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## Using *Drosophila* to Teach Genetics

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The objectives of this unit are to take basic concepts of genetics and apply them to an organism, easily raised and observed in the classroom. It has been a challenge in the teaching of High School Biology, to take the abstract ideas of genes, chromosomes, heredity, and make them visible and tangible for the students. *Drosophila melanogaster* offers a way for teachers to help students make connections between populations, the organism, the cell, the chromosome, the gene, and the DNA. As a part of a unit on genetics, this unit on *Drosophila* can give students the opportunity to get to know an organism well, observing closely its development and physical characteristics, and then questioning how it is that the fly came to be this way.

### Unit Format:

- I. Discussion of *Drosophila*
  - 1. Review of genetics concepts
  - 2. Thomas Hunt Morgan and the historical frame
  - 3. *Drosophila* chromosomes, characteristics, and developmental stages
  - 4. Mutations
  - 5. Homeobox genes
  - 6. Homologs in other vertebrates
  - 7. Making a map
  - 8. DNA Sequencing
- II. Activities:
  - 1. Observations of the individual flies,
  - 2. Doing genetic crosses, and predicting offspring outcomes in the Lab,
  - 3. Salivary gland extraction, and staining of chromosomes
  - 4. Creating a genetic map
  - 5. DNA sequencing simulation,
  - 6. Virtual Fly Lab.
- III. Appendix
- IV. Bibliography and resources

Chromosomes are the structures that contain the hereditary material of an organism. During mitosis (the division of the nuclear material prior to cell division) the chromosomes shorten and thicken, and become visible under the light microscope. Sections of the chromosomes which code for individual traits are genes, (although many traits are coded for by several genes., and some genes may have more than one function at different times). Deoxyribonucleic acid, DNA, is the double stranded master molecule which provides the cell directions for assembling structural and functional proteins. Chromosomes occur in pairs, which in most animals stay separate except during meiosis. Meiosis is the cell division that allows gametes (sex cells) to have the haploid (half normal) number of chromosomes. In the salivary gland chromosomes of fruit flies however, the two homologous chromosomes of a pair do not ever separate. Sometimes genes are not passed on in the correct form. These mutated, or changed genes produce differences that caught the interest of Thomas Hunt Morgan.

Thomas Hunt Morgan (1866-1945), was the first to observe the white eye mutation in *Drosophila* in 1910. The wild type normal eye color is red. What caused this mutation? And how was this change transmitted? During the decade prior to this, *Drosophila* had been used in experiments such as fly reactions to light and gravity, and effects of rearing conditions on fecundity (amount of eggs produced), but not yet for genetic study. In his 1925 book, *The Genetics of Drosophila*1 , Morgan raised questions: "All of the heritable characters about which we have definite information at present are referable to the genes, [but] whether genes are active all the time or whether their activity is correlated with certain phases of development we do not know at present...[And] whether the gene is a chemical molecule or a collection of molecules is guess work at present." It was not until the 1950's that it was clear that the hereditary material of the chromosomes was DNA, and what the structure of this molecule entailed. The double helix model of DNA won James Watson and Francis Crick the Nobel Prize in 1962.

It is interesting for students of science to know that scientific understanding is cumulative, with present discoveries and understanding built upon the work of past scientists. It may also give science students working with *Drosophila* in their classrooms a smile to read Morgan's statement from *Genetics of Drosophila*, "It may be estimated that at least 13,000,000 flies have been etherized and examined by competent observers.",... and to know that the numbers are much higher now, and that *they* can count themselves among those observers.

*Drosophila melanogaster* , or the red-eyed pomace fly, is classified in the family Drosophilidae, and order Diptera ( which also includes flies, mosquitoes and midges.) Scientists study simple model systems in hopes of understanding principles that can apply to complex systems. *Drosophila* has been such a model organism for several reasons. They are small, easy to raise in the Lab, have a short life-cycle, have only 4 pair of chromosomes, and contain large polytene chromosomes. Polytene chromosomes, found in the salivary glands of organisms in the order, Diptera, are actually 1,000's of copies of each chromosome lined up in register. Areas of dark and light bands contain various concentrations of DNA and protein in the chromatin, and can be seen under a light microscope at a magnification of 450X. (Salivary glands are important for flies in the production of material to make the pupal case; this may account for the giant size polytene chromosomes.) Each band has been assigned a number.

Some *Drosophila* genes have homologs (corresponding genes with similar structure and functions), in other animals, even vertebrates. Some vertebrate genes when introduced into flies, do the job of the homologous fly gene. It seems that there are more similarities of design among organisms than there are differences. This universality helps Biologists understand the complexities of living things.

Some background Biology of *Drosophila* : *Drosophila melanogaster* was first recorded in New York City in 1875, and in New Haven in 1879, introduced again and again in fruit shipments from the south. They do not probably hibernate in the Northern cold, except perhaps in fruit stores. They are found near ripe fruit. In the wild, adults and larvae feed on yeasts and bacteria growing on rotting fruit. In the Lab they can be fed on the yeast cells growing on a high carbohydrate prepared diet.

The average life span of *Drosophila* depends on the environmental conditions. There are records of 80-153 days, but the average life span of a Lab fly is 26 days for a female, and 33 days for a male. (Under crowded conditions this may be reduced to 12 days. Also mutant flies generally have a shorter life span.) Temperature greatly effects the rate of development. At room temperature (25 oC): 10 days from egg to adult; at 20 oC: 13 days; at 15 oC: 90 days. There are four phases to the life cycle: egg / 3 larval (instar)stages/pupa/adult.

Courtship begins by the male tapping the abdomen of the female with his foreleg. This is his means of identifying his own species. He approaches the female fly from the front, and circles around her making half turns. He sticks a wing out, vibrating it for several seconds. If the female is receptive, copulation results. Sperm from the male are deposited in the female's uterus, and held there, in the seminal receptacle, and spermathecae. There may be more than one mating. Eggs are .5 mm long, and fertilized eggs are laid generally after the third day of the female's adult life. Initially in embryogenesis, there are 13 nuclear divisions in a single cytoplasm. Then these nuclei become separated into cells by membranes, to form a blastoderm of 6,000 identical cells around a large yolk mass.

Larvae hatch out in 22 hours, and grow and feed for four days, (longer at lower temperatures). The larvae are transparent, and one can see the inside organs such as the coiled intestines, whitish fat bodies, and gonads (visible in the male but very small in the female). During the third instar stage of larval development, observers of *Drosophila* will notice the larvae crawling up the sides of the culture container in preparation for pupation. At this time one can see dark projections called pupal horns, off the anterior end. These are the spiracles (outside opening of the respiratory tubes) turned inside out. (It is important to notice this when doing the salivary gland extraction, because this procedure is best done during the third instar, but before the appearance of pupal horns.)

The pupal case forms, darkens and hardens. Sexing of the pupae can be done by looking for tarsal sex combs of the male, visible from the ventral side. The sex combs in the adult males, are tufts of dark hair found on the most proximal tarsal joint of the foreleg, used for holding the female during mating. Even in the pupal phase, these dark patches are visible in males.

The pupal stage lasts for 4 - 6 days, during which time metamorphosis occurs. Larval tissues are broken down (except for the brain and a few other tissues), and imaginal discs (pockets of cells stored in the larvae) develop into adult organs. There is a disc for each leg, wing, eye, antennae etc. The understanding of how genes act to control the development of imaginal discs, has gotten clearer with molecular cloning.

How is the embryo given the information to tell how to build body parts in the right position? Mother flies deposit into eggs, the genetic information to direct the embryo which way is up (dorsal), down (ventral), front (anterior), and back (posterior). The sperm do not carry directions for embryonic development. Embryo cells which at first are structurally the same, begin expressing different combinations of genes due to a system of regulatory genes. Mutations in these genes may produce larvae with strange changes, such as no head or thorax. It used to be thought that genes worked at only one stage of development, and had one specific developmental function, but it is now known that genes are reused at different times.

Finally the pupa is ready to eclose (emerge) into the adult stage. At first, before the cuticle has darkened and hardened, the newly emerged female adult looks pale and puffy. (When crossing flies of different genotypes, it is important to use virgin females, and this is one way to visually separate out virgins in a Lab population.) Adult *Drosophila* males and females can be easily distinguished. Males are smaller, with a rounded, blackened tip to their abdomen (posterior segment). Females have a pointed abdomen, with a pattern of even dark bands.

*Drosophila* Chromosomes: In *Drosophila melanogaster* there are 4 homologous pairs of chromosomes: 2 pairs of large autosomes, ( one slightly smaller than the other pair), 1 pair of very small autosomes, and a pair of sex chromosomes. Females normally have two X chromosomes; males have one X and one tiny Y chromosome. But sex is determined by the X to autosome ratio, not merely the presence or absence of the Y chromosome. (One of chromosome #2, #3, #4 = one set.) 2 X chromosomes to 2 sets of autosomes = 1:1 ratio = female. 1 X chromosome to 2 sets of autosomes = 1:2 ratio = male. For this reason, a fly with only one X and no Y chromosome ( XO) is a male. This male is sterile because it lacks a Y chromosome, however externally it appears normal. A XXY fly is a normal female.

The salivary gland chromosomes (dissected from the third instar larvae) are large and easily visible. When a chromosomal squash is made, (see activity #3), the chromosomes can be seen radiating out from a common center called the chromocenter, forming 5 arms. The whole *Drosophila* genome is divided into physical units of distance up to 102. The X chromosome makes up the units 1-20. The 2nd chromosome is divided into the left and right sides, 2L having units 21-40, and 2R units 41-60. The 3rd chromosome has units 61-80 on the left, (3L), and 81-100 on the 3R side. The 4th chromosome is very small with only a few genes, and has units 100-102.

The relative position of many *Drosophila* genes along the chromosomes have been identified and mapped out based on frequencies of recombination with neighboring genes. This is called a linkage or genetic map (see appendix).

### **Mutations:**

Thomas Hunt Morgan described 61 mutations in his 1925 book, *The Genetics of Drosophila* . Mutations are changes in the chromosomes, genes, or part thereof, resulting in a change in the characteristics of an organism. Mutations caused by large changes in chromosome arrangement, such as inversions and deletions, can be observed under the microscope in the banding of a chromosome squash, and the position of breakpoints noted. Such chromosomal rearrangements often affect more than one gene.

There may also be a missing or duplicated section of a specific gene of a DNA sequence, which can be located with DNA sequencing. A point mutation is a single base pair change. Mutations occur in nature spontaneously. They can also be initiated under Lab conditions with X-rays, gamma rays, and chemical mutagens such as ethylmethane sulfonate (EMS). Studying the phenotypes of flies bearing chromosomal rearrangements and point mutations can give information about gene function. In the decades following Morgan's first investigations, knowledge of *Drosophila* gene location and function has expanded. In Lindsley and Zimm's book, *The Genome of Drosophila melanogaster* 2 a.k.a. "The Red Book", some 4,000 genes and 9,000 chromosomal arrangements are described in detail. (As the librarian in the reserve section of Yale's Kline Biology library said while handing over volumes of books, "At least you can take comfort in the fact that *Drosophila* has been well studied." True. Wouldn't Morgan be amazed and delighted!)

Most mutations produce weaknesses as observed in the Lab, in such ways as body morphology, shortened life

span, sterility, or death. (Most mutated types would die off in a natural world, but have been maintained over many generations in Lab conditions.) Many genes have a phenotype only when both genes in the pair are affected. This would be a recessive mutation. Dominant mutations are denoted with capital letters, recessive are noted in small case letters. Examples of recessive lethal mutations are : wingless (wg) location: Chromosome 2 position 30.0, and congested (cgd) location: Chromosome 1 position 37.6 and 41.7 (causes the first instar larva to not be able to escape the egg case.) Examples of dominant mutations are: Curly Wings (Cy) location: Chromosome 2 position 6.1; Stubble Bristles (Sb) location: Chromosome 3 position 58.2. Some nonlethal recessive mutations include: purple eyes (pr) location: Chromosome 2 position 54.5, and ebony body (e) location: Chromosome 3 position 70.7. (For a more complete list of mutations, see Appendix.)

Sex-linked mutations are carried on the X chromosome (Chromosome 1). Examples of these are white eyes (w) position 1.5, miniature wings (m) position 36.1, cut wings (ct) position 20.0, and vermilion eyes position 33.0.

The work with *Drosophila*, which Thomas Hunt Morgan began at his Lab at Columbia University in 1909, set the stage for his discovery of sex linkage in fruit flies. The genetic cross activity in this unit is based on these experiments. He found that there is a mutation which affects eye color, changing it from the normal “wild type” red found in nature, to unpigmented and therefore white eyes. When a white-eyed male was crossed with a wild type female, all the offspring were red-eyed. This would show red-eyes to be dominant over white. When these red-eyed offspring were mated, the results followed a predictable 3:1 Mendelian ratio of red to white. But the interesting thing about these results were that all the white-eyed flies were males. Half of the males were red-eyed, and all of the females were red-eyed. Morgan realized that these results could be explained if the gene for red vs white eye color were located on the X chromosome. In this experiment the parent (P) females have two X chromosomes each with a dominant red-eyed gene (homozygous for this trait) Males have only one X, in this case carrying the white-eyed mutation, and no gene for eye color on the Y. (This is termed hemizygous because the male can only have one possible copy of a gene, since the Y carries none.) The first generation (F1) offspring would be females all be carrying a red-eyed gene, and would therefore be red-eyed. In the F2 generation, all females would be red-eyed because male gametes could only have the red-eyed gene. Males would have a 50/50 chance of being red or white-eyed because half of the female gametes carry a white-eyed gene.

A rather strange and interesting mutation is Antennapedia (Antp) located on chromosome 3 position 47.5. This mutation may cause death, but if the fly survives to adulthood, they may have an extra pair of legs where the antennae should be. In some cases wing tissue also develops in the eyes. Antennapedia mutations are a good lead in to the concept of the Homeobox.

### **Homeobox Genes:**

These are a series of genes that are mostly transcription regulators. In the 1920's a group of mutations called homeotic genes were first discovered. They change one structure into another, such as antennae into feet, with the Antp mutation, and a front part of the third thoracic segment transformed into an area of the second segment in the bithorax (bx) mutation. It was not until 10-20 years later in the 1940's at Cold Spring Harbor with the beginnings of molecular biology, that scientists began to understand the structure of these homeotic genes. This earlier work led to the sequencing and cloning of these genes in the 1970's. Through the years it has been found that homeotic mutations were not caused by one gene, but by a complex of several genes that are very close to each other on the chromosome (so much so that crossing over doesn't happen). The homeobox is a short stretch (180 nucleotides long) on the DNA sequence that is almost the same in each of these homeotic-complex genes. These homeotic genes seem to be in a linear order. When one gene is

mutated, the expression domain of the next gene is expanded, leading to changes in the ultimate fate of cells. For example, legs will grow where antennae should be. Humans have complexes of homeobox genes similar to the ones in fruit flies that regulate anterior posterior development of the central nervous system.

### Homologs in Humans and other Vertebrates:

Many vertebrates have genes that are homologs (have structural and functional likenesses) of *Drosophila* genes. Vertebrates and flies after all have common ancestors, and genes transmitted 500 million years ago would have had a similar code for directing the development of front and back, up and down. Some of the homologs are structural, for example the singed bristle gene (located on *Drosophila melanogaster* X chromosome, position 21.0) directs the cross linking of actin filaments, the same as in human muscle. Other homologs are functional, such as the daughterless gene (da), (location: chromosome 2, position 41.5). Flies that have this mutation have no daughters or few daughters. It codes for a transcription factor, and has a homolog in humans. Another functional homolog is the Notch gene (*Drosophila* location : chromosome 1-X, position 3.0), which is involved in signal pathways for cell communication in both flies and humans.

### Making a Chromosome Map:

During meiosis, each homologous pair of chromosomes becomes doubled, forming 2 sets of sister chromatids. A synapsed (joined) bundle of 4 chromatids is called a tetrad. During Prophase I, the first phase of meiosis, the strands of the tetrad sometimes twist around each other. Non-sister chromatids may exchange segments at this point. This is crossing-over. In *Drosophila* it is only the females in which crossing over may occur. (The reason for this is not yet known.)

Maps of relative positions, (the orders) of linked genes on a chromosome can be constructed by noting the frequencies of crossing-over between genes. The closer two genes are together, the less likely they will show crossing-over. Conversely, the greater the distance between two genes on a chromosome, the greater the chance that a cross-over will happen between them. The probability of crossing over can be expressed as a distance or value (the % of crossing-overs that occurs between 2 points on the chromosome. One map unit, (m.u.) is the distance between linked genes in the space where 1% of crossing-over occurs, or is the distance between genes for which one result of meiosis out of 100 is recombinant. (A map unit is sometimes called a centimorgan [cM] in honor of Thomas Hunt Morgan.) The position on the map where a gene is located is called the gene locus. On *Drosophila* chromosome 1 (X), for instance, the locus of the cross-veinless wings (cv) is 13.7. The locus of cut wings (ct) is 20.0, so the distance is 6.3 m.u. The relationship could be shown like this

cv ct

—|—————|

6.3

If the recombination frequency between cv and ct is 6.3, and ct and vermilion eyes (v) is 13, the order on the chromosome could either be cv-ct-v, or ct-cv-v. We can determine which of these is correct by measuring the recombination frequency between cv and v. If cv and v are found to recombine with a frequency of 19.3 %, then we deduce that ct is located between them.

### DNA Sequencing:

Recombinant DNA technology has made it possible to understand the specific nucleotide sequences of sections of a gene, whole genes, and in some cases, entire chromosomes. It was the discovery of restriction



enzymes which made it possible to cut DNA at specific points, to create reproducible fragments. Restriction enzymes are naturally produced by bacteria (and other microorganisms such as yeast). They have evolved as a defense mechanism which enables the bacterium to cut up the DNA of an invading viral particle or cell, while preserving their own DNA. Their names usually come from the first two letters of the species name of the bacteria in which they occur. There are now restriction enzymes which can be used to cut DNA at 150 or more specific sequence sites. Each restriction enzyme recognizes one sequence usually 4-6 base pairs long. For instance the restriction enzyme EcoR I(from *Escherichia coli* ) cuts DNA at the sequence:

G|A-A-T-T- C

C -T-T-A-A | G

Agarose gel electrophoresis is used to separate out the fragments of DNA cut by a restriction enzyme. In this process, an electrical field is used to move the negatively charged DNA through an agarose gel. The solution of sample DNA is injected into one or more wells at the top end of a gel. The gel is porous, and pieces of DNA which are the same base pair length, will move through the pores in the gel at the same rate along a lane. Bands are formed on the gel, with the smaller sized pieces (which move faster) at the far end of the gel lane, and larger pieces ( which move more slowly), closer to the start of the lane. Ethidium bromide, which fluoresces under UV light, can be used to stain the DNA for viewing of the gel.

One method of DNA sequencing developed by Fred Sanger in 1977, is the dideoxy chain termination method, (on which the DNA sequencing simulation activity (#5) is based.) The enzyme DNA polymerase normally uses triphosphate derivatives (dNTP's) to construct polynucleotides. The four dNTP's are: deoxyadenine triphosphate (dATP), deoxythymine triphosphate (dTTP), deoxycytosine triphosphate (dCTP), and deoxyguanine triphosphate (dGTP). When dideoxynucleotide triphosphates (ddNTPs) are added, those nucleotide analogs become a part of the nucleotide chain, which then terminates. In the Sanger procedure: 1) An unknown DNA sequence is introduced into a plasmid,( a loop of DNA found in yeasts and bacteria.) A short primer polynucleotide is added, which will hybridize (join with) the plasmid just at the beginning of the unknown sequence. DNA polymerase, is added which directs the making of a complimentary strand of the unknown sequence, and many copies will be made. Into this mixture is added all four dNTP's, (dATP, dTTP, dGTP, and dCTP), then is heated to denature (separate )the DNA, forming single-stranded DNA. A radioactive label is added. 2) This mixture is divided into 4 test tubes. To tube "A" will be added ddATP. To tube "T" will be added ddTTP. To tube "C" will be added ddCTP. To tube "G" will be added ddGTP. 3. A small amount of each tube is loaded into 4 separate wells of an acrylamide gel, and the fragments separated by electrophoresis. 4. The gel is exposed to X-ray film. The resulting autoradiogram shows a pattern of bands that gives the DNA sequence. The gel is read from bottom to top as shown below:

fragment

size longer A T C G

\*\*\*\* T

\*\*\*\* G

\*\*\*\*A

\*\*\*\* C

size shorter

## II. Student Activities:

1. Observation of *Drosophila* individuals

### **Introduction :**

A. What is *Drosophila* ? Where have you seen it before?

B. Why is *Drosophila* used for genetics?

### **Materials :**

*Drosophila* population, males and females

culture tubes with food at the bottom

breathable cotton material for tube stopper

ether (and eye dropper for transferring ether to sleep box)

sleep box

dissecting microscope or hand lens

soft, small paint brush for moving etherized flies

white paper (index card) on which to view the flies

### **Procedure :**

CAUTION! Fruit Flies FLY and will quickly do so if given the chance.

Work with care so that you do not end up with more flies around the classroom than in your tubes!

#### *A. Putting the flies to sleep:*

1. Put a couple drops of ether on the sponge part at the bottom of the plastic 'sleep box'. (Note: A Q-tip swab soaked in ether can also be inserted into the tube with flies instead of using the sleep boxes as described below.) BEWARE: too much ether will KILL your flies.

2. Open plastic sleep box

3. Tap tube on desk to get flies to the bottom.

4. Invert tube.

5. Remove cotton tube plug, and hit the bottom of the tube to get as many flies as possible into the sleep box. QUICKLY put the plastic top onto the sleep box, and put the cotton stopper back onto the tube.

6. WAIT until you see that the flies are not moving around.



7. Dump flies onto white surface for viewing. CAUTION! If your flies start waking up, place them in the sleep box, or put the sleep box over them *briefly* again.

B. Observations:

1. What do you see? What is the basic structure of these organisms? (For example number of legs, body segments, antennae, eyes etc.)

2. There are some easily identifiable differences between male and female *Drosophila* . Put your flies into two general groups, and describe the major differences. DRAW a male and female from what you see. (Consult with your teacher's drawing of the male and female to label sex of each.) Use the back of this paper for your drawings.

3. List and carefully describe, any differences you see between individual flies. (For example color, shape, size and placement of body structures.) What do you think accounts for these variations? Explain why each fly is not identical.

2. *Drosophila* Genetic Crosses:

## Set-Up : Part I

In this activity you will be setting up a genetic cross.

Your class will be divided up into teams A and teams B,( two different groups of parent phenotypes.) You will need to first separate out etherized males and females then red and white-eyed phenotypes of each sex.

### **Materials : (See activity 1 for other materials)**

Starting population of *Drosophila* , in two tubes: A:Heterozygous wild-type (red-eyed) females, and white-eyed males. (Capitol "R" denotes the dominant wild-type/red eye color gene, small "r", the white-eyed mutation. Remember: eye color is a sex-linked trait in *Drosophila*, carried on the X chromosome.) B. Heterozygous wild-type females and wild-type males.

New tubes (with food in the bottom) in which to put your mated flies, and raise the offspring.

TEAM A: Pick out 2 red-eyed females ( $X^R X^r$ )

4 white-eyed males ( $X^r Y$ )

Place them in the same new tube. Label the tube clearly: "A CROSS" and then add your initials.

TEAM B: Pick out 2 red-eyed females ( $X^R X^r$ )

4 red-eyed males ( $X^R Y$ )

Place them in the same tube. Label the tube clearly: B CROSS", and then add your initials.

ALL TEAMS: Using a Punnet Square to show your reasoning, give your hypothesis as to the phenotypic ratio of the offspring of the flies in both Team A and B's tubes. Will they be the same? Explain why/why not.

It takes about 10-14 days, depending on the temperature, for *Drosophila* to develop from egg to adult. Make several observations during the development of your selected flies' offspring. Include drawings, descriptions, and the date of each observation.

### ***Drosophila* -Cross Project Results : Part II**

**Note to teacher:** Raising *Drosophila* in the classroom is easy, and inexpensive. Ashburner's book, *Drosophila: A Laboratory Handbook*<sup>3</sup>, give detailed discussion of fly husbandry. Biological supply companies, such as Carolina, or Conn Valley also have both information, materials, growth medium/food, and fly cultures. It is wise to keep the cultures at room temperature, and to keep the culture vials or tubes humidified. ( A small, moistened piece of filter paper, or water at the bottom of the rack in which the culture tubes rest will do this job.) Subcultures can be made, but adults should be transferred to fresh medium every 20-30 days to avoid problems with mites and mold. Overcrowding can also affect the outcome of crosses, slowing developmental time because of competition for food and a place along the side of the tube to pupate. Virgin females are needed for most experiments. *Drosophila melanogaster* adults do not mate for about 10 hours after eclosion (emerging from the egg). So to collect virgins, one can tap out all of the adults from a population tube, then remove all of the females within 8 hours. Females can be held for 3-4 days until enough are collected for an experiment, but should not be too crowded (less than 50 in an 8-dram vial) Female fertility usually peaks between 4 and 10 days old. In the following activity, two sets of prepared cultures were used, with the phenotype of red vs white eye color to be studied.

#### *First Observations:*

1. Roughly estimate how many alive flies you see. Circle the closest answer:  
1-10 10-20 20-30 30-40 Many more
2. How many larvae (white "maggots") do you see?  
1-10 10-20 20-30 30-40 Many more
3. Has the food gone down? Describe how it has changed in form and amount.
4. If there are not many alive flies: What do you think could have gone wrong? Think about what could have gone wrong at each stage of the project. Write down one reason for each:  
Putting the flies to sleep:  
Picking and handling the flies:  
Storing and feeding the flies:

Time between setting up the cross and observing the flies today:

*Counting the Flies:*

1. Put your flies to sleep.
2. Look at the flies one at a time. Write down your observations on your tally table: number ( 1 through to the last fly you see), whether it's alive or dead, male (red or white-eyed) or female,( red or white-eyed). Add up the numbers from your columns.
3. Results: (Record your team's results first, then compile the entire classes results for teams A and B in a large data table.)

Team\_\_\_\_\_

a) How many of your flies were:

Female and red-eyed:\_\_\_\_\_ Female and white-eyed:\_\_\_\_\_

Male and red-eyed:\_\_\_\_\_ Male and white-eyed:\_\_\_\_\_

b)How are your results different from your hypothesis? List several possible reasons for this.

c) From the class data recorded on the large data table, calculate the ratio of each phenotype.

What are the advantages of several teams doing the same genotypic cross?

3. Salivary Gland Extraction, and Staining of Chromosomes:

**Note to teacher:** As described in the text section, the polytene chromosomes in the salivary glands of *Drosophila* are useful for chromosomal study. In the third and last larval stage, the larvae wanders up the side of the culture tube in preparation for pupation. The salivary glands have reached their maximum size, and are translucent white. The chromosome strands have attained their greatest width, and the characteristic banding can be seen. At this third instar stage the chromosomal strands stretch well in smear preparations. This Lab activity is perhaps best offered as an optional activity for small pairs of focused students. The dissection of the salivary glands requires much fine motor, and hand-eye coordination, using fine forceps and a dissecting microscope. One idea would be for selected students to prepare the glands and slides, then present their work to the entire class for viewing and discussion.

This is best viewed using a phase contrast microscope. The standard classroom brightfield microscopes can be used at 450X, although the chromosomes will be small, and banding patterns will not be easily seen.

**Chromosome Squash:**

**Materials**

## *Drosophila*

microscope slides, (depression and flat), and coverslips

fine forceps

isopropyl alcohol (for cleaning slide)

Saline solution

Silon wash (such as "Rain-X")

45% Acetic acid ( a fixative)

aceto-orcein stain

Kimwipes

### **Procedure**

1. Pick the largest third instar larvae you can find. Don't chose one in which you can see the pupal horns.
2. Drop the larvae on a depression slide onto which you have placed a few drops of saline solution. Leave it there briefly to wash the larvae.
3. Clean one slide and coverslip with alcohol, then rinse with silicon wash.
4. Prepare the slide and the coverslip with three drops of liquid arranged like this:

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X (Saline sol.)(aceto-orcein

---

Xstain on top

---

\_\_\_\_\_of cover slip)

---

\_ X ( Acetic acid)

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5. Take the larvae from the wash slide and drop it into the saline solution drop.
6. Examine the larvae under the dissecting scope. Find the dark mouth parts moving in and out at

the anterior end.(These structures need to be poking out for you to do the dissection; if your larvae is still, then gently nudge it.)

7.The idea is to use your forceps to grab the extended mouth part. Time your grab so that you've grabbed the mouth part when it is extended. Make sure that you only grab the mouth part and not any part of the body. (This takes time and patience and practice!) After you've grabbed the mouth part, hold the body about half way, and pull apart. The mouth part should pull cleanly from the body. The salivary glands are the symmetric extended bag-like things hanging off the mouth part.

8. Hold the salivary glands at the root near the mouth part,and gently move them to the drop of acetic acid. (You are not so much holding the glands as "floating" them between your forceps tips.)

9. The glands are now being fixed in the acetic acid. Usually 1 minute is enough. Do not fix them too long or they will become brittle. While the glands are fixing, try to remove the fatty bodies attached to the glands. (These are small strips of greyish opaque bodies hanging off the glands.)

10. Move the glands to the drop of orcein stain on the coverslip. Wait 30 seconds for staining.

11.Take a clean slide and gently place it over the coverslip with the drop of orcein stain. The coverslip will stick "up" to the slide because of atmospheric pressure. In this way, lift the cover slip off the slide on which you have been dissecting.

12. Turn the slide over so that the cover slip is up. Take a piece of Kimwipe and gently blot off any excess stain. Squeeze gently over the cover slip and blot at the edges.

13. Take a blunt instrument and tap on the cover slip starting from the center. Make your way out towards the sides tapping in a concentric circle. This action loosens the chromosomes.

14.Turn the slide over with the coverslip facing down, and place on a Kimwipe. Place your thumb on one edge halfway over the coverslip and press and roll your thumb at the same time. The idea is to create a flow of the stain from one side of the slide to the other.

15. Turn the slide over again so that the coverslip is facing up. Place a Kimwipe over the coverslip. Put your thumb squarly over the coverslip, (holding it in place with the other hand,) and press really hard. (This is the "squash" part of this Lab activity.) Blot off excess liquid. Observe under the microscope.

Draw and describe what you see.

#### *4. Creating a Genetic Map:*

A genetic map, which shows the order of and relative distances between genes on a chromosome, can be made by noting the frequency of crossing over between genes on sister chromatids. The unit of distance in a genetic map is called a map unit: one map unit is equal to one percent recombination.

In *Drosophila*, the genes for these recessive mutations are linked on the 1st or X chromosome: vermillion eyes (v), cross-veinless wings (cv), and cut wings (ct). In this activity, we will observe the phenotypes of offspring from a genetic cross (based on the experimental data of Thomas Hunt Morgan). The parents will have some normal, (wild-type) genes, (denoted with a "+"), and one or more mutations (denoted with a "v", "cv", or "ct"). Consider the following data for the percents of crossing over between the genes:

Genotype of parents: v + + (female) X + cv ct (male)

v + +Y

Genotypes of F1 generation: v + + (female) X v cv ct (male)

+ cv ct ( \* )

(\*Remember the male has only one X, and the Y doesn't contain these genes.)

Look at the genotypes of the offspring (listed in this table by the phenotypes shown), to see the amount of crossing over that has occurred.

Offspring Number of offspring with each

phenotype phenotype

v + + 580	(vermillion eyes)
+ c v ct 592	(cross-veinless and cut, but not vermillion)
v cv + 45	
+ + ct 40	
v cv ct 89	
+ + + 94	
v + ct 3	
+ cv + 5	

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1. What is the total number of individuals? \_\_\_\_\_ (\* Teacher note: 1448)
2. Calculate the % of offspring in which recombinations/cross-overs occurred that result in pairs of linked genes different than those in the parents. (% = # of each genotype/total number of individuals. ) Fill in these percentages below.

(\*Teacher note/answer key)

Recombinations that are v cv or + +:

(\*45 + 89 + 40 + 94

45 + 89 + \_\_\_ + \_\_\_ = \_\_\_ / \_\_\_ x 100 = \_\_\_ % = 268/1448 x 100 = 18.5%)

**Recombinations that are v ct or + +:**

(\*3 + 89 + 94 + 5

\_\_\_ + \_\_\_ + \_\_\_ + \_\_\_ = \_\_\_ / \_\_\_ x 100 = \_\_\_ % = 191/1448 x 100 = 13.2%)

**Recombinations that are cv + or + ct:**

(\*40 + 3 + 45 + 5 = 93/1448 x 100 = 6.4%)

\_\_\_ + \_\_\_ + \_\_\_ + \_\_\_ = \_\_\_ / \_\_\_ x 100 = \_\_\_ %

3. What is a map unit (m.u.) equal to?

4. How many m.u. are there between ct and cv? \_\_\_\_\_ (\*6.4)

ct and v? \_\_\_\_\_ (\*13) cv and v? \_\_\_\_\_ (\*18.5 Note: More replicate crosses and data would likely mean a m.u. closer to the actual 19.3 between cv and v.)

5. What is the sequence of these genes on the X chromosome? Make a sketch on the back of this paper.

6. One laboratory mouse costs about 20 cents a day to keep, and the gestation is about 3 weeks. What are the advantages, and disadvantages of using *Drosophila* vs mice in genetic studies?

What are the advantages of doing gene mapping from a three-point testcross (using three pairs of alleles in a chromosome) as we did in this activity, vs a single cross-over between two alleles?

(\*You can get the same information by doing a cross between two markers instead of three, but you'd have to do three sets of recombination-cross experiments instead of one.)

## 5. DNA Sequencing Simulation:

In this activity, you will be reading the sequence of *Drosophila* DNA separated by gel electrophoresis. This 36 base sequence is only a small part of the actual 1,000+ base pair code for the *Drosophila* muscle protein,



actin. Read the gel from the bottom to the top, and record the sequence of the bases at the right hand side. Use a ruler to be sure you're going line by line. The first 4 are done for you.

(figure available in print form)

Now, what are these bases coding for in the fruit fly? Remember, the DNA nucleotides in groups of three make up the triplet codons, (the universal code of protein-synthesis.). Each triplet codon codes for one out of 20 possible amino acids. The codon ATG is a DNA start codon. (In RNA the start codon would be AUG, because in RNA, Thymine is replaced with Uracil.) UAA, UGA, and UAG are stop codons, signaling the end of a particular protein molecule. (This activity is using the codons for a protein coded for by many more codons than shown here; the middle section was cut out to conserve space, leaving the start and end intact.)

1. On your list at the right, circle and label the start and stop codons.
2. Now list each of the triplet codons starting with the start codon, and ending with the stop.  
(\*Teacher answer key:  
ATG [start] TGT GAC GAA GAA GTT GCT GCT CTG GTT TGA [stop].)
3. Carefully consider the two charts below: The first shows the amino acids (denoted in abbreviations), for each codon. The second gives the full amino acid name with each abbreviation. Write the single (capital) letter for each of the amino acid in this *Drosophila* sequence.

(These are for RNA, so T is replaced with U)

(figure available in print form)

4. Following the identification of a sequence of DNA (which is transcribed into RNA and translated into protein), you may then identify the protein(s), and consequently the gene(s). To do this, it is common to compare these sequences to known sequences by computer. This information is accessible via the Internet at the database called "Gen Bank", at the National Institute of Health. Here is a table of four known sequences. Compare yours to these. What protein does your DNA sequence code for?

MHLLTFALLFS \_\_\_\_ (\*Teacher note: this is part of a rat neurotransmitter receptor)  
MGTMRLFLLAV \_\_\_\_ (\*...Frog neurotransmitter receptor)  
MCDEEVAALV \_\_\_\_ (\*... *Drosophila* actin)  
MKVASGSAAA \_\_\_\_ (\*...Mouse gene regulating protein)

## 6. Virtual Fly Lab:

### Note to Teacher:

With Virtual Fly Lab you can play the part of a research geneticist. It is

accessible through the Internet at the following address:

<http://vflylab.calstatela.edu/edesktop/VirtApps/VflyLab/IntroVflyLab.html>

The following introductory paragraph from the Virtual Fly Lab opening page, describes its goals:

“The philosophy behind the development of Virtual Fly Lab is to allow students to learn the principles of genetic inheritance through the design and analysis of genetic ‘experiments’. An exposure to the techniques of scientific reasoning is at least as important a goal as learning the genetic principles themselves. For this reason, Virtual Fly Lab provides no information regarding the nature of the genetic mutations; it is up to the students to discover these for themselves.”

For more information, you can contact Robert A. Descharnais:

Associate Professor Biology Phone: (213) 343-2056

Cal State LA Fax: (213) 343-2095

Los Angeles, CA 90032-8201

Email: [bobbio@next.calstatela.edu](mailto:bobbio@next.calstatela.edu)

Include: your name, your institution, your email address, class or course which you’re teaching, and how you’d like to use Virtual Fly Lab for instruction.

### To start your activity:

1. First you will pick two flies, choosing (from a menu,) mutations for them such as eye color, wing form, placement and type of bristles. (To simplify this process, assign a mutation to only one parent at a time, giving the other the normal “wild-type” [+], gene for that trait. Describe your parent flies and selected traits here:

female: \_\_\_\_\_

male: \_\_\_\_\_

2. Click the “Mate” button.

3. The computer will generate a picture of parents and offspring, and a list of the numbers of offspring which are carrying the selected traits. Record the results of your cross on the back of this paper.

4. Although recessive mutations are typically denoted by abbreviations with small case letter(s), Virtual Fly Lab capitalizes all the letters of abbreviations so that it’s up to you to determine (based on the ratios of the offspring) which traits are dominant or recessive.

5. What is your hypothesis about your cross results? Are your selected mutations dominant? recessive? sex-linked? lethal? How could you tell? Explain your reasoning on the back side of this

paper.

## Appendix

This is an abbreviated genetic map of *Drosophila melanogaster*, showing some of the genes that have been mapped to date. Dominant mutations are shown with capitalized symbols. Lethal mutations are marked with an \*.

1.5—white eyes, w	0.0 aristaless antennae, al	19.2—javelin bristles, jv	—grooveless scutellum, gvl
3.0—facet eyes, fa	1.3—Star eyes, S*	26.0—sepia eyes, se	—bent wings, bt
5.5—echinus eyes, ec	4.0—held-out wings, ho	26.5—hairy body, h	2.0—eyeless, ey
7.5—ruby eyes, rb	6.1—Curly wings, Cy*	35.5—eye gone, eyg	
13.7—crossveinless wings, cv	13.0—dumpy wings, dp	41.0—Dichaete wings/bristles, D*	
20.0—cut wings, ct	16.5—clot eyes, cl	44.0—scarlet eyes, st	
21.0—singd bristles, sn	21.9—spade wings, spd	47.5—Antennapedia, Antp*	
27.5—tan body, t	31.0—dachs tarsi, d	48.0—pink eyes, p	
33.0—vermillion eyes, v	41.5—daughterless, da	50.0—curled wings, cu	
36.1—miniature wings, m	48.5—black body, b	58.2—Stubble bristles, Sb*	
43.0—sable body, s	51.2—reduced bristles, rd	58.5—spineless, ss	
44.0—garnet eyes, g	54.5—purple eyes, pr	58.7—bithorax body, bx	
51.5—scalloped wings, sd	55.9—light eyes, lt	62.0—stripe body, sr	
56.7—forked bristles, f	57.5—cinnabar eyes, c	63.1—glass eyes, gl	
57.0—Bar eyes, B	67.0—vestigial wings, vg	66.2—Delta veins, Dl	
59.5—fused veins, fu	72.0—Lobe eyes, L	69.5—Hairless, H	
62.5—carnation eyes, car	75.5—Curved wings, C	70.7—ebony body, e	
66.0—bobbed bristles, bb	91.5—smooth abdomen, sm	74.7—cardinal eyes, cd	
	104.5—brown eyes, bw	88.0—mahogany eyes, mah	
	107.0—speck body, sp	106.2—Minute bristles, M	

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## Unit Synopsis:

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### *Using Drosophila to Teach Genetics*

This is a unit designed for High School Biology students. Sections of it may also be suitable for middle school students. The objectives are to take basic concepts of genetics and apply them to an organism easily raised and observed in the classroom. *Drosophila melanogaster* offers a way for teachers to help students make connections between populations, the organism, the cell, the chromosome, the gene, and the DNA. The activities in this unit follow this progression. First students carefully observe the organism, noting variations, and generating questions. Secondly they set up and perform genetic crosses, making predictions about traits of the offspring. During the 2 weeks or so of development from egg to adult, the fruit flies provide students with a means to witness and discuss insect metamorphosis. A protocol for salivary gland extraction, and staining of chromosomes is included that enables students to see the chromosomes that are responsible for the traits they have observed. There is an activity on making a genetic map based on gene cross-over data provided, and a DNA sequencing simulation based on *Drosophila* protein data from Gen Bank. Finally there is information on using the “Virtual Fly Lab” program available on the Internet. A genetic map of *Drosophila melanogaster*, and bibliography, and resource list is included.

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