Laboratory Two

Genetic Variation: Meiosis, Recombination

Prelab

Study this Genetic Variation lab description and textbook figures 12.4, 12.5, 13.4, 13.7, 13.8, and 13.9, with accompanying text; then do the Lab 2 Prelab linked to the course website. The next week's prelab link is posted each Thursday afternoon. See "Submitting Catalyst Exercises" in this course manual; prelabs are due by Tuesday at 8:00 AM. (2 points)

Personal Data Questionnaire

Read the questions below, make the required measurements, then complete the online Personal Data Questionnaire linked to the course website. Questionnaire results will be used in *Laboratory Three*, but the questionnaire is **due by 9:00 AM Friday of the second week of lab**.

To make the required measurements, use a ruler or a tape measure. If you don't have either, use an 8.5 x 11" or 8.5 x 14" piece of paper and fold it evenly several times to get smaller increments. Please **do not guess!** The information you submit is anonymous: It is de-identified so that no one will know who submitted the data; we know only whether or not you completed the questionnaire. If any of the requested information is unavailable or confidential, simply leave that part of the questionnaire blank.

1. What is your height? (as total number of inches, with fractions expressed as decimals)
2. What is your weight? (in pounds, with fractions expressed as decimals)
3. What is your mother's height? (as total number of inches, with fractions expressed as decimals)
4. What is your father's height (as total number of inches, with fractions expressed as decimals)
5. What is your sex? (enter M for male, F for female)
6. What is your lucky number? (If you don't have one, answer N for none.)
7. Are you right-handed or left-handed? (Enter R for right, L for left)
8. What is your "wingspan"? (With both arms outstretched to the side, measure from the tip of your left middle finger to the tip of your right middle finger, in inches, with fractions expressed as decimals.)
9. What is the length of your right lower arm plus hand? (Measure from the tip of the bone at your elbow to the tip of your middle finger, in inches, with fractions expressed as decimals.)
10. How long is your right hand? (Measure the palm side, from the crease at your wrist to the tip of your middle finger, in inches, with fractions expressed as decimals.)
11. How long is your right foot? (Measure yourself barefoot, from the back of your heel to your longest toe, in inches, with fractions expressed as decimals.)
12. What is the circumference of your waist? (Measure all the way around your abdomen, at the level of your navel, standing up straight with muscles relaxed, in inches, with fractions expressed as decimals.)

Learning Objectives

The goal of this lab is to **resolve Darwin's dilemma by understanding how the meiotic process explains Mendelian patterns of inheritance**. By the end of the lab, students should be able to:

- Explain the relationship between chromosomes and chromatids
- Identify homologous chromosomes and state what is similar and what is different about them
- Define ploidy and haploid number, and identify haploid and diploid cells
- Demonstrate the stages of meiosis
- Describe crossing over and predict its consequences for the genetic make-up of daughter cells
- Describe how chromosome movements during meiosis explain Mendel's principles of segregation and independent assortment

I. Introduction to mitosis and meiosis

Biologists like to point out that mutation is the ultimate source of genetic variation in populations. By this they mean that mutation is the only process that can create *new* genes and alleles.

However, mutation occurs at a slow rate. In fact, if you compared your genetic make-up with that of a sibling or any other human, relatively few of the genetic differences that you observe would be due to new mutations. Why, then, are the individuals in most populations so diverse in their genetic make-up? For example, why do you and your siblings have different alleles for the genes involved in traits like height, hair color, and aspects of personality?

The answer is that meiosis creates genetic diversity by continually reshuffling existing alleles into new *combinations*. You and your siblings are unlike genetically because you inherited different combinations of alleles from your mother and father. The purpose of this lab is to explore how meiosis creates genetic variation and why mitosis and meiosis produce fundamentally different types of daughter cells. The issue is important: genetic variation is the raw material of evolution.

II. Mitosis: Growth, Repair, and Asexual Reproduction

Mitosis is a type of nuclear division that, barring mutation, yields two genetically identical daughter cells. It is important in many biological processes:

1) Growth of a multicellular organism from one cell, a fertilized egg. Multiple rounds of mitosis produce an individual with perhaps billions or trillions of cells, all with the same genetic material.
2) Wound repair, in which cells adjacent to the damaged area produce replacement cells of the same type.
3) Asexual reproduction. In single-celled organisms, each daughter cell is a new individual. In multicellular species, a few reproductive cells produce new individuals by mitosis.

Some organisms can reproduce both sexually and asexually. For example, populations of *Daphnia*, small freshwater crustaceans abundant in Lake Washington and other bodies of water around the world, are made up almost entirely of females during the spring and summer. These females reproduce asexually, forming diploid eggs by mitosis. The eggs develop in a brood pouch, are released when the female molts (sheds her exoskeleton), and eventually develop into adult *Daphnia* that are genetically identical to the parent. In the photograph, the dark roundish structures are diploid eggs.

We will not teach mitosis in lab, but a knowledge of mitosis is an important foundation for understanding meiosis. Be sure to study textbook figures 12.4 and 12.5 and the accompanying text before coming to lab. Don't worry too much about the nuclear envelope, spindle, or centrioles; instead, focus on the chromosomes: How many are present in the original cell? Is that cell haploid or diploid? When does replication occur? Which chromosomes move to each daughter cell, a random subset or a particular combination? How many chromosomes are present in each daughter cell? Is each daughter cell haploid or diploid? Are those daughter cells genetically the same, or different; why?

Exercise 1: Sexual reproduction in *Daphnia*

After reproducing asexually during the spring and summer, *Daphnia* begin reproducing sexually in the autumn. In response to short daylengths, food shortage, and changes in water quality produced by high population densities, some *Daphnia* females begin producing male offspring by mitosis. Meiosis in these males leads to the production of haploid **sperm**. In females, meiosis produces haploid **eggs**. When sperm fertilize eggs, the resulting diploid cells develop a tough case and settle to the bottom of the lake or pond. In spring these cells develop into diploid females that begin reproducing asexually.

QUESTION: In terms of genetic make-up, are sexually produced offspring like their parents or unlike them?

To answer this question, perform the following steps:

1. The tray on your lab bench contains pipe cleaner "chromosomes." You have three types of chromosomes, short, medium, and long. There are white and green versions of each length; one set of chromosomes (white) came from one parent, the other set (green) came from the other parent.

2. Draw a circle on your lab bench with chalk, to represent a nucleus about to undergo meiosis.

3. Make a diploid nucleus–one that has two versions of each type of chromosome, one white and one green. Note that for the purposes of this exercise, we'll suppose that diploid cells in *Daphnia* have six chromosomes ($n = 3$; $2n = 6$).

4. Use the pipe cleaners to model the steps in meiosis described below. When you are ready, you and your lab partner should demonstrate your mastery of the steps to an instructor and have him/her initial "Meiosis" on the sign off sheet at the end of this lab description. Your TA will collect that page at the end of lab.

Summary – Phases of Meiosis I

Interphase: Chromosomes replicate, so each chromosome consists of a pair of sister chromatids.

Prophase I: Homologous chromosomes synapse (come together) to form pairs. Crossing over occurs. The nuclear envelope breaks down. (Erase it on the bench top.) The spindle apparatus forms and attaches to the chromosomes.

Metaphase I: Spindle fibers have pulled synapsed pairs of homologous chromosomes to the metaphase plate.

Anaphase I: Homologous chromosomes separate and are pulled toward opposite poles of the cell. Sister chromatids remain attached. (The chromosomes are still replicated.)

Telophase I: The chromosomes finish moving to opposite poles.

Cytokinesis: Once meiosis I is complete, the cell divides along its midline to form two daughter cells.

Phases of Meiosis II

(NOTE: The two daughter cells from Meiosis I undergo Meiosis II in exactly the same way.)

Prophase II: A new spindle apparatus forms, a spindle fiber attaches to each chromosome, and chromosomes begin to move toward the metaphase plate.

Metaphase II: Chromosomes are lined up on the metaphase plate.

Anaphase II: Sister chromatids separate to form individual chromosomes; spindle fibers pull the chromosomes toward opposite poles of the cell.

Telophase II: Chromosomes finish migrating to opposite poles of the cell and nuclear envelopes form.

Cytokinesis: Once meiosis II is complete, the cell divides along its midline to form two daughter cells, resulting in a total of four daughter cells from the original single cell.

Exercise 2: Linkage, Crossing over and Recombination

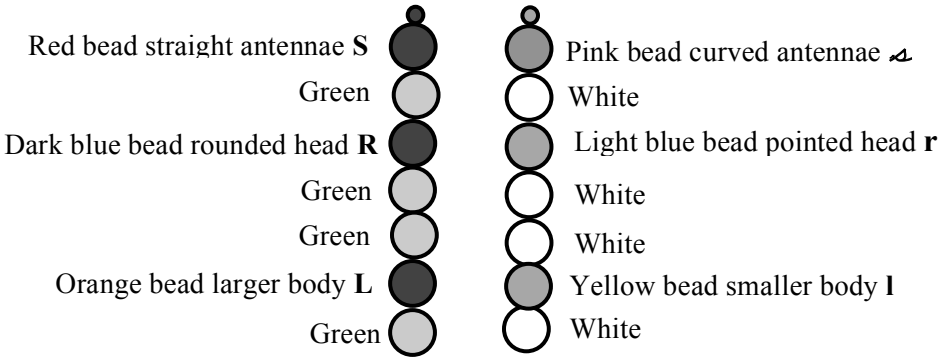
QUESTION: How does crossing over, the exchange of segments of chromatids in a pair of homologous chromosomes, affect allele combinations?

To answer this question, we will "zoom in" on one pair of homologs to model crossing over. (The other two pairs of homologs are still there, we just aren't describing them.)

To begin, suppose that genes that influence antenna shape, head shape and body size are all found on the same chromosome. Genes on the same chromosome are said to be **linked** to each other; genes on different chromosomes are **unlinked**.

For this exercise, we'll use pop beads of different colors to represent the two alleles of each gene. Assume that the alleles for straight antennae, rounded head and larger body are dominant to the alleles for curved antennae, pointed head and smaller body. Darker beads represent the dominant alleles.

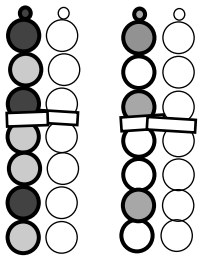
1. Start by assembling two homologous chromosomes of seven beads each as shown in the drawing. The green and white beads represent additional genes not being considered here.



When writing genotypes, we use a special notation to indicate linkage. For example, the genotype of the chromosome on the left would be written SRL; the genotype of the chromosome on the right would be written ⊿rl. If a cell contained *two* copies of the chromosome on the left, the genotype of that cell would be written $\frac{\text{SRL}}{\text{SRL}}$ (or SRL/SRL). In this notation, the line separates the homologs.

Write the genotype of the individual with the chromosomes shown above:

2. Next, replicate each homolog, using the appropriate bead colors. Assume that the centromere is just below the head shape gene; to simulate a centromere, wrap a twist-tie loosely around sister chromotids as shown. Ignoring crossing over for the moment, write the genotypes of the gametes produced when this cell undergoes meiosis:



3. Now suppose that during prophase of meiosis I, a single crossing-over event involving non-sister chromatids occurs somewhere between the locus for head shape (R or r) and the locus for body size (L or l). To simulate this event, first align the homologs in synapsis. Then separate the beads at the appropriate locations on two non-sister chromatids, switch segments, and re-attach. Finally, model the remaining steps of meiosis and examine the resulting four bead chromosomes. Notice that two of the chromosomes have the same allele combinations as those we started with; those are the **parentals**.

   The chromosomes with new combinations are called **recombinant**. Draw those chromosomes below and write the genotype of each one.

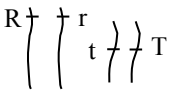
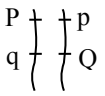
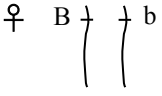
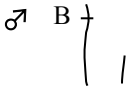
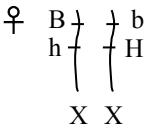
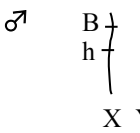
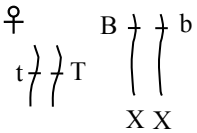
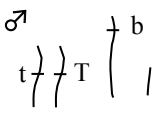
4. When you have completed the above steps, you and your lab partner will demonstrate crossing over for your TA or other instructor. They will initial the sign off sheet.

Biology 180 Handy Genetics Guide

The many possible combinations of dominance, linkage (genes on the same chromosome) and sex-linkage (genes on a sex chromosome) can get confusing. Use the chart below to sort it all out.

Suggestions: Be consistent with notation. In Biology 180, we use uppercase letters (**A**) for a dominant allele, lowercase (**a**) for recessive. To avoid confusion for both yourself and readers of your work, use upper- and lower case characters that *differ by more than just size*!

Example: **S♂** is better than **Ss**. Group the alleles for each trait (e.g. **S♂Tt**, not **ST♂t**) and list them alphabetically unless you know the order in which they occur.

For this combination …	… the chromosomes would look something like this:	Use this notation for a diploid individual …	… and this for the (haploid) gametes[1]:
Autosomal, one trait	One pair of homologs, each with one allele: 	**Rr**	**R, r**
Autosomal, two unlinked traits	Two pairs of homologs, each pair with alleles for one trait: 	**RrTt**	**RT**, **Rt**, **rT**, **rt**
Autosomal, two linked traits	One pair of homologs, each chromosome with alleles for *both* traits: 	Can be written any of four ways; all mean the same thing. The line(s) separate the homologs. **Pq/pQ** **Pq** **pQ** **Pq//pQ** **Pq** **pQ**	**Pq**, **pQ** Note that the underline notation indicating linkage should be used for gametes, too.
X-linked[2], one trait	♀  ♂ 	♀: X^BX^b ♂: X^BY	♀: X^B, X^b ♂: X^B, **Y**
X-linked[2], two traits	♀  ♂ 	♀: $X^{Bh}X^{bH}$ ♂: $X^{Bh}Y$	♀: X^{Bh}, X^{bH} ♂: X^{Bh}, **Y**
X-linked[2] *and* autosomal traits in the same cross	♀  ♂ 	♀: TtX^BX^b ♂: TtX^bY	♀: TX^B, TX^b, tX^B, tX^b ♂: TX^b, tX^b, **TY**, **tY**

Notes:

[1] The gametes shown assume no recombination between linked alleles.

[2] In most cases, only the X chromosome has alleles for the gene(s) of interest. Recall that ♀ = female, ♂ = male.

Now, what if there are *three* traits, or four, or five ….?

Meiosis and Crossing Over Sign Off Sheet

Student names: (1) _____ (2) _____

Meiosis _____
(TA initials)

Crossing Over _____
(TA initials)

Your TA or other instructor will initial above after you demonstrate each exercise. When both are initialed, give this sheet to your TA.