mutagen and probably a carcinogen. You must wear gloves when handling the gel, and the gel must be disposed of appropriately.
2. DNA visualization is done on a UV light box, which can cause damage to the retina of the eye. In our case the UV light box is enclosed, and we use digital camera to view our gels, so there is no danger to you.

Exercise: Determination of Genotype

Your task today, is to run your PCR samples on an agarose gel.

The gel you will be loading in this lab will be prepared for you. Here is a brief overview of the steps involved (your TA may be able to demonstrate this if time permits):

Materials:

- PCR samples
- Loading dye
- Molecular marker
- 1% agarose gel
- 0.5X TBE Buffer for the gel tank
- Gel apparatus and power supply
- P200 and/or P100 micropipettors
- box of 20-200µl tips

1. Agarose Gel Electrophoresis

Procedure:

1. Thaw your samples.
2. Wipe the water and ice from the outside and place the tubes in a microfuge. Remember to balance your tubes.
3. Spin down your samples for 30sec at maximum speed.
    - There may still be some condensation along the sides of your tubes. A brief spin in a microfuge will collect all that condensation back at the bottom of the eppitube.
4. Add 5ul of loading dye (LD) to each tube. The total volume in each tube will now be 30µl.
5. Spin down your samples for 30sec at maximum speed.
6. Pipette 25µl of each sample into the wells of the gel. Your TA will load the molecular marker.
7. Run the gel at 100V for about 30-45min

Project Work Clean Up

If you haven't done so already, please make sure you've cleaned up all of your fly cultures. Do not simply release the flies – etherize them and put them in the morgue jar.