

# Biology 2250

# Principles of Genetics

Revised September 2008

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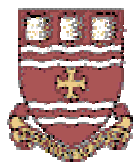
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<p style="text-align: center;"><b>INTRODUCTION TO BIOLOGY 2250</b> <b>PRINCIPLES OF GENETICS</b></p>
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**Biology 2250 (Principles of Genetics)** is an introductory course in genetics and molecular biology that deals with the the molecular basis of heredity, the laws of Mendelian inheritance, and how these combine to produced organismal phenotypes.

The major laboratory investigations emphasize hands-on experience with basic genetic techniques, including computer analysis of patterns of DNA sequence variation, construction and analysis of genetic crosses with several organisms, preparation and analysis of chromosome material, examination of protein structure by electrophoresis, and construction of physical gene and DNA 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 2005 edition has been prepared in PDF format, to reduce costs and to make it more directly accessible to students. This also allows us to make changes during the course of the semester. 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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Dr. David Innes  
Dr. Steve Carr

St. John's, Newfoundland  
September 2005

This 2008 revision has been reduced to core material on *Drosophila* genetics and laboratory safety. Exercises and Investigations will be provided individually.

## 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* 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<sup>+</sup>**. Thus, the genotype of a wild-type homozygote would be designated **e<sup>+</sup>e<sup>+</sup>** (or ++), a mutant homozygote **ee**, and a heterozygote **e<sup>+</sup>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<sup>+</sup>**.



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:

$P_1$	<b>RR</b> (red)	x	<b>rr</b> (white)
$F_1$	<b>Rr</b> (pink)		
$F_2$	1 <b>RR</b> (red)	:	2 <b>Rr</b> (pink) : 1 <b>rr</b> (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<sup>+</sup>** is wild-type body colour  
**vg** is vestigial wing shape  
**vg<sup>+</sup>** 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
	<b>ee vg<sup>+</sup>vg<sup>+</sup></b>		<b>e<sup>+</sup>e<sup>+</sup> vgvg</b>
gametes	<b>e vg<sup>+</sup></b>		<b>e<sup>+</sup>vg</b>
$F_1$	<b>e<sup>+</sup>e vg<sup>+</sup>vg</b> (all wild-type)		
gametes	<b>e<sup>+</sup>vg<sup>+</sup>, e<sup>+</sup>vg, evg<sup>+</sup>, e vgvg</b> $F_2$ genotype combinations:		

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

F<sub>2</sub> Phenotype ratio: 9 wild-type: 3 ebony: 3 vestigial: 1 ebony vestigial

In a dihybrid cross, each of the F<sub>1</sub> 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 <sub>1</sub>	<b>st<sup>+</sup>cu<sup>+</sup>/st<sup>+</sup>cu<sup>+</sup></b> (wild-type)	x	<b>st cu / st cu</b> (scarlet eye, curled wing)
gametes	<b>st<sup>+</sup>cu<sup>+</sup></b>		<b>st cu</b>
F <sub>1</sub>	<b>st<sup>+</sup>cu<sup>+</sup>/st cu</b> (all wild-type, <i>cis</i> arrangement)		
gametes	<b>st<sup>+</sup>cu<sup>+</sup> , st cu</b>		
F <sub>2</sub>	<b>1 st<sup>+</sup>cu<sup>+</sup>/st<sup>+</sup>cu<sup>+</sup> : 2 st<sup>+</sup>cu<sup>+</sup>/st cu : 1 st cu/st cu</b> 3 wild-type : 1 scarlet curled		

*Trans* arrangement

P <sub>1</sub>	<b>st<sup>+</sup>cu/st<sup>+</sup>cu</b> (curled wing)	x	<b>st cu<sup>+</sup> / st cu<sup>+</sup></b> (scarlet eye)
gametes	<b>st<sup>+</sup>cu</b>		<b>st cu<sup>+</sup></b>
F <sub>1</sub>	<b>st<sup>+</sup>cu/st cu<sup>+</sup></b> (all wild-type, <i>trans</i> arrangement)		
gametes	<b>st<sup>+</sup>cu , st cu<sup>+</sup></b>		
F <sub>2</sub>	<b>1 st<sup>+</sup>cu/st<sup>+</sup>cu : 2 st<sup>+</sup>cu/st cu<sup>+</sup> : 1 st cu<sup>+</sup>/st cu<sup>+</sup></b> 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 <sub>1</sub> (from above) x homozygous recessive	
	<b>st<sup>+</sup>cu<sup>+</sup> / st cu</b>	x <b>st cu / st cu</b>
	wild-type	scarlet curled
gametes	<b>st<sup>+</sup>cu<sup>+</sup>, st cu</b>	<b>st cu</b>
F <sub>2</sub>	<b>1 st<sup>+</sup>cu<sup>+</sup>/st cu : 1 st cu / st cu</b> 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*:

P<sub>1</sub>            **X<sup>+</sup>X<sup>+</sup>** (standard)    x    **X<sup>g</sup>Y** (goggle-eyed)

F<sub>1</sub>            **X<sup>+</sup>X<sup>g</sup>, X<sup>+</sup>Y** (standard)

F<sub>2</sub>            **X<sup>+</sup>X<sup>+</sup>, X<sup>g</sup>X<sup>+</sup>, X<sup>+</sup>Y, X<sup>g</sup>Y**

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

Reciprocal

P<sub>1</sub>            **X<sup>g</sup>X<sup>g</sup>** (goggle-eyed)    x    **X<sup>+</sup>Y** (standard)

F<sub>1</sub>            **X<sup>+</sup>X<sup>g</sup>** (standard)            **X<sup>g</sup>Y** (goggle-eyed)

F<sub>2</sub>            **X<sup>g</sup>X<sup>+</sup>, X<sup>g</sup>X<sup>g</sup>, X<sup>+</sup>Y, X<sup>g</sup>Y**

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 F<sub>2</sub>s. If the P<sub>1</sub> female were homozygous dominant, as in the first instance, an allele of the gene can be present in the F<sub>2</sub> 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.

## Appendix A

### The Chi-Square ( $\chi^2$ ) Test in Genetics

With infinitely large sample sizes, the ideal result of any particular genetic cross is exact conformation to the expected ratio. For example, a cross between two heterozygotes should produce an exact 3:1 ratio of dominant to recessive phenotypes. In any particular real- world experiment, with limited and sometimes very small sample sizes, results are expected to deviate somewhat from the exact theoretical ratio, due simply to chance. In order to evaluate a genetic hypothesis (for example, that a particular trait is due to a recessive allele segregating at a locus), we need a means to distinguish an experimental result that is consistent with the hypothesis within the bounds of simple chance deviations, apart from one that is intrinsically unlikely (“wrong”), given the data. Statistical tests are a means of quantifying the results of an experiment as evidence for or against a particular hypothesis.

One of the simplest statistical tests is **Chi-Square ( $\chi^2$ ) Analysis**, which compares the "**goodness of fit**" between observed and expected counts. An hypothesis is developed that predicts how a set of observations will fall into each of two or more categories (the **expected** result). These counts are compared with the experimental data (the **observed** result). Allowing for the sample size, the differences among the observed and expected results are reduced to a single number, the **chi-square value**. Because larger deviations from expectation are expected with more categories, the test also takes into account the **degrees of freedom** in the experiment. Comparison with a **table of probability values** shows the probability that the observed deviation could have been obtained by chance alone. [See the website for Bio2900 (Principles of Evolution) for further discussion of the concepts of null hypothesis, significance testing, and Type I & II error: [http://www.mun.ca/biology/scarr/2900\\_Hypothesis\\_testing.htm](http://www.mun.ca/biology/scarr/2900_Hypothesis_testing.htm)].

### CHI-SQUARE CALCULATIONS

The chi-square formula is:

$$\chi^2 = \sum [(O - E)^2 / E]$$

**O** = observed # of individuals with phenotype

**E** = expected # of individuals with phenotype

$\sum$  = sum of deviations for all phenotypes

- i) The chi-square test should be used *only* on the numerical data themselves, *not* on ratios or percentages derived from the data.
- ii) In experiments where the expected frequency in any phenotypic class is less than five, the true probability is usually slightly larger than the **p** given in the table.
- iii) Expected values should be adjusted to the closest integer [you would not expect a fraction of an individual]; the sum totals of observed and expected values should not differ more than one individual.
- iv) These calculations neglect a number of corrections, including those for small expected classes, multiple simultaneous tests, and the "one-tail" or "two-tail" nature of the test. These will be discussed in your statistics course.

## INTERPRETATION OF THE CHI-SQUARE TABLE

The table of chi-square values (below) can be used to determine the probability that any particular result (observed deviation) could have been obtained by chance alone.

- i) Horizontal rows indicate the number of **degrees of freedom (df)**. In general,  $df = (n - 1)$  where  $n$  is the number of observed classes or phenotypes. For each row, the **p** value at the top of the column is the probability that a particular chi-square value could have been obtained by chance alone. This is called the **critical value** for that level of probability.
- ii) To use the table, enter it on the row corresponding to the number of degrees of freedom. Look for the column with the critical value *closest to and less than* the chi-square obtained in your calculations. The probability of your result is *less than* the **p** value for that column.
- iii) For a biological experiment, we typically set the **level of significance** at  $p = 0.05$ . If the observed chi-square is *greater* than the critical value for  $p = 0.05$ , we conclude that the result could have been obtained by chance *less than* 5% of the time, and we reject the **null hypothesis** that chance alone is responsible for the result. We say that this result is **statistically significant**.

Example #1:  $\chi^2 = 15.0$  and  $n = 8$  phenotypes.

The observed chi-square of 15.0 with  $df = 7$  is greater than the **critical value** of 14.07 for  $p < .05$ . This means that the observed deviation represented by this chi-square value would be expected to occur by chance less than 5% of the time. The result is **statistically significantly**, and the hypothesis (that the data are consistent with the expected ratio) can be rejected.

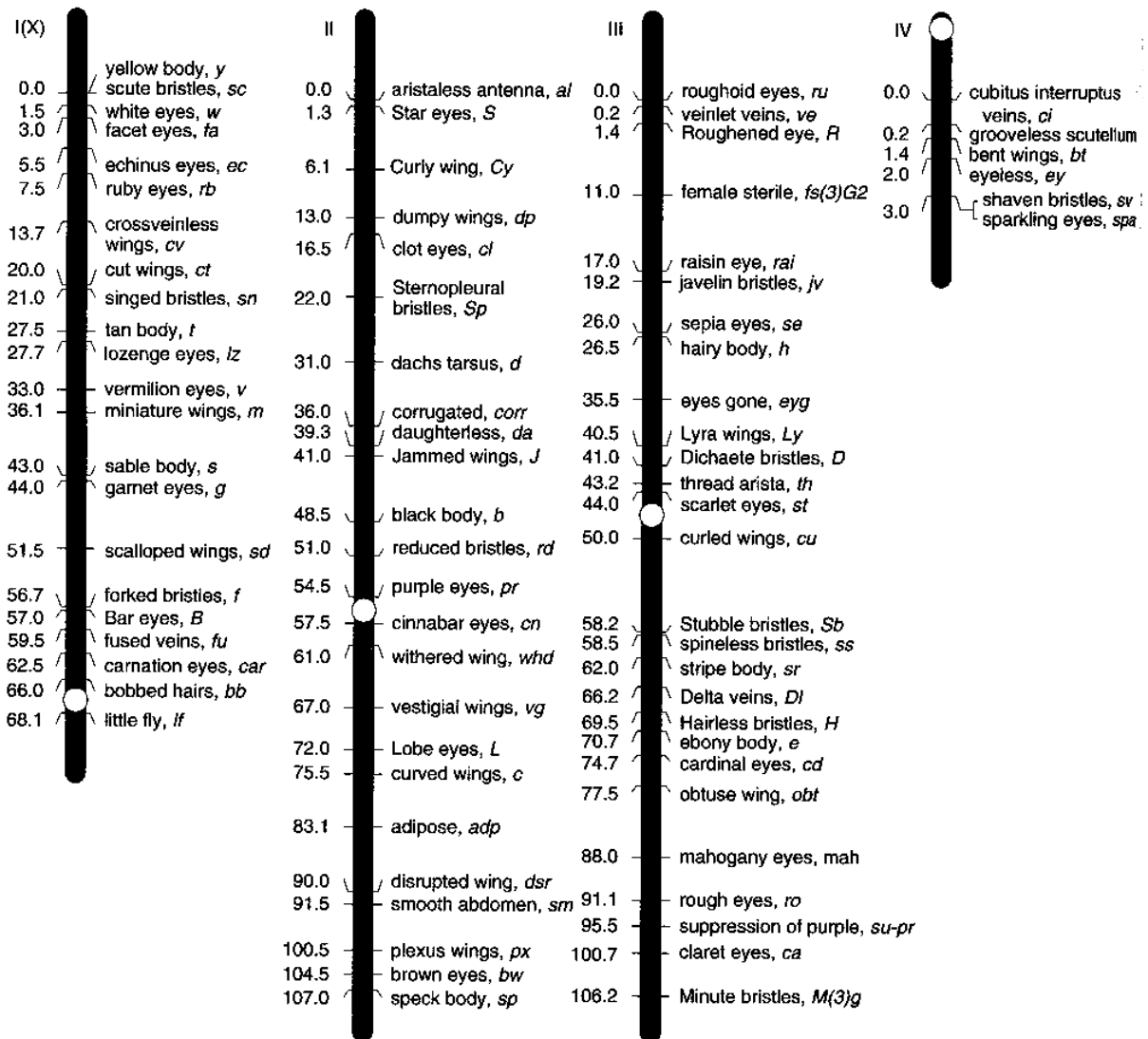
Example #2:  $\chi^2 = 5.0$  and  $n = 8$  phenotypes.

The observed chi-square of 5.0 with  $df = 7$  lies between the values 2.83 and 6.35, which correspond to  $.90 > p > .50$ . A deviation as large as that observed would be expected to occur by chance more than 50% of the time. The difference between the observed and expected results is **not statistically significant**, and the null hypothesis (the ratio being tested) cannot be rejected.

p =	0.9	0.50	0.20	0.05	0.01	0.001
df = 1	0.02	0.46	1.64	3.84	6.64	10.83
2	0.21	1.39	3.22	5.99	9.21	13.82
3	0.58	2.37	4.64	7.82	11.35	16.27
4	1.06	3.36	5.99	9.49	13.28	18.47
5	1.61	4.35	7.29	11.07	15.09	20.52
6	2.20	5.35	8.56	12.59	16.81	22.46
7	2.83	6.35	9.80	14.07	18.48	24.32
8	3.49	7.34	11.03	15.51	20.09	26.13
9	4.17	8.34	12.24	16.92	21.67	27.88
10	4.87	9.34	13.44	18.31	23.21	29.59
15	8.55	14.34	19.31	25.00	30.58	37.30
25	16.47	24.34	30.68	37.65	44.31	52.62
50	37.69	49.34	58.16	67.51	76.15	86.6

## Appendix B

### Gene Map of *Drosophila melanogaster*



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## 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<sup>+</sup>e<sup>+</sup>**. 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<sup>+</sup>B<sup>+</sup>**, wild-type eye male is **B<sup>+</sup>→**.

#### 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<sup>+</sup>vg<sup>+</sup>e<sup>+</sup>e<sup>+</sup>**.

#### 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<sup>+</sup> e<sup>+</sup> / cu<sup>+</sup> e<sup>+</sup>**.

#### 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<sup>+</sup>f<sup>+</sup> / B<sup>+</sup>f<sup>+</sup>**, wild-type male is **B<sup>+</sup>f<sup>+</sup> / →**.

#### 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<sup>+</sup>B<sup>+</sup>vg<sup>+</sup>vg<sup>+</sup>**, wild-type male is **B<sup>+</sup>→ vg<sup>+</sup>vg<sup>+</sup>**.

## Appendix D

### WHMIS for Undergraduate Laboratories

The **Workplace Hazardous Materials Information System** (WHMIS) is a Canada-wide information system for ensuring that industrial workers are informed about the chemicals and other hazardous materials they use. Although student laboratories are not included in the legislation, the Memorial University Safety Office encourages the idea of making the same kind of information available to graduate and undergraduate students as to employees of the university, and we have therefore included this material in the lab manual.

#### *Hazard Classifications*

A **controlled product** is a material that may have characteristics which would put it into one or more of the hazard classes on the attached table - *Hazard symbols and classes*.

A controlled product can be recognized if its label:

- has any of the WHMIS hazard symbols,
- has the WHMIS hatched border, or
- makes reference to a **Material Safety Data Sheet** (MSDS).

#### *WHMIS Labels*

There are two basic types of WHMIS labels:

- **supplier labels**
- **workplace labels**

**Supplier labels** are attached to all packages of controlled products by suppliers. These labels give the identity of the product and its supplier, risk phrases, precautionary measures, first aid measures, hazard symbols, and reference to a material safety data sheet (MSDS). The labels have the WHMIS hatched border.

**Workplace labels** are produced in the workplace and are attached to containers of controlled products which do not have supplier labels such as when products are decanted from supplier containers, old containers which have been around since before WHMIS became effective, or other containers which do not have supplier labels for whatever reason. Workplace labels need only a product identifier, safe handling procedures and reference to an MSDS..

## *Material Safety Data Sheets (MSDS)*

A material safety data sheet is a technical document relating the health effects of exposure to a product, hazard evaluation, protective measures and emergency procedures. MSDS's are sent by suppliers of controlled products or may be generated in the workplace.

MSDS's have nine categories of information:

- Name of product and its use, supplier address and phone.
- Name and concentration of all hazardous ingredients.
- Physical characteristics of the product.
- Fire or explosion hazard.
- Reactivity hazards.
- Toxic hazards.
- Actions required to prevent injury or accident.
- First aid procedures.
- Identity of organization which prepared MSDS and date it was prepared.

Students should be aware that MSDS's are available for the controlled products being used in laboratories and that these may be consulted for specific information on the controlled products.









In some cases, MSDS's will not be sent by suppliers of laboratory chemicals when the appropriate safety information is given on the container labels.

### ***Exemptions***

There are other products used in various workplaces, including laboratories, which might be considered hazardous but are exempted from WHMIS regulations. These include manufactured articles, products made of wood or tobacco, products packaged for consumer use, hazardous waste, and products governed by the federal acts for: explosives, food and drugs, pest control products, and radioactive materials. Radioactive material are covered under regulations of the Nuclear Regulatory Commission.



## Hazard symbols and classes

The symbol represents ...	It means that the material ...	And that you should ...
 <p><b>Class A – Compressed gas</b></p>	<input type="checkbox"/> poses an explosion danger because the gas is being held in a cylinder under pressure <input type="checkbox"/> may cause its container to explode if heated in a fire <input type="checkbox"/> may cause its container to explode if dropped	<input type="checkbox"/> handle with care; do not drop cylinder <input type="checkbox"/> keep cylinder away from potential sources of ignition <input type="checkbox"/> store the containers in the area designated by your supervisor
 <p><b>Class B – Combustible and flammable material</b></p>	<input type="checkbox"/> is one that will burn and is therefore a potential fire hazard <input type="checkbox"/> may burn at relatively low temperatures; flammable materials catch fire at lower temperatures than combustible materials <input type="checkbox"/> may burst into flame spontaneously in air or release a flammable gas on contact with water <input type="checkbox"/> may cause a fire when exposed to heat, sparks, or flames or as a result of friction	<input type="checkbox"/> keep the material away from heat sources and other combustible materials <input type="checkbox"/> never smoke when working with or near the material <input type="checkbox"/> store the material in a cool, fire-proof area, as designated by your supervisor
 <p><b>Class C – Oxidizing material</b></p>	<input type="checkbox"/> poses a fire and/or explosion risk in the presence of flammable or combustible material <input type="checkbox"/> may cause fire when it comes into contact with combustible materials such as wood <input type="checkbox"/> may react violently or cause an explosion when it comes into contact with combustible materials such as fuels <input type="checkbox"/> may burn skin and eyes upon contact	<input type="checkbox"/> keep the material away from combustible materials and store in the areas designated by your supervisor <input type="checkbox"/> keep the material away from sources of ignition <input type="checkbox"/> never smoke when working near the material <input type="checkbox"/> wear the proper protective equipment, including eye, face and hand protection and protective clothing
 <p><b>Class D, Division 1 – Poisonous and infectious material: immediate and serious toxic effects</b></p>	<input type="checkbox"/> is a potentially fatal poisonous substance <input type="checkbox"/> may be fatal or cause permanent damage if it is inhaled or swallowed or if it enters the body through skin contact <input type="checkbox"/> may burn eyes or skin upon contact	<input type="checkbox"/> handle the material with extreme caution <input type="checkbox"/> avoid contact with the skin or eyes by wearing the proper protective equipment, including eye, face and hand protection and protective clothing <input type="checkbox"/> avoid inhaling by working in well-ventilated areas and/or wearing respiratory equipment <input type="checkbox"/> wash and shower thoroughly after using <input type="checkbox"/> store the material in designated areas only
 <p><b>Class D, Division 2 – Poisonous and infectious material: other toxic effects</b></p>	<input type="checkbox"/> is a poisonous substance that is not immediately dangerous to health <input type="checkbox"/> may cause death or permanent damage as a result of repeated exposures over time <input type="checkbox"/> may be a skin or eye irritant <input type="checkbox"/> may be a sensitizer, which produces a chemical allergy <input type="checkbox"/> may cause cancer <input type="checkbox"/> may cause birth defects or sterility	<input type="checkbox"/> avoid skin and eye contact by wearing all protective equipment necessary, including eye, face, and hand protection and protective clothing <input type="checkbox"/> avoid inhaling by working in well-ventilated areas and/or wearing respiratory equipment as designated by your supervisor <input type="checkbox"/> store the material in designated places only
 <p><b>Class D, Division 3 – Poisonous and infectious material: biohazardous infectious material</b></p>	<input type="checkbox"/> may cause a serious disease resulting in illness or death	<input type="checkbox"/> take every measure to avoid contamination <input type="checkbox"/> handle the material only when fully protected by the proper, designated equipment <input type="checkbox"/> handle the material in designated areas where engineering controls are in place to prevent exposure
 <p><b>Class E – Corrosive material</b></p>	<input type="checkbox"/> causes severe eye and skin irritation upon contact <input type="checkbox"/> causes severe tissue damage with prolonged contact <input type="checkbox"/> may be harmful if inhaled	<input type="checkbox"/> keep containers tightly closed <input type="checkbox"/> avoid skin and eye contact by wearing all necessary protective equipment, including eye, face and hand protection and protective clothing <input type="checkbox"/> avoid inhaling by using in well-ventilated areas only and/or wearing the proper respiratory equipment, as designated by your supervisor
 <p><b>Class F – Dangerously reactive material</b></p>	<input type="checkbox"/> is very unstable <input type="checkbox"/> may react with water to release a toxic or flammable gas <input type="checkbox"/> may explode as a result of shock, friction or increase in temperature <input type="checkbox"/> may explode if heated when in a closed container <input type="checkbox"/> undergoes vigorous polymerization	<input type="checkbox"/> keep material away from heat <input type="checkbox"/> open containers carefully; do not drop them <input type="checkbox"/> store the material in a cool, flame-proof area, as designated by your supervisor

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