Biology 164 Laboratory

Transmission Genetics: Inheritance of Mutant Traits in *Drosophila* Fruit Flies

**Introduction**

To reinforce your understanding of basic eukaryotic genetic principles, you will study the inheritance of mutant traits of the fruit fly, *Drosophila melanogaster*. *Drosophila* is a genus of small flies, widely used in genetic studies. This genus is included in the Phylum Arthropoda, Class Insecta, Order Diptera, and Family Drosophilidae. The normal chromosome complement of this species consists of three pairs of autosomes and a pair of sex chromosomes, XX for females and XY for males.

You will study transmission genetics using wild (normal) and mutant strains of flies. Stock cultures of these strains have been maintained in the lab as pure-breeding lines, meaning that, if the flies breed among themselves, the normal flies will produce only normal progeny and the mutant flies will produce only mutant progeny. After considering how the mutant flies differ from the normal (wild type) flies by visually identifying the mutant traits, the following three questions will be addressed:

1.) How many loci are responsible for the mutant characteristics?

2.) Are the genes for the mutant characteristics recessive or dominant to their alleles for wild type.

3.) Are the loci responsible for the expression of the traits part of an autosomal chromosome or the X chromosome. (Neither trait is associated with the Y chromosome.)

4.) If more than one locus is identified, are the loci linked or unlinked?

Your goal is to design a series of crosses that will allow you to answer the above questions in the shortest amount of time possible. In conjunction with these crosses you will make predictions about their expected outcomes based upon the different possibilities for the inheritance of the mutant traits.

When preparing your predictions consider what you would expect to be the distribution of each mutant trait among the progeny of the crosses you make if the allele responsible for the mutant trait is:

1.) autosomal and recessive?
2.) autosomal and dominant?
3.) X-linked and recessive?
4.) X-linked and dominant?

If multiple mutant traits are observed, you need not make predictions on all the possible combinations of outcomes. Simply base your predictions on one of the mutant traits.
A Guide to Using Drosophila for Genetic Studies

Derived in large part from:

A. Developmental Cycle

There are four phases in the life cycle of the fruit fly: egg, larva, pupa and adult. The entire developmental cycle from egg to adult takes about 14 days at 21°C (close to lab room temperature). At higher or lower temperatures, the cycle is proportionally shorter or longer.

Eggs are tiny, oval, white objects about 1 mm long with two long filaments at one end. Larvae hatch from the eggs about a day after they are laid. Larvae are white, segmented, worm-like burrowers with black mouthparts at their anterior ends and for tracheal breathing, a pair of spiracles (short tubes that project) at both their anterior and posterior ends. Larvae vary from about 1 mm in length when newly hatched to 6 or 7 mm when ready to pupate. Since their cuticles (skin) will not stretch, the larvae periodically molt (shed cuticle, mouthparts and spiracles) to reach adult larval size. There are two such molts during Drosophila development. Before and after molts, larvae are called instars; thus, the fruit fly has three instars. After the second molt, the third larval instars crawl from the medium onto the sides of the culture vessel and pupate. Metamorphosis occurs during the next several days. The pupae darken about a day before the emergence (eclosure) of the adult flies. At first, the adults are light in color and their wings are not expanded, but within a few hours, the wings expand and the flies darken.

Adult flies may mate 8-12 hours following eclosure, and females can start laying eggs two days after emerging. After reaching sexual maturity, fruit flies are fertile for the remainder of their lives. It is important to note that female fruit flies can store and use sperm from a single insemination for much, if not all, of their reproductive lives.

B. Morphological Characteristics

(Refer to illustrations found in the laboratory)

Adults are about 2 mm long. The body is divided into head, thorax, and abdomen. The head bears one pair of antennae that have a feathery distal part emerging from a heavy basal segment. Hemispherical compound eyes are set far apart on the head.

The thorax bears three pairs of legs, each having the following segments (beginning at the distal end): 1. a tarsal segment bearing two claws, 2) additional small tarsal segments, 3) a somewhat larger metatarsal segment (bearing a sex comb in the male), 4) a long lower leg segment or tibia, 5) a long upper leg segment or femur, 6) a tiny trochanter, and 7) a coxa (the proximal segment). The thorax bears one pair of wings and one pair of rudimentary wings (halteres). All wing veins are named and the general venation pattern should be noted.

The abdomen consists of a series of segments (apparently one or two more in the female than in the male). Dorsally, the abdominal plates are called tergites; ventrally they are called sternites.

The sex of the flies can be distinguished using several criteria. The male is smaller than the female. In dorsal view the male’s abdomen is more rounded at the posterior tip, and the banding pattern of the abdomen is less conspicuous at the tip, making it appear solid black. The male has a small black sex comb on the metatarsal segments of the first pair of legs. The
male genitalia have conspicuous, brown, forked claspers on the ventral surface of the tip of the abdomen. It is best to use the presence or absence of the sex comb as the primary criterion when sexing Drosophila.

Because their abdomen usually is filled with eggs, females are larger than the males. A dorsal view of the abdomen shows five or six clearly separate, dark bands. A tuft of bristles emerges from a conspicuous anal papilla located on the dorsal posterior tip of the abdomen. The female lacks sex combs.

C. Preparing Culture Medium

1. Culture Vessels and Medium
   a. Use sterile, foam plugged culture vessels (small milk bottles or glass vials). To avoid unwanted contaminants, keep the foam plugs in place except when you are placing something into the vessels.
   b. Add a portion of dry "Instant Drosophila Medium" to each each glass vial sufficient to fill vial 2 cm. from bottom.
   c. Add exactly 15 ml distilled water from dispenser to the vial. Keep the sides of the vessels above the medium dry and free of medium.
   d. When the medium has jelled, sprinkle 1 grain of dry yeast onto the surface of the medium.
   e. Using lab tape, label each culture vessel with appropriate information that will include date, your names, and phenotypes and gender of flies used for the cross.

D. Making Crosses

1. Since Drosophila melanogaster females can store and use sperm from one insemination for a large part of their reproductive lives, virgin females must be used in making crosses between different strains. To insure virginity, females must be collected before they are 8 hours old. This is done as follows:
   a. Remove (clear) all adult flies from a culture. Darkening of pupae is a good indication that adults will eclose soon. Since flies tend to eclose in greater numbers early in the day, clear adults from the culture as late in the evening or as early in the morning as practical.
   b. Collect, anesthetize and sex newly eclosed flies within 8 hours of culture clearing. The females can be presumed to be virgin.
2. Flies for Setting Up P1 Cross
   
a. You will be provided with plastic bottles of anesthetized flies. Handle flies very gently at all times, and avoid exposing them or the culture vial to heat from a desk lamp.

b. Working with only one phenotype at a time, transfer anesthetized flies to an index card that has been labeled with phenotype, and divided into female and male sections. Using a stereomicroscope, sort flies by gender into either section of the card. Presence of sex combs on the front legs of male flies is a sure indication of their sex; females do not have sex combs.

c. Have your instructor or TA confirm that you have sexed the flies properly. ONE FLY OF THE WRONG GENDER COULD RUIN YOUR CROSS!!

d. Obtain anesthetized males and females of the types required for the cross and place them on a dry wall of a culture vessel laid on its side. Use at least one healthy pair of flies per culture vessel. If sufficient flies are available for all lab groups, the cultures will do better if three females and two males are used. Keep the culture vessel on its side until the flies recover from anesthesia.

e. Cultures will be incubated between 20 and 25° C. in a heated insulated styrofoam box.

E. Collecting Data

1. About 7 days after starting cross, remove parents to prevent breeding between generations and to insure data collection from one generation only.

2. Data collection from an experimental cross is begun the day after the progeny first emerge. Usually flies are phenotyped and counted every other day for about 8 days to insure inclusion of mutants and the sex with slower developmental rates (females often appear sooner than males).

3. Anesthetize flies with FlyNap by bumping flies from their inverted culture vessel into an ABSOLUTELY DRY AND EMPTY vessel, inserting a FlyNap dipped wand into the dry, empty vessel and waiting until the flies are anesthetized- usually ~ 2 min. Since these flies will not be used for a subsequent cross, it is OK to heavily anesthetize them.

4. When the flies have stopped moving, remove the wand, and tap the vessel gently so that the flies drop to the bottom. If they cannot right themselves, they are adequately anesthetized.

5. Record phenotypes of flies and transfer data to chalkboard to be collated with other lab groups.

6. Once examined, flies should be discarded in the morgues provided.
F. Genetic Notation Used in Describing Crosses

A fly with red eyes and other normal traits is called wild type and is designated by a +. The + refers here to all the traits (the entire phenotype). However, a + can refer to an allele (locus).

A fly with a heritable trait different from wild type is considered a mutant. Mutations at particular loci are designated by letters derived from the descriptive name of the mutation. Abbreviations for recessive mutations are written entirely in lower case letters, whereas abbreviations for dominant mutations begin with capital letters.

During the initial stages of an inheritance study when the dominance relationships of alleles are unknown, the problem of deciding how to abbreviate the name for the mutation can be avoided by using a combination of letters and superscripts to designate a particular allele. For example, a mutant autosomal trait can be denoted as A\textsuperscript{m}, while its wildtype counterpart can be denoted as A\textsuperscript{+}. Similarly, an X-linked trait can be denoted as X\textsuperscript{m} for the mutant allele, and X\textsuperscript{+} for the wild type allele.

The symbols can be combined to designate the specific genotypes involved in a particular cross as shown below,

first for **autosomal** alleles:

\[
\begin{array}{ccc}
\text{Female parent} & \times & \text{Male parent} \\
P1 \text{ generation:} & A^m A^m & A^+ A^+ \\
\downarrow & & \\
F1 \text{ generation:} & A^m A^+ \text{ (all progeny have same genotype)},
\end{array}
\]

and secondly, for **X-linked** alleles:

\[
\begin{array}{ccc}
\text{Female parent} & \times & \text{Male parent} \\
P1 \text{ generation:} & X^m X^m & X^+ Y \\
\downarrow & & \\
F1 \text{ generation:} & X^m X^+ \text{ (female progeny genotype differs from } \text{male progeny genotype)}
\end{array}
\]

Note that because males only have only a single X chromosome they can never be heterozygous for an X-linked allele. What are the genetic and phenotypic consequences of this fact?