

Biology 2250 Fall 2003

Laboratory Manual

Laboratory 1 (Sept. 15 – 18; Sept. 22 – 25)

INTRODUCTION TO BIOLOGY 2250 PRINCIPLES OF GENETICS

Biology 2250 (Principles of Genetics) is an introductory course in genetics and molecular biology that deals with the laws of Mendelian inheritance and their chromosomal and molecular basis.

The major laboratory investigations emphasize hands-on experience with basic genetic techniques, including construction and analysis of genetic crosses with several organisms, preparation and analysis of chromosome material, examination of protein structure by electrophoresis, calculation and analysis of patterns of DNA sequence variation, and construction of physical gene maps. Many of the exercises involve *Drosophila melanogaster*, the classic organism of experimental genetics.

The exercises have been selected to give you additional experience with the means by which the principles of genetics have been established, to reinforce the discussion of these topics in lecture, to acquaint you with modern biotechnology, and to help you appreciate the origins of the diversity of life on the planet.

The current edition represents is the latest in a series of revisions of a volume that has been developed, modified, and (hopefully) improved by several generations of lecturers, lab instructors, and, not least, students. We are in particular grateful to Ms. Sylvia Kao, who developed an earlier version of the manual, and to Dr. Brian Stavely and Ms. Anika Heywood for improvements to the *Drosophila* exercise and investigations.

This edition has been prepared in PDF format, to reduce costs and to make it more directly accessible to students. Blank pages have been retained, where answers are to written on one side only, or drawings submitted, or in order to start each new exercise or investigation on a facing page. The entire manual may thus be printed in two-sided format, to save paper.

We welcome your comments and suggestions on the format, presentation of ideas, and on the exercises and experiments themselves.

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September 2003

LAB SAFETY

1. Some of the experiments in laboratory present potential hazards with chemicals, electricity, and/or short-wave radiation. These are described in the lab manual and you will be alerted to the possibilities at the start of the relevant laboratory. *Read the laboratory instructions before coming to lab to anticipate these.*
2. WHMIS sheets on major chemicals used in lab are available (see Appendix C).
3. We *recommend* that you wear eyeglasses or safety goggles on lab days. Eyeglasses provide some degree of eye protection. Some people with contact lenses experience eye irritation from the chemicals used in the lab. Daily-wear soft contacts may absorb fumes permanently. Lab coats may also be advisable when stains/chemicals are being used.
4. Broken glass, used razor blades, and other sharp objects should be disposed of in the containers provided, **NOT** in the regular garbage. *Immediately* report any cuts, accidents, spills, etc. to the lab instructor.
5. If the fire alarm sounds: turn off all electrical equipment and proceed to the nearest fire exit. Do not return to the building until instructed to do so by the Fire Wardens.

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* consists of 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. [The only place you will find a “pair of genes” is the clothing store]. 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”.

Most species that we will deal with in this course, like *Drosophila*, 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 are different from each other, the individual is a **heterozygote** and is described as **heterozygous**. If

the gene occurs on a sex chromosome, a male fly 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 F_2 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 F_2 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 F_2 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 vgvg F_2 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] x [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 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 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 **Xs** 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 starting a cross, obtain a bottle of fresh culture media. Check that the media is firm, free of mold, and does not shake loose from the bottle when tapped gently.
7. When observations are complete, place any flies needed for a cross in the fresh culture jar and leave the jar on its side until the flies are moving about. If the flies are not needed by anyone else, discard them in the morgue. *Never return flies to the stock bottle!*
8. Label the culture bottles used for the crosses and place them in the appropriate part of the incubator, where they will be at a constant temperature of approximately 25°C.
9. Leave the stock culture bottles on the front bench.
Return the refrigerant packs to the freezer.

DISTINGUISHING SEX

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*.

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 darkened posterior segments in male, & pointed abdomen in female

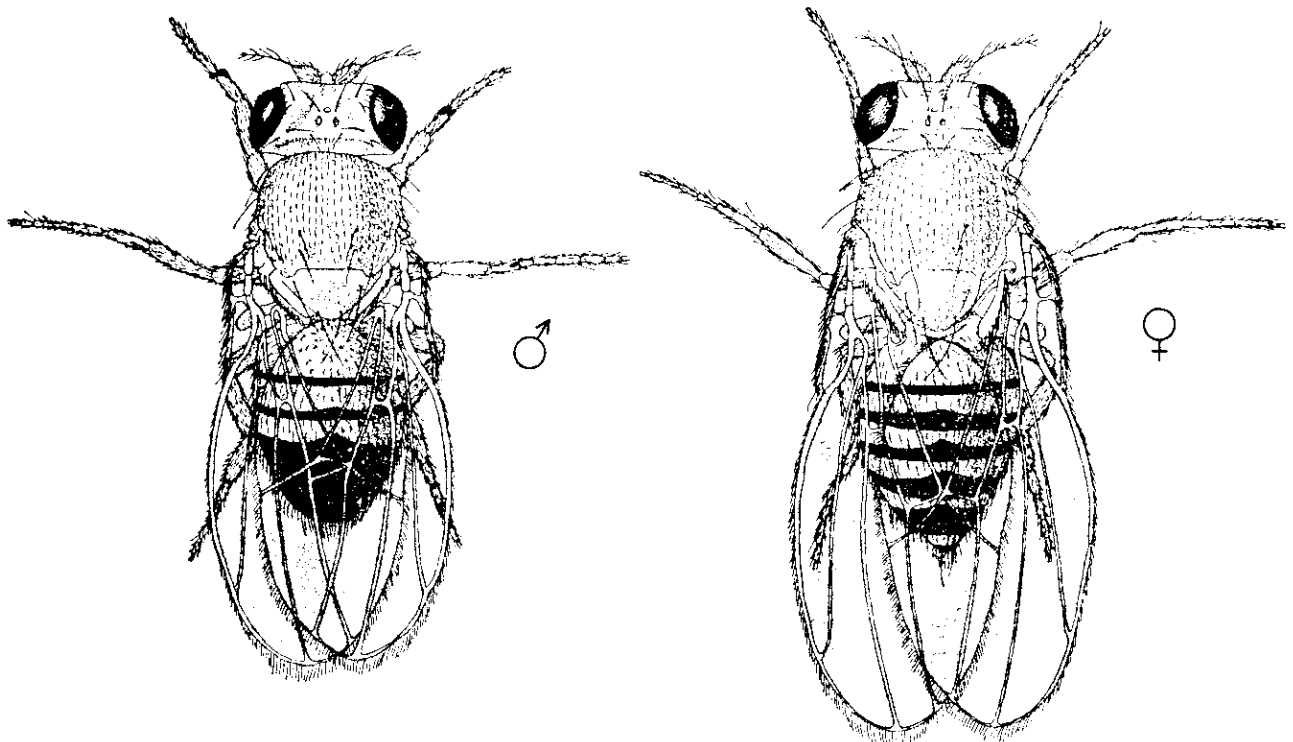


Figure 2. Ventral view of genitalia of male (left) and female (right) *Drosophila*

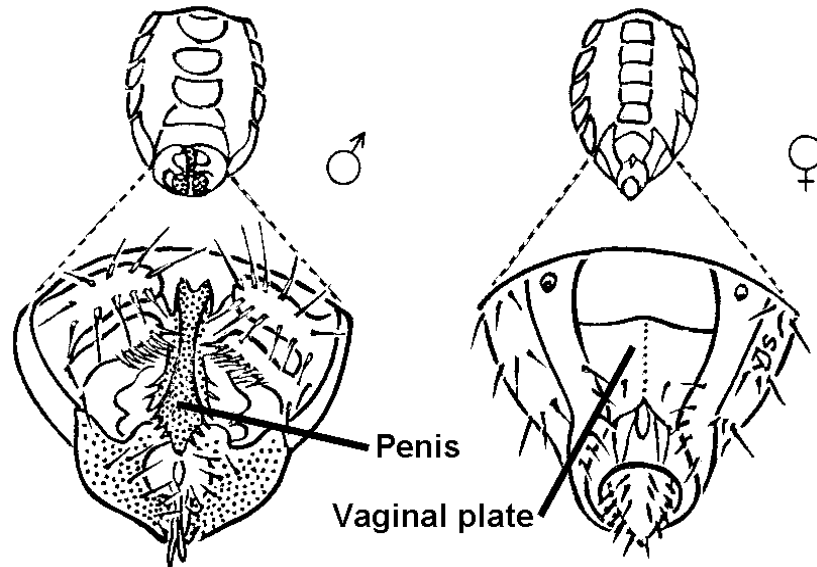
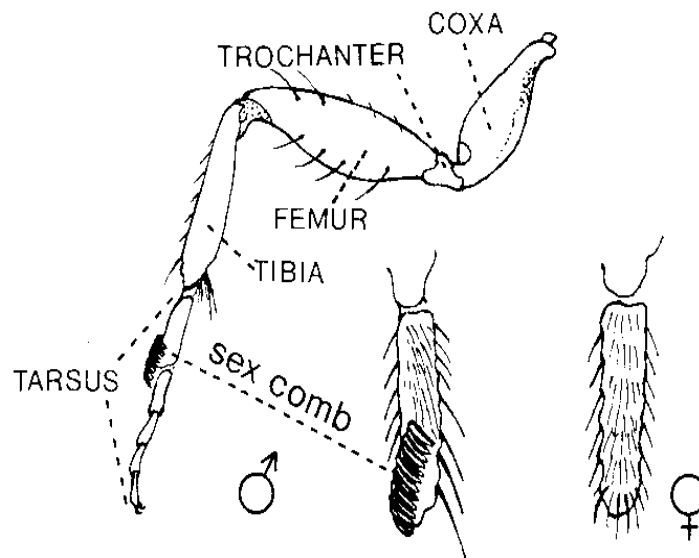


Figure 3. Left foreleg of male (left) and female (right) *Drosophila*. Note sex-comb on tarsus of male fly, absent in female. (drawn by W. Hewitt)



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

EXERCISE 2 - OBSERVATION OF <i>DROSOPHILA</i> MUTANTS
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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 overpage. For any **one** of these, make an accurate biological drawing (choose from among those that have a ✓ beside them). 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	✓

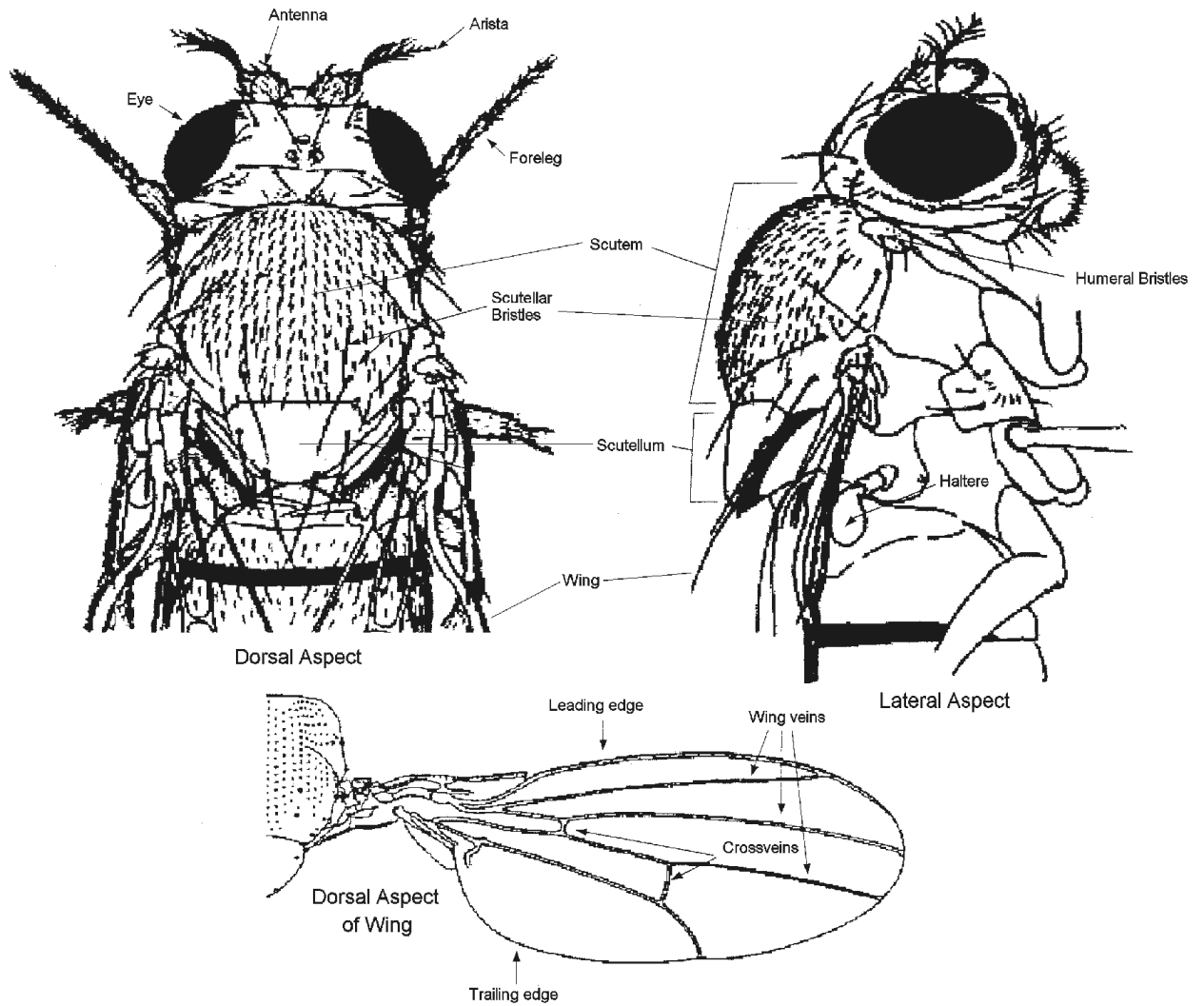


Figure 1: *Drosophila melanogaster* in dorsal, lateral, and wing views. Note morphological variation in features of the head, thorax, and wings in the various mutant strains available in the lab

Name _____
MUN # _____
Lab Slot _____

Exercise 2: Observation of *Drosophila* morphology

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 are red or orange (the normal colour). What do you think may have caused this?

EXERCISE 3 - VIRTUAL FLY LABORATORY

The Virtual Fly Laboratory uses a computer program that simulates crosses for 29 common morphological mutations (bristles, eye colour, body colour, wing size, etc.) based on the actual dominant / recessive inheritance patterns and linkage relationships known for these traits *Drosophila melanogaster*.

The principle advantages of a computer simulation for our purposes is that crosses and the resulting progeny can be produced in only a few seconds (instead of several days), and that large numbers of progeny can be produced in each cross, which increases the statistical reliability of experimental results. Further advantages are that computer simulations are not subject to escaped flies, attempts to mate flies of identical sex, misidentification of mutations, and do not require preparation of malodorous media, or any of the other joys that afflict experiments with living organisms. The Virtual Fly Website can be found at

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

For each cross, follow the instructions on the computer to select the phenotypes of the parents. Record phenotypes and results in the tables provided.

1. Click on the box ***Design a Cross Between Two Flies***

Select the appropriate mutation(s) for your cross. All other mutations are automatically set as homozygous wild-type for both parents. Note that the program does not use the standard notation for *Drosophila* mutants: you are expected to interpret the dominance or recessiveness of particular variants from the crosses. **Use the correct notation (lower & upper case, and "+") to describe your results.** (See Appendix A).

2. Click on the box ***Mate Designed Flies***

You will be given the F₁ results of the cross: record the phenotypes of the F₁ males and females.

3. To produce the second generation click on the circles below the diagrams of the F₁ offspring, then click on the box ***Mate Selected Flies***

You will be given the results of the F₂ generation: record them in the table provided. If any calculations are required, they should be done before performing another cross. If you repeat a cross, the computer will give you a new set of data.

Use the ***BACK*** icon to return to the beginning of the program to perform another cross.

Virtual Fly
Part 1
Due at end of lab period

Name _____

Student ID _____

Lab Slot _____

1 - MONOHYBRID CROSS - AUTOSOMAL
PURPLE EYE x WILD-TYPE EYE

PARENTS PHENOTYPE	CROSS A		CROSS B	
	MALE	FEMALE	MALE	FEMALE
	PURPLE EYE	WILD-TYPE EYE	WILD-TYPE EYE	PURPLE EYE
F ₁ PHENOTYPE				
F ₂ PHENOTYPE	# MALES	# FEMALES	# MALES	# FEMALES
WILD-TYPE EYE				
PURPLE EYE				

Observed F₂ Phenotype Ratio (round to one decimal place):

Cross A _____ Wild-type: _____ Purple

Cross B _____ Wild-type: _____ Purple

QUESTIONS

- Which of the alleles of this gene for eye colour is dominant? _____
- Are the results of a cross involving an autosomal trait the same for reciprocal crosses? _____
- Is there any difference between results for males and results for females -
in the F₁? _____
in the F₂? _____
- Does it make any difference to the F₂ generation if the mutant allele is carried by the male or female parent? _____

5. Refer to Appendix B and C for the correct way to write genotypes and using the **correct symbols** for the mutants involved complete the cross diagram to determine the EXPECTED F₂ PHENOTYPE RATIOS

PARENT PHENOTYPE	CROSS A		CROSS B	
	MALE	FEMALE	MALE	FEMALE
	PURPLE EYE	WILD EYE	WILD EYE	PURPLE EYE
P ₁ GENOTYPE				
P ₁ GAMETES				
F ₁ PHENOTYPE				
F ₁ GENOTYPE				
F ₁ GAMETES				

PUNNETT SQUARES using the F₁ gametes

♀ \ ♂	CROSS A			♀ \ ♂	CROSS B	

EXPECTED F₂ PHENOTYPE RATIOS
(don't forget to write the phenotype with the number ratio)

2 - MONOHYBRID CROSS - SEX-LINKED
WHITE EYE x WILD-TYPE EYE

PARENTS PHENOTYPE	CROSS A		CROSS B	
	MALE	FEMALE	MALE	FEMALE
	WHITE EYE	WILD-TYPE EYE	WILD- TYPE EYE	WHITE EYE
F ₁ PHENOTYPE				
F ₂ PHENOTYPE	# MALES	# FEMALES	# MALES	# FEMALES
WILD-TYPE EYE				
WHITE EYE				

Observed F₂ Phenotype Ratio (round to one decimal place):

Cross A: _____ wild-type male: _____ wild-type female: _____ white female: _____ white male

Cross B: _____ wild-type male: _____ wild-type female: _____ white female: _____ white male

QUESTIONS

- Which of the alleles of this gene for eye colour is dominant? _____
- Are the results of a cross involving a sex-linked trait the same for reciprocal crosses? _____
- Is there any difference between results for males and results for females -
in the F₁? _____
in the F₂? _____
- Does it make any difference to the F₂ generation if the mutant allele is carried by the male or female parent? _____
- Look for the mutants purple eye and white eye on the gene map.
Which chromosome is the white gene on? _____
Which chromosome is the purple gene on? _____

6. Refer to Appendix B and C for the correct way to write genotypes and using the **correct symbols** for the mutants involved complete the cross diagram to determine the EXPECTED F₂ PHENOTYPE RATIOS

PARENT PHENOTYPE	CROSS A		CROSS B	
	MALE	FEMALE	MALE	FEMALE
	WHITE EYE	WILD EYE	WILD EYE	WHITE EYE
P ₁ GENOTYPE				
P ₁ GAMETES				
F ₁ PHENOTYPE				
F ₁ GENOTYPE				
F ₁ GAMETES				

PUNNETT SQUARES using the F₁ gametes

♀ \ ♂	CROSS A			♀ \ ♂	CROSS B	

EXPECTED F₂ PHENOTYPE RATIOS

(don't forget to write the phenotype with the number ratio and include sex)

3 - DIHYBRID CROSS - AUTOSOMAL INDEPENDENT
EBONY BODY x VESTIGIAL WING

PARENTS PHENOTYPE	CROSS A		CROSS B	
	MALE	FEMALE	MALE	FEMALE
	EBONY BODY	VESTIGIAL WING	VESTIGIAL WING	EBONY BODY
F ₁ Phenotypes				
F ₂ Phenotypes	# MALES	# FEMALES	# MALES	# FEMALES
WILD TYPE				
EBONY				
VESTIGIAL				
EBONY & VESTIGIAL				

Observed F₂ Phenotype Ratio (round to one decimal place) excluding sex:

Cross A ____ wild-type: ____ ebony: ____ vestigial: ____ ebony & vestigial

Cross B ____ wild-type: ____ ebony: ____ vestigial: ____ ebony & vestigial

4 - DIHYBRID CROSS - AUTOSOMAL LINKED (trans)
PURPLE EYE x VESTIGIAL WING

PARENTS PHENOTYPE	CROSS A		CROSS B	
	MALE	FEMALE	MALE	FEMALE
	PURPLE EYE	VESTIGIAL WING	VESTIGIAL WING	PURPLE EYE
F ₁ Phenotypes				
F ₂ Phenotypes	# MALES	# FEMALES	# MALES	# FEMALES
WILD-TYPE				
PURPLE				
VESTIGIAL				
PURPLE & VESTIGIAL				

F₂ Phenotype Ratio excluding sex.

Cross A _____ wild-type: _____ purple: _____ vestigial: _____ purple & vestigial

Cross B _____ wild-type: _____ purple: _____ vestigial: _____ purple & vestigial

QUESTIONS

- Look at the F_1 results of crosses 3 and 4. For each of the three genes involved state whether the mutant allele of that gene is dominant or recessive.
 Ebony gene and the mutant allele is _____
 Purple gene and the mutant allele is _____
 Vestigial gene and the mutant allele is _____
- Can you tell from the F_1 results if two autosomal loci are on separate chromosomes or linked on the same chromosome?
 Cross 3: _____
 Cross 4: _____
- Is there a difference in the phenotypes from the males and females of the F_2 generation?
 Cross 3: _____
 Cross 4: _____
- For an autosomal trait (*e.g.* vestigial wing) does it make any difference in F_2 results which parent carries the mutant allele? _____
- Can you tell from the F_2 results if two autosomal loci are on separate chromosomes or linked on the same chromosome? _____
 How? _____

- Find the locus for each of these genes on the gene map

Gene	Chromosome #	Position	Notation
Ebony	_____	_____	_____
Purple	_____	_____	_____
Vestigial	_____	_____	_____
- Did the results obtained in the above crosses correspond to the linkage shown by the gene map? _____
- Did the dominant/recessive alleles correspond with the notation given on the gene map?

- Use the information from questions 1-3 and both Appendix B and C to determine the correct genotypes and complete the cross diagrams below to find the EXPECTED PHENOTYPE RATIO for each cross.

3 - DIHYBRID CROSS - AUTOSOMAL INDEPENDENT

PARENT PHENOTYPE	EBONY BODY	VESTIGIAL WING
P GENOTYPE		
P GAMETES		
F ₁ GENOTYPE [♂ & ♀]		
F ₁ PHENOTYPE		
F ₁ GAMETES		

PUNNETT SQUARE

♀ \ ♂				

Expected F₂ Phenotype Ratio excluding sex:

4 - DIHYBRID CROSS - AUTOSOMAL LINKED

PARENT PHENOTYPE	PURPLE EYE	VESTIGIAL WING
P GENOTYPE		
P GAMETES		
F ₁ GENOTYPE[♂ & ♀]		
F ₁ PHENOTYPE		
F ₁ GAMETES		

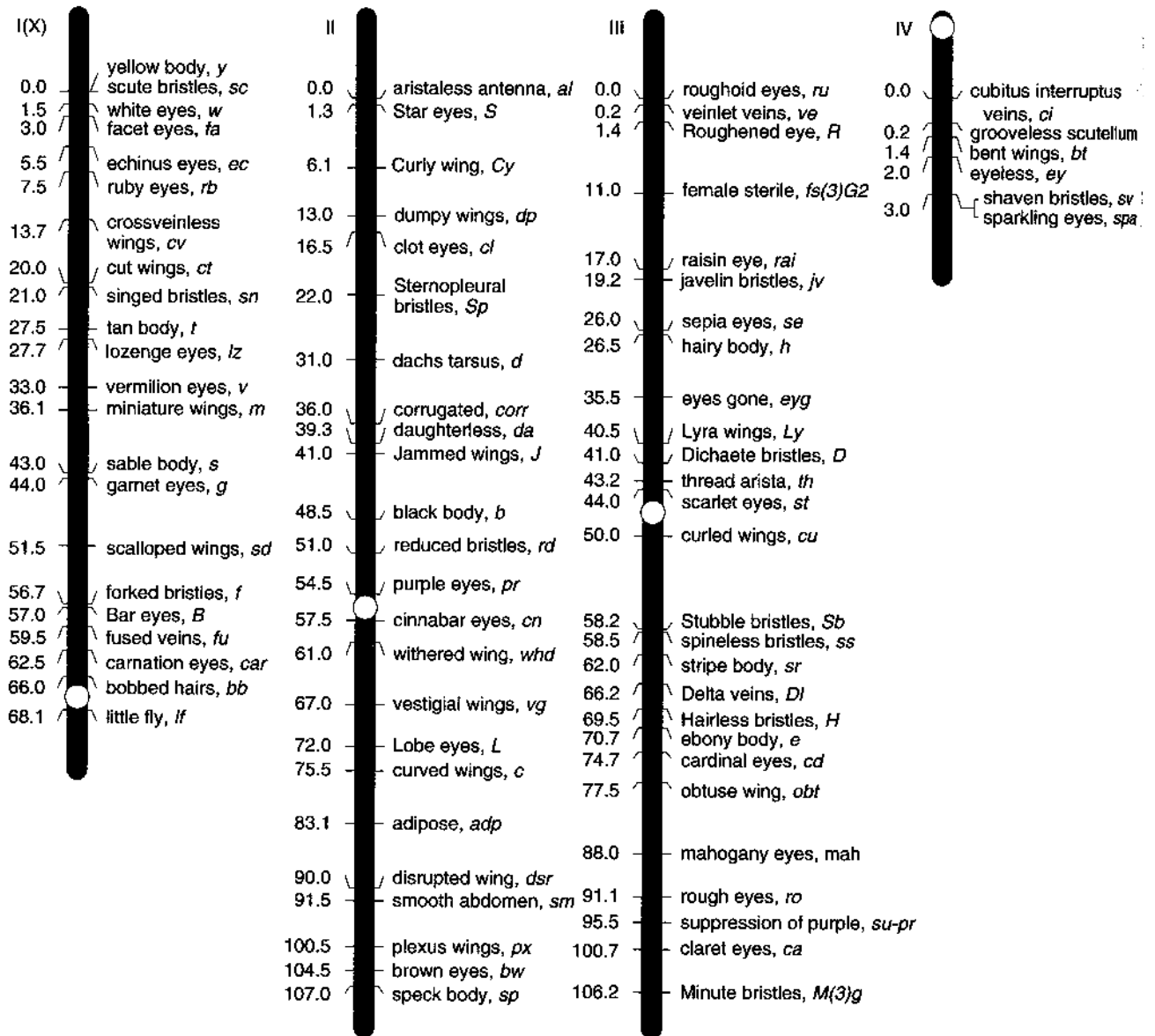
PUNNETT SQUARE

♀ ♂		

Expected F₂ Phenotype Ratio

Appendix B

Gene Map of *Drosophila melanogaster*



Appendix C

Genetic Nomenclature & Notation for *Drosophila*

Clear notation for any *Drosophila* genotype will indicate whether the **locus** involved is on an **autosomal** (II, III, or IV) or **sex** chromosome (I(X) or Y), and in the case of two (or more) **loci**, whether they are the same or different chromosomes (**linked** or **unlinked**, respectively). **Dominant** alleles at a locus are indicated by a capitalized symbol, **recessive** alleles by a lower-case symbol. Examples of such notation are as follows.

1) One autosomal locus:

e.g. The genotype for ebony body on Chromosome III is **ee**, for wild-type body at that locus **e⁺e⁺**. For autosomal genes the genotype is the same for male and female, and can be homozygous or heterozygous.

2) One sex-linked locus:

In *Drosophila* alleles may be present on the **X** chromosome but not on the **Y** chromosome, therefore the genotypes for male and female are different. The symbol (**→**) indicates a male **Y** sex-chromosome and therefore the presence of only one allele.

e.g. Bar eye on Chromosome I. Bar eye female has genotype **BB**, Bar eye male is **B→**. Wild-type eye female is **B⁺B⁺**, wild-type eye male is **B⁺→**.

3) Two unlinked autosomal loci

e.g. vestigial wing (II) and ebony body (III) would have genotype **vgvg ee** and wild-type (wing and body at these loci) would have **vg⁺vg⁺ e⁺e⁺**.

4) Two linked autosomal loci

e.g. curled wing (III, 50.0) and ebony body (III, 70.7). The genotype is written to show the alleles on each homologue **cu e / cu e**. Wild-type would be **cu⁺ e⁺ / cu⁺ e⁺**.

5) Two sex-linked loci

e.g. Bar eye (I 57.0) and forked bristle (I, 56.7). Female is **Bf / Bf**, male is **Bf / →**. Wild-type female is **B⁺f⁺ / B⁺f⁺**, wild-type male is **B⁺f⁺ / →**.

6) One sex-linked & one autosomal loci

e.g. Bar eye (I) and vestigial wing (II). Female is **BB vgvg**, male is **B→ vgvg** wild-type female is **B⁺B⁺vg⁺vg⁺**, wild-type male is **B⁺→ vg⁺vg⁺**.