

INTRODUCTION TO *DROSOPHILA* GENETICS

DROSOPHILA CULTURE

We will study basic principles of Mendelian inheritance with the use of the fruit fly, *Drosophila melanogaster* [the name means “black-bodied fruit-lover”]. *Drosophila* was one of the first organisms to be studied genetically: its small size, short life cycle (10 ~14 days at 25°C), high reproductive rate (an adult female can lay 400-500 eggs in 10 days), and ease of culture and genetic manipulation have made it perhaps the best understood animal genetic system. Many different species, and a large number and wide variety of naturally-occurring and artificially-induced genetic variants are available. The partial genetic map in Appendix B describes the location of all the mutations used in crosses and lab questions.

VIRGIN FEMALES

All female flies used in controlled genetic crosses must be “virgins”. Female flies are capable of mating as early as 8 hours after emerging from the pupae stage and are **polyandrous**, that is, capable of mating with several males. Once mated, females can retain viable sperm for several days and this will confuse the results of a subsequent controlled mating. To prevent this, all adult flies are removed from the culture bottle about 7 hours prior to lab time, so that all newly hatched flies will remain virgin.

BASIC GENETICS

The **karyotype** of *Drosophila* comprises four pairs of chromosomes, of which three pairs are **autosomes** and one pair are **sex chromosomes**. Female *Drosophila* are **XX**, and males **XY**.

A **gene** is a heritable factor that controls the expression of some trait, which may be morphological, behavioural, molecular, etc. Each such gene occupies a specific physical **locus** (pl. **loci**) on a particular chromosome. Variant forms of these loci are termed **alleles**. *Gene*, *locus*, and *allele* are often used more or less interchangeably, and this can lead to confusion. *Gene* is the popular and most general term, and is most appropriate when the inherited basis of a trait is emphasized, e.g., a “gene” for eye colour. *Locus* is most appropriate when the physical nature or position of a gene, especially with respect to other genes, is emphasized, as for example in gene mapping and linkage studies. *Allele* is most appropriate when the particular form(s) of a gene found in any particular individual or chromosome is(are) emphasized: e.g., there are “brown” and “blue” alleles of the eye colour gene. It is therefore inaccurate to say, for example, “He has the gene for sickle-cell anemia,” and more accurate to say “He has two **HbS** alleles at the beta-globin locus on Chromosome 6.” We all have the “gene” for every genetic condition, some of us have the particular allele(s) that result in the condition being expressed. In the technical literature, “locus” and “allele” are probably more common than “gene”.

Drosophila, like most species we will deal with in this course, are **diploid**, with two sets of chromosomes and therefore two alleles at each autosomal locus. If both alleles are identical, the individual is a **homozygote** and is described as **homozygous**. If the alleles differ from each

other, the individual is a **heterozygote** and is described as **heterozygous**. If the gene occurs on a sex chromosome, females may be either homozygous or heterozygous, but a male fly with only one allele at a locus will be a **hemizygote** and would be described as **hemizygous**.

Drosophila of typical appearance are said to show the “**wild-type**” forms (**phenotypes**) of genetically-controlled traits for body colour, eye colour, wing shape, etc. Naturally-occurring or artificially-induced genetic variants (**mutations**) of the alleles that control these traits produce flies with different morphologies, according to the dominant or recessive nature of the alleles involved in the **genotype**. Such mutant alleles are designated by symbols that are typically abbreviations of the mutant name. For example, the typical body colour phenotype is grey. One mutant produces an **ebony** (shiny black) body colour. Because this allele is **recessive**, it is symbolized by a lower-case letter, **e**. The wild-type allele is symbolized by a “+” sign, used either alone (if there is no ambiguity) or in combination with the mutant allele symbol, in this case **e⁺**. Thus, the genotype of a wild-type homozygote would be designated **e⁺e⁺** (or ++), a mutant homozygote **ee**, and a heterozygote **e⁺e** or **e+** [Use of the term “wild-type” derives from an early assumption that most flies are homozygous for a ‘standard’, usually dominant, allele. As we will see, this is not the case, but the terminology is still used].

It is important to remember that not all mutants are recessive. A mutation that is **dominant** to the wild-type is symbolized by a capital letter. For example, the typical eye shape is round. One mutant produces a narrow “**bar eye**”: the allele is dominant, symbolized by a capital letter **B**, and the wild-type (round) eye is **B⁺**.

In cases such as the above example, the F₂ phenotype ratio of 3:1 indicates a case of **complete dominance**. That is, one allele completely masks the expression of the other (**recessive**) allele.

In cases of **incomplete dominance**, on the other hand, neither allele masks the other, and heterozygous individuals express new phenotypes that are intermediate between the homozygous parents. This may arise for example if the dominant homozygous phenotype results from the expression of a double-dose of gene product, and the heterozygous phenotype from a single dose. The F₂ phenotype ratio of 1:2:1 is characteristic. A non-*Drosophila* example of this is seen in red- and white-flowered snap dragons:

P ₁	RR (red)	x	rr (white)
F ₁	Rr (pink)		
F ₂	1 RR (red)	:	2 Rr (pink) : 1 rr (white)

When both alleles are expressed the effect is known as **codominance**. Heterozygous individuals express gene products from both alleles: unlike incomplete dominance, the phenotype need not be intermediate. This sort of interaction is seen in the **ABO** blood group system of humans. One allele controls the production of **A** antigen while the other controls the **B** antigen (a third allele **O** produces no antigen). Heterozygotes carrying the allele for antigen **A** and the allele for antigen **B** have blood type **AB** in which both proteins are present in equal quantities. The F₂ shows a ratio of 1:2:1, as in the case of incomplete dominance.

DIHYBRID CROSS

Dihybrid crosses involve manipulation and analysis of two traits controlled by pairs of alleles at different loci. For example, in the cross ebony body x vestigial wing

e is ebony body colour
e⁺ is wild-type body colour
vg is vestigial wing shape
vg⁺ is wild-type wing shape:

where the loci for ebony body colour and vestigial wing are on separate autosomes. Therefore the genotypes and gametes are the same for male and female.

CROSS DIAGRAM

Autosomal Independent

P ₁	ebony body	x	vestigial wing
	ee vg⁺vg⁺		e⁺e⁺ vgvg
gametes	e vg⁺		e⁺vg
F ₁	e⁺e vg⁺vg (all wild-type)		

gametes $e^+vg^+, e^+vg, evg^+, e\ vg$ F₂ genotype combinations:

♀ \ ♂	e^+vg^+	e^+vg	$e\ vg^+$	$e\ vg$
e^+vg^+	$e^+e^+vg^+vg^+$	$e^+e^+vg^+vg$	$e^+e\ vg^+vg^+$	$e^+e\ vg^+vg$
e^+vg	$e^+e^+vg^+vg$	e^+e^+vgvg	$e^+e\ vg^+vg$	$e^+e\ vgvg$
evg^+	$e^+e\ vg^+vg^+$	$e^+e\ vg^+vg$	$ee\ vg^+vg^+$	$ee\ vg^+vg$
evg	$e^+e\ vg^+vg$	$e^+e\ vgvg$	$ee\ vg^+vg$	$ee\ vgvg$

F₂ Phenotype ratio: 9 wild-type: 3 ebony: 3 vestigial: 1 ebony vestigial

In a dihybrid cross, each of the F₁ parents can produce four different gamete types, so there are 16 (= 4 x 4) possible offspring combinations. Because the two traits show complete dominance and separate independently of each other (**Law of Independent Assortment**), the expected genotypic and phenotypic ratios from an analysis of these 16 possibilities can be calculated.

Phenotype	Genotype
(9:3:3:1)	(1:2:1:2:4:2:1:2:1)

These ratios can be derived from the results of a monohybrid ratio. A basic principle of probability theory is that the probability of two independent events occurring together is equal to the *product* of the two independent probabilities.

For example, the expected proportions of flies with wild-type and ebony body colours in a monohybrid cross are 3/4 and 1/4, respectively. Likewise, in a monohybrid cross involving vestigial wings, the proportions are 3/4 wild-type and 1/4 vestigial-winged. In a dihybrid cross, the proportions of flies with various combinations of *both* characters can be calculated as:

$$\begin{aligned}
 \text{wild-type \& wild-type} &= 3/4 \times 3/4 = 9/16. \\
 \text{ebony} &= 1/4 \times 3/4 = 3/16 \\
 \text{wild \& vestigial} &= 3/4 \times 1/4 = 3/16 \\
 \text{ebony \& vestigial} &= 1/4 \times 1/4 = 1/16
 \end{aligned}$$

This produces the familiar 9:3:3:1 ratio. In a similar manner, the expected genotype proportions

can be predicted because each monohybrid cross produces a 1:2:1 genotype ratio. The product $[1:2:1] \times [1:2:1] = [1:2:1:2:4:2:1:2:1]$ then gives the results of the dihybrid cross.

AUTOSOMAL LINKAGE

Mendel's work on peas was done before the discovery of chromosomes, and his **Law of Independent Assortment** postulated that each trait would segregate independently of every other. We know now that loci are arranged in linear fashion on chromosomes, and that loci that are physically close to each other will not segregate completely independently of each other. This phenomenon is called **genetic linkage**. Linkage may be **complete** (loci are so close that crossing-over rarely if ever occurs between them, and only the parental type gametes are produced) or **incomplete** (where crossing over occurs between the two loci and produces some recombinant type gametes).

[*Pisum* has seven pairs of chromosomes. Because Mendel worked on just seven characters, one of the leading urban myths of genetics is that he must have "cheated" to have found seven characters, each of which occurred on a different chromosome pair. In fact, we know now that his seven traits occur on just four chromosome pairs, and that only one of the 21 possible dihybrid crosses involves loci close enough to affect the expected 9:3:3:1 ratio for unlinked traits. His 1867 paper shows clearly that Mendel did not attempt to perform all 21 possible dihybrid crosses, and that the one anomalous cross was *not* one he performed. Mendel did *not* cheat].

The chance that a cross-over will occur between the loci depends on the genetic distance between them. Loci located far enough apart on the same chromosome act as though they are unlinked and produce equal proportions of parental and recombinant gametes.

When the loci in a dihybrid cross are linked, it is necessary to indicate clearly the specific allelic combinations that are present on the two chromosomes in each of the parents, because these alleles will tend to stay together and not assort independently. In the case of a double heterozygote, a chromosome in which the two linked loci show alternately the recessive and dominant alleles is called the *trans* (**repulsion**) arrangement (a^+b/ab^+). A chromosome in which the two linked loci show either both recessive or both dominant alleles is called the *cis* (**coupling**) arrangement (a^+b^+/ab).

The phenotypic expressions of *cis* and *trans* arrangements of heterozygous dihybrids are typically identical, but will produce different arrangements of alleles in their respective offspring.

Cis arrangement

P_1	st^+cu^+/st^+cu^+ (wild-type)	x	$st\ cu / st\ cu$ (scarlet eye, curled wing)
gametes	st^+cu^+		$st\ cu$
F_1	$st^+cu^+/st\ cu$ (all wild-type, <i>cis</i> arrangement)		
gametes	st^+cu^+ , $st\ cu$		
F_2	$1\ st^+cu^+/st^+cu^+ : 2\ st^+cu^+/st\ cu : 1\ st\ cu/st\ cu$ 3 wild-type : 1 scarlet curled		

Trans arrangement

P_1	st^+cu/st^+cu (curled wing)	x	$st\ cu^+ / st\ cu^+$ (scarlet eye)
gametes	st^+cu		$st\ cu^+$
F_1	$st^+cu/st\ cu^+$ (all wild-type, <i>trans</i> arrangement)		
gametes	st^+cu , $st\ cu^+$		
F_2	$1\ st^+cu/st^+cu : 2\ st^+cu/st\ cu^+ : 1\ st\ cu^+/st\ cu^+$ 1 curled : 2 wild-type : 1 scarlet		

The simplest mechanism for assessing linkage is a **test cross** (a mating in which one of the individuals is homozygous recessive for all traits considered). A non-linked dihybrid test cross will give a 1:1:1:1 ratio.

Linked Dihybrid test cross (*cis* arrangement)

	F_1 (from above) x homozygous recessive	
	$st^+cu^+ / st\ cu$ x $st\ cu / st\ cu$	
	wild-type	scarlet curled
gametes	st^+cu^+ , $st\ cu$	$st\ cu$
F_2	$1\ st^+cu^+/st\ cu : 1\ st\ cu / st\ cu$ 1 wild-type : 1 scarlet curled	

The expected result of a dihybrid test cross with completely linked loci is a 1:1 ratio.

SEX CHROMOSOMES

Sex-determination mechanisms vary among different organisms. In species such as humans and fruit flies, females are described as **homogametic** (**XX**: all gametes will carry the **X** chromosome) and males as **heterogametic** (**XY**: half the gametes carry the **X** and half the **Y** chromosome). We have made a distinction between the genes carried on the **X** and those carried on the **Y**. Since the law of segregation applies to sex chromosomes as well as to autosomes, it follows that genes on the **X** chromosome are passed on independently from genes on the **Y** chromosome.

As an example of an **X-linked cross**, we will look at **goggle-eye** (unusually prominent eyes), an X-linked recessive trait (**g**) in *Drosophila*:

P₁ **X⁺X⁺** (standard) × **X^gY** (goggle-eyed)

F₁ **X⁺X^g, X⁺Y** (standard)

F₂ **X⁺X⁺, X^gX⁺, X⁺Y, X^gY**

Ratio 2 standard ♀ : 1 standard ♂ : 1 goggle-eyed ♂

Reciprocal

P₁ **X^gX^g** (goggle-eyed) × **X⁺Y** (standard)

F₁ **X⁺X^g** (standard) **X^gY** (goggle-eyed)

F₂ **X^gX⁺, X^gX^g, X⁺Y, X^gY**

Ratio 1 standard ♀ : 1 goggle-eyed ♀ : 1 standard ♂ : 1 goggle-eyed ♂

The reciprocal cross shows an example of **criss-cross inheritance**, where the trait is passed from the mother to the sons, and can then appear in both male and female F₂s. If the P₁ female were homozygous dominant, as in the first instance, an allele of the gene can be present in the F₂ females, but it will be masked by a maternal dominant allele. The ratio will be similar to that of a monohybrid cross.

In humans, a small number of loci are known to be **Y-linked** or **holandric** (located on the **Y** chromosome). Such genes are expressed only in males. One such gene is a mutation that causes excess growth of hair on the outer ear.

In a sex-linked cross, the principles are similar but the notation differs. Instead of showing the alleles on the **X** or **Y** chromosome, simply use the symbol for the gene that is on the **X**, for example

w^+w^+ is a female red-eyed fly.
 $w \rightarrow$ is a hemizygous white-eyed male.

The (\rightarrow) denotes the **Y** chromosome, which in *Drosophila* carries only a few genes. Keep in mind that w^+ is completely dominant to w , and that this is a case of complete sex-linkage.

In crosses with X-linked loci in *Drosophila*, males or females of an unexpected phenotype occasionally appear in the F_2 . This happens when the two **X** chromosomes do not separate during oogenesis: the result is an egg with two **X**s and an egg with none. The failure of the **X** chromosomes to separate is known as **non-disjunction**. Fertilization with typical **X** or **Y** sperm gives **XXY**, **XXX**, and **XO**, **YO** offspring, respectively. **XXY** is a typical female; **XXX** and **YO** die, and **XO** is sterile.

EXERCISE 1 - HANDLING AND SEXING *DROSOPHILA*

FLY HANDLING

To set up a cross, you need to recognize traits and to distinguish between the sexes. Moving flies are very difficult to deal with: you must first anaesthetize them.. Often this is done with ether: because 30 or more students working with ether in a confined space produces an explosion (not to mention sleep) hazard, we will use instead cold treatment.

1. Obtain a bottle of flies. Each bottle is labelled with the symbol for the mutation.
2. Place the bottle of flies in the cooler for approximately 5 minutes. *Drosophila* are cold-sensitive and become immobile at low temperatures, the flies should show definite signs of sluggishness (inability to fly) after this period. Do not leave too long as the flies will become trapped in condensation that forms on the inside of the bottle.
3. Remove a frozen refrigerant pack from the freezer and place a petri dish on top of it. Remove the cover of the petri dish to avoid condensation in which the flies can drown. Use a paper towel to absorb any condensation that does form.
4. When the flies have become sluggish, remove the culture bottle plug and gently tap some flies into the petri dish. Do not tap the bottles so hard as to shake loose the culture medium, which can block the mouth of the culture bottle or squash the flies. Replace the cover of the petri dish and the culture bottle.
5. Place the petri dish and refrigerant pack on the stage of the dissecting microscope for observation. Use a small brush to sort the flies. As long as the flies remain in the dish on top of the refrigerant pack they will stay immobile. They should recover very quickly when the temperature rises.
6. When observations are complete, place flies back in the culture jar. If the flies are not needed by anyone else, discard them in the morgue.
7. Leave the stock culture bottles on the front bench.
Return the refrigerant packs to the freezer.

DISTINGUISHING SEX

If you were to set up specific crosses, you must first be able to distinguish between males and females. As well, sex-linked loci show different patterns of inheritance in males and females. Therefore, to determine how a genetic trait is inherited, it is necessary to record the sex of the flies in each phenotype.

Observe wild-type flies from the stock bottle marked "+". There are several morphological differences between male and female *Drosophila*. **Make sure you can distinguish males from females as you will have to do so when you do Investigation 1.**

The abdomen of the female has seven segments, several dark transverse stripes and is pointed at the tip. The abdomen of the male has only five segments, two dark stripes, and a more rounded, heavily pigmented tip (Fig. 1). In immature males the pigmentation may not be developed.

The most dependable characteristic is the external genitalia. Flip the fly on its back and look at the end of the abdomen. The (mature) male will show dark genitalia, while the female abdomen will be pale in colour and relatively smooth (Fig. 2).

Male flies have a secondary sex characteristic called a **sex comb**, which is a small tuft of about 10 black bristles at the front of the last large segment (#3 counting from the body) (Fig. 3). This is visible even in immature males.

Figure 1. Dorsal view of male (left) and female (right) *Drosophila*.
Note segments in male, & pointed abdomen in female

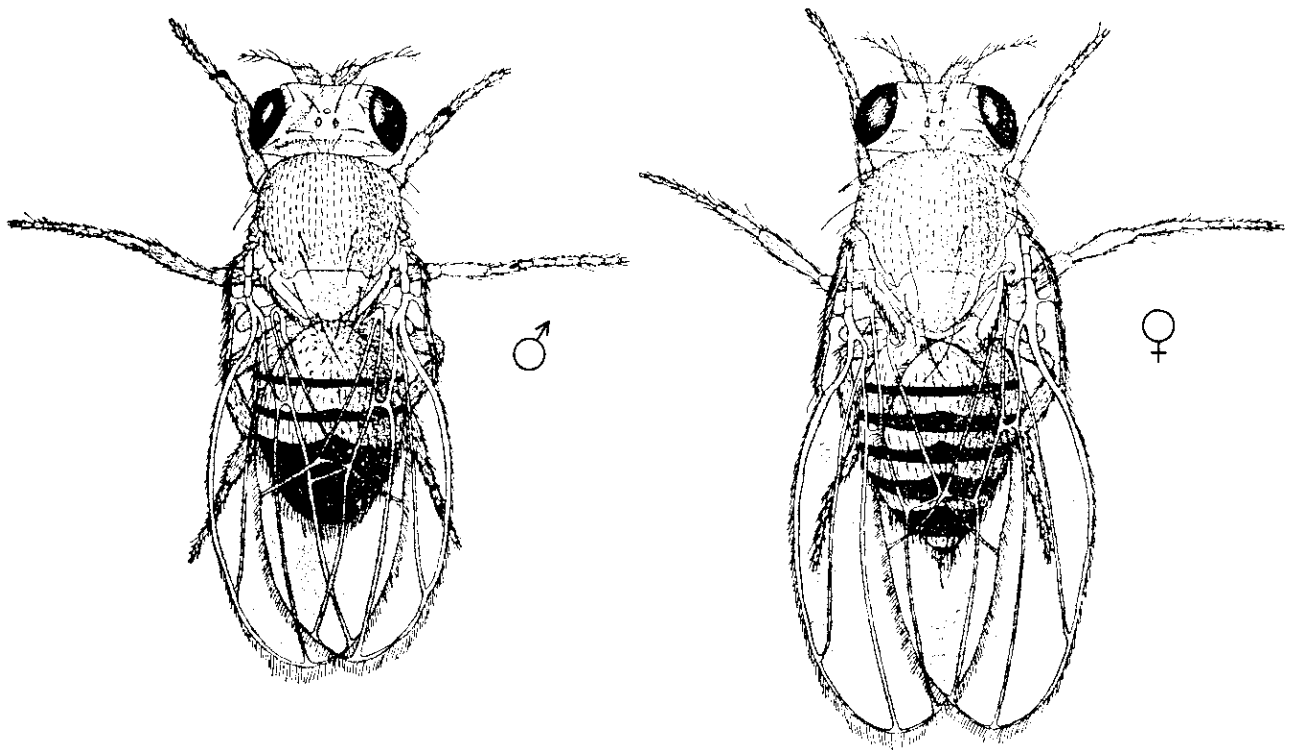


Figure 2. Ventral view of genitalia of male (left) and female (right) *Drosophila*

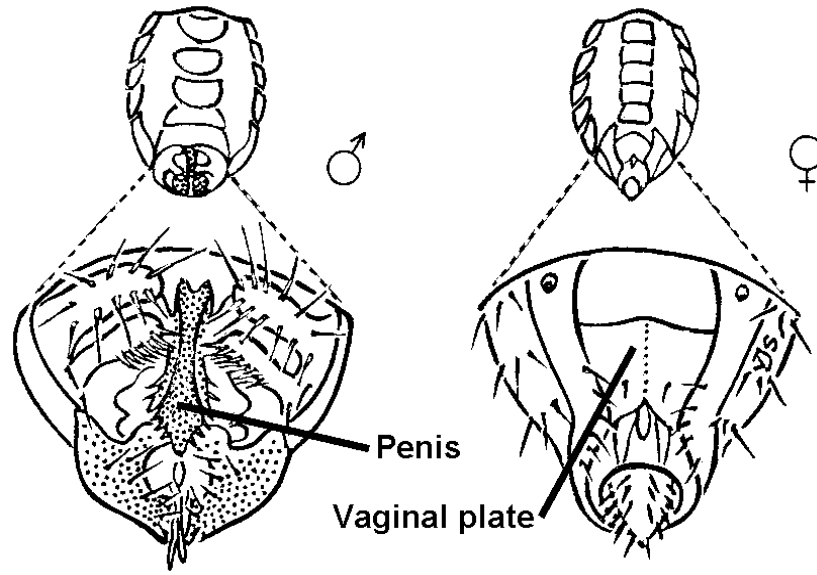
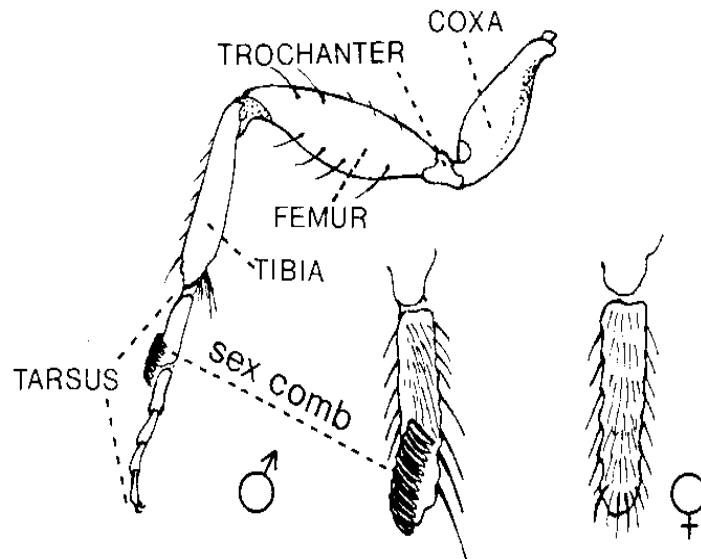


Figure 3. Left foreleg of male (left) and female (right) *Drosophila*. Note sex-comb on tarsus of male fly, absent in female. (drawn by W. Hewitt)



Redrawn with permission after M. Demerec, B. P. Kaufman, *Drosophila Guide*, Carnegie Institute of Washington, ©1967, 8th edition, p. 7.

EXERCISE 2 - OBSERVATION OF *DROSOPHILA* MUTANTS

To carry out the *Drosophila* crosses in Investigation 1, and to more fully appreciate *Drosophila* genetics generally, it is necessary to familiarize ourselves with the appearance of wild type flies, and some of the more common or interesting phenotypic mutant phenotypes. This exercise will allow you to examine some of these.

Observe as many of the following phenotype as time permits. Compare the morphology with that of a wild-type fly, shown on the next page. For any **one** of these, make an accurate biological drawing (choose from among those that have a ✓ beside them). **Refer to Appendix E for a review of how to do biological drawings.** Label clearly the main structures. Note the differences compared to wild type flies.

The drawing and answers to questions are due at the end of the lab period.

Gene Name	Description	Symbol	
<i>Scutoid</i>	bristles missing from the scutellum	Sco	
<i>cut</i>	end of wings “cut off”, possible antenna mutation	ct	✓
<i>crossveinless</i>	cross veins absent in wings	cv	✓
<i>garnet</i>	eye mutation, eyes have garnet colour	g	
<i>net^{ske}</i>	extra wing veins	net^{ske}	✓
<i>white</i>	eye mutation, white eyes	w	
<i>wingless</i>	no wings or tiny buds, no halteres	vg^w	✓
<i>Roughened eye</i>	eye mutation, rough eyes	Roe	
<i>Stubble</i>	hairs on the scutum are short and thick	Sb	
<i>ebony</i>	body colour is black or very dark	e	
<i>Ultrabithorax</i>	extra large halteres	Ubx	
<i>Bar</i>	eyes small slithers or kidney shaped when heterozygous	B	✓
<i>Lyra</i>	wings have the edges cut away	Ly	✓
<i>scute</i>	bristles missing from the scutellum	sc	✓
<i>Curly</i>	wings are curled at the ends	Cy	✓
<i>Humeral</i>	extra hairs on the “shoulders”	Hu	
<i>Lobe</i>	very small round or absent eyes	L	✓

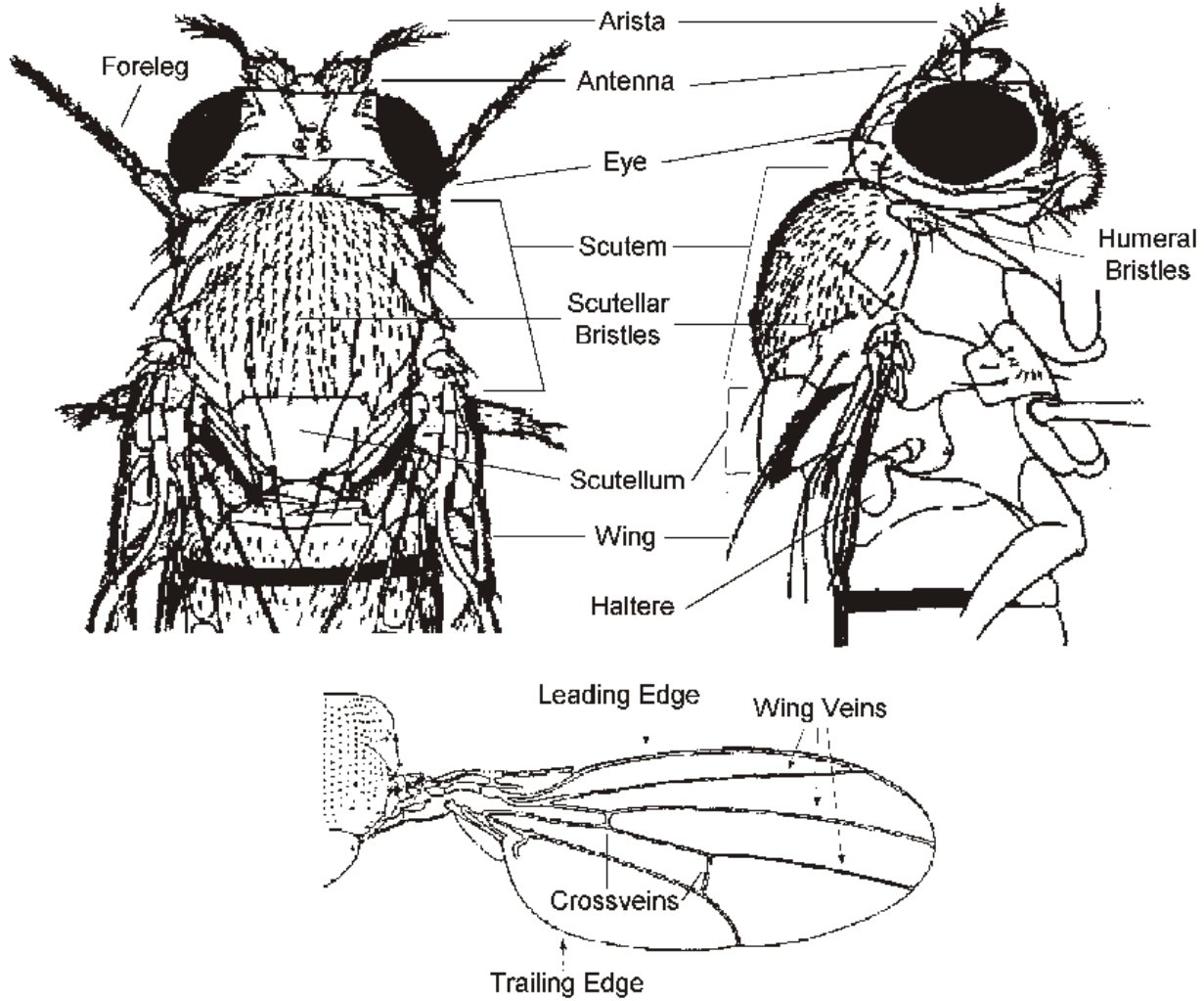


Figure 1: *Drosophila melanogaster* in dorsal, lateral, and wing views. Note morphological variation in features of the head, thorax, and wings in the various mutant strains available in the lab

Name _____
MUN # _____
Lab Slot _____

Exercise 2: Observation of *Drosophila* morphology

10.0 **1. On the reverse side of this page, make an accurate biological drawing** of any **one** of the listed phenotypes (use those that have a ✓ beside them). Label clearly any main structures. Note the differences compared to wild type flies.

2. Answer the following questions:

4.0 A. Why do some symbols start with small letters and other with capital letters?

4.0 B. Why do some of the symbols have extra superscript symbols?

2.0 C. If the gene for eye colour (called the white gene) is interrupted by the insertion of a transposable element (a mobile piece of DNA), this prevents the gene from being expressed and hence the flies eyes are white. However, under rare circumstances, these flies' eyes return to a red or orange (the normal colour). What do you think may have caused this?