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*+. 
GENETIC CROSSES

An “X” is used to indicate that two individuals have been mated together. The parents are designated as P (for parental) and the offspring as F (for filial). When several generations are involved, subscripts are added to designate the generations.

P₁ give rise to F₁ (first filial) progeny.
If the F₁ are crossed together they become P₂ and their progeny F₂.

A cross between members of the F₁ and members of the P₁ is a backcross.
A cross between members of the F₁ and the true breeding recessive P₁ is a test cross.

MONOHYBRID CROSS

The simplest form of a cross is a monohybrid cross, which analyses a single trait and its associated variations. The diagram below shows the progression of a pair of alternative alleles for a single gene through two generations.

CROSS DIAGRAM

<table>
<thead>
<tr>
<th>P₁</th>
<th>ee (ebony body) x e⁺e⁺ (wild-type body) [homozygous parents]</th>
</tr>
</thead>
<tbody>
<tr>
<td>gametes</td>
<td>e⁺</td>
</tr>
</tbody>
</table>

F₁ | e⁺e (wild-type body) [heterozygous offspring] |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>gametes</td>
<td>e⁺ &amp; e</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>&amp;</th>
<th>e⁺</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>e⁺</td>
<td>e⁺e⁺</td>
<td>e⁺e</td>
</tr>
<tr>
<td>e</td>
<td>e⁺e</td>
<td>ee</td>
</tr>
</tbody>
</table>

F₂  Phenotype ratio  3 wild-type: 1 ebony body
Genotype ratio  1 e⁺e⁺ : 2 e⁺e : 1 ee

During gamete formation, the members of a pair of alleles are duplicated and then segregated from one cell into four separate gametes so that each contains only one member of the pair (Law of Segregation).

A Punnet Square diagram can be used to calculate the various combinations. The gametes from one parent are written across the top, and those from the other go down the side. Each one of these gametes has an equal chance of combining with either of the gametes from the other parent.
(Random Union of Gametes).

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-\textit{Drosophila} example of this is seen in red- and white-flowered snap dragons:

\[
P_1 \quad RR \text{ (red)} \times \quad rr \text{ (white)}
\]
\[
F_1 \quad Rr \text{ (pink)}
\]
\[
F_2 \quad 1 \quad RR \text{ (red)} : 2 \quad Rr \text{ (pink)} : 1 \quad rr \text{ (white)}
\]

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

\[
e \text{ is ebony body colour} \\
e^+ \text{ is wild-type body colour} \\
v g \text{ is vestigial wing shape} \\
v g^+ \text{ is wild-type wing shape}
\]

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

<table>
<thead>
<tr>
<th>Autosomal Independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>P$_1$ \quad ebony body \times \quad vestigial wing</td>
</tr>
</tbody>
</table>
\[
ee \quad v g^+ \quad v g^+ \quad e^+e^+ \quad v g v g
\]
\[
gametes \quad e \quad v g^+ \quad e^+v g
\]
\[ F_1 \quad e^+e\, vg^+\, vg \quad (\text{all wild-type}) \]

Gametes \( e^+vg^+, e^+vg, evg^+, e\, vg \) \( F_2 \) genotype combinations:

<table>
<thead>
<tr>
<th>&amp;</th>
<th>( e^+vg^+ )</th>
<th>( e^+vg )</th>
<th>( e, vg^+ )</th>
<th>( e, vg )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( e^+vg^+ )</td>
<td>( e^+e^+vg^+vg^+ )</td>
<td>( e^+e^+vg^+vg )</td>
<td>( e^+vg^+vg^+ )</td>
<td>( e^+e^+vg^+vg )</td>
</tr>
<tr>
<td>( e^+vg )</td>
<td>( e^+e^+vg^+vg )</td>
<td>( e^+e^+vgvg )</td>
<td>( e^+e^+vg+vg )</td>
<td>( e^+e^+vgvg )</td>
</tr>
<tr>
<td>( evg^+ )</td>
<td>( e^+e^+vg^+vg^+ )</td>
<td>( e^+e^+vg^+vg )</td>
<td>( ee, vg^+, vg^+ )</td>
<td>( ee, vg^+, vg )</td>
</tr>
<tr>
<td>( evg )</td>
<td>( e^+e^+vg^+vg )</td>
<td>( e^+e^+vgvg )</td>
<td>( ee, vg^+, vg )</td>
<td>( ee, vg vg )</td>
</tr>
</tbody>
</table>

\( F_2 \) Phenotype ratio: 9 wild-type: 3 ebony: 3 vestigial: 1 ebony vestigial

In a dihybrid cross, each of the \( F_1 \) 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.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
</table>

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:

\[
\text{wild-type} \, & \, \text{wild-type} = \frac{3}{4} \times \frac{3}{4} = \frac{9}{16}.
\]

\[
\text{ebony} = \frac{1}{4} \times \frac{3}{4} = \frac{3}{16}
\]

\[
\text{wild} \, & \, \text{vestigial} = \frac{3}{4} \times \frac{1}{4} = \frac{3}{16}
\]

\[
\text{ebony} \, & \, \text{vestigial} = \frac{1}{4} \times \frac{1}{4} = \frac{1}{16}
\]

5
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

P₁  \( \text{st}^{+}\text{cu}^{+}/\text{st}\text{cu}^{+} \) (wild-type)  x  \( \text{st cu} / \text{st cu} \) (scarlet eye, curled wing)

gametes  \( \text{st}^{+}\text{cu}^{+} \)  \( \text{st cu} \)

F₁  \( \text{st}^{+}\text{cu}^{+}/\text{st cu}^{+} \) (all wild-type, cis arrangement)

gametes  \( \text{st}^{+}\text{cu}^{+} , \text{st cu} \)

F₂  \( 1 \text{ st}^{+}\text{cu}^{+}/\text{st cu}^{+} : 2 \text{ st}^{+}\text{cu}^{+}/\text{st cu}^{+} : 1 \text{ st cu}/\text{st cu}^{+} \)

3 wild-type : 1 scarlet curled

Trans arrangement

P₁  \( \text{st}^{+}\text{cu}/\text{st}^{+}\text{cu} \) (curled wing)  x  \( \text{st cu}^{+} / \text{st cu}^{+} \) (scarlet eye)

gametes  \( \text{st}^{+}\text{cu} \)  \( \text{st cu}^{+} \)

F₁  \( \text{st}^{+}\text{cu}/\text{st cu}^{+} \) (all wild-type, trans arrangement)

gametes  \( \text{st}^{+}\text{cu} , \text{st cu}^{+} \)

F₂  \( 1 \text{ st}^{+}\text{cu}/\text{st cu}^{+} : 2 \text{ st}^{+}\text{cu}/\text{st cu}^{+} : 1 \text{ st cu}/\text{st cu}^{+} \)

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

\[ \text{st}^{+}\text{cu}^{+} / \text{st cu} \times \text{st cu} / \text{st cu} \] wild-type scarlet curled

gametes  \( \text{st}^{+}\text{cu}^{+} , \text{st cu} \)  \( \text{st cu} \)

F₂  \( 1 \text{ st}^{+}\text{cu}^{+}/\text{st cu}^{+} : 1 \text{ st cu} / \text{st cu}^{+} \)

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*:

\[
\begin{array}{c}
P_1 \quad X^+X^+ \text{ (standard)} \times \quad X^gY \text{ (goggle-eyed)} \\
F_1 \quad X^+X^g, X^gY \text{ (standard)} \\
F_2 \quad X^+X^+, X^gX^+, X^+Y, X^gY \\
Ratio \quad 2 \text{ standard } \& \text{ 1 goggle-eyed } \\
\end{array}
\]

Reciprocal

\[
\begin{array}{c}
P_1 \quad X^gX^g \text{ (goggle-eyed)} \times \quad X^+Y \text{ (standard)} \\
F_1 \quad X^+X^g \text{ (standard)} \quad X^gY \text{ (goggle-eyed)} \\
F_2 \quad X^gX^+, X^gX^g, X^+Y, X^gY \\
Ratio \quad 1 \text{ standard } \& \text{ 1 goggle-eyed } \& 1 \text{ standard } \& \text{ 1 goggle-eyed } \\
\end{array}
\]

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.
\[ w\pi \] is a hemizygous white-eyed male.

The (\(\pi\)) 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 and Observations

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. Examine the flies to distinguish males from females and examine the different mutants available using the dissection microscope.
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)

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 mutant phenotypes that are available. Compare the morphology with that of a wild-type fly.

**Examples of some common mutants:**

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