

# Laboratory III

## Linkage

### Introduction

Genes are arranged as units in a linear order along chromosomes. The position of each gene along the chromosome is called its locus. Each gene has a unique chromosomal position and therefore a unique locus. In fact, geneticists often use the term genetic locus interchangeably with the term gene.

The relative position of genes along a chromosome can be analyzed by determining their linkage relationship. The concept of genetic linkage is outlined in this handout and will be discussed in class in the coming weeks. The position of a gene on a chromosome can be established by its identity.

### Specific Goals

1. Conduct appropriate genetic crosses with a mutant *D. melanogaster* to determine the following:
  - a. Segregation
  - b. Independent Assortment/Linkage
  - c. Sex Linkage

### Complete Linkage

The law of independent assortment is easiest to understand as a product of meiosis if the two genes are on different chromosomes. Then the random assortment of homologous chromosomes during meiosis I can account completely for the independent assortment of alleles of the two different genes. But early on it became clear that there were more genes than there were chromosomes. For example, thousands of corn genes have been identified, but there are only ten pairs of homologous chromosomes in corn. Therefore, if two genes are chosen randomly there is a one in ten chance they will have loci on the same chromosome. When genes are on the

same chromosome, the meiotic assortment of chromosomes alone would not predict the independent assortment of genes on those chromosomes. In the absence of gene assortment, we would predict that the progeny could only have the allelic combinations that were present in the parent chromosomes. Genes that do not assort are said to show complete linkage. Alternatively, genes that independently assort are said to be unlinked. For the following two examples, compare the genotypes and phenotypes generated in a dihybrid crosses of unlinked and completely linked genes.

Example 1: A dihybrid cross for genes A and B, two unlinked genes.

Generation	Genotypes	Phenotypes
P1	AABBx aabb	AB and ab
F1	AaBb	AB
F2	1AABB:2AABb: 2AaBB:4AaBb:1AAAbb: 2Aabb:1aaBB:2aaBb:1aabb	9AB:3Ab:3aB:1ab

Phenotypic ratio from dihybrid cross for unlinked genes:

9:3:3:1

Example 2: A dihybrid cross for genes A and C, which are completely linked.

Generation	Genotypes	Phenotypes
P1	AACCx aacc	AC and ac
F1	AaCc	Ac
F2	1AACC:2AaCc:1aacc	3AC:1ac

Phenotypic ratio from dihybrid cross for completely linked genes

3:1

In example 1, alleles at the A and B genes are found in all possible allelic combinations in the F2 generation because alleles at these loci assort independently. However, in example 2 A and C are linked and always found in parental combinations. For example whenever there is an A allele there is always its linked C allele.

While a dihybrid cross can demonstrate the phenomenon of linkage, it is more common to test for linkage using a test cross. The advantage of the test cross is that the phenotypes of the offspring show the genotype of the gametes contributed by the tested parent. A testcross of the dihybrid for unlinked genes, AaBb, would result in a 1:1:1:1 phenotypic ratio. A testcross of the dihybrid for completely linked genes AaCc would result in a 1:1 ratio. See the following examples.

(Note the different notation system used to keep track of allelic combinations from each gamete. The genotype of each gamete contributed by the P1 generation is separated by a line. The parental class of offspring represents gamete genotypes that were produced by the original parental generation. The recombinant class represents the gamete genotypes not produced by the parental generation.)

Example 3: Test cross of dihybrid for unlinked genes.

Testcross	Genotypes of offspring			
	Parental Class		Recombinant Class	
$\frac{AB}{ab} \times \frac{ab}{ab}$	$\frac{AB}{ab}$	$\frac{ab}{ab}$	$\frac{Ab}{ab}$	$\frac{aB}{ab}$

Phenotypic ratio for test cross of dihybrid for unlinked genes:

1:1:1:1

Example 4: Test cross of dihybrid for completely linked genes.

Testcross	Genotypes of offspring	
	Parental Class	Recombinant Class
$\frac{AC}{ac} \times \frac{ac}{ac}$	$\frac{AC}{ac}$	$\frac{ac}{ac}$

Phenotypic ratio for test cross of dihybrid for unlinked genes:

1:1

## Partial Linkage

Complete linkage is very rare. This is because of the phenomenon of crossing over (or recombination) that occurs during prophase I where regions of chromatids are exchanged between homologous chromosomes. Crossing over is a mechanism that allows for the assortment of genes on the same chromosome. Whenever there is a crossing over event between two genes on the same chromosome, gametes are going to be generated that resemble the recombinant type (see previous examples). If the frequency of crossing over is as common as 50%, then alleles at the two genes show independent assortment as if they were on different chromosomes. However in many cases the frequency of crossing over is less than 50%. In this situation, the parental combination of alleles will be more frequent in the next generation than the recombinant combination of alleles. Genes that show this biased assortment are said to show partial or incomplete linkage.

## Determining Linkage

An example.

In a test cross of the dihybrid BD/bd, offspring with the following phenotypes were generated: 30 BD:30bd:20Bd:20bD.

The expected ratio of phenotypes is 1:1:1:1. If we tested this ratio, 1:1:1:1, with chi-square we would simultaneously be testing for independent assortment and random segregation. If we just want to test for independent assortment alone, then we must test the expected ratio of 1:1, parental:recombinants.

The parental types are BD and bd, 60 individuals

The recombinant types are Bd and bD, 40 individuals.

$$R_f = 40/100 = 0.40 \text{ or } 40 \text{ map units}$$

Chi-square analysis

Hypothesis: B and D are not linked and assort independently

H<sub>0</sub>: The expected ratio of parental:recombinant types is 1:1.

H<sub>1</sub>: The ratio is not 1:1.

Test: Chi-square

Degrees of freedom: 1

Significance Level: 0.05

Critical value: 3.84

Allelic Combinations	O	E	(O-E) <sup>2</sup> /E
Parental	60	50	2
Recombinant	40	50	2
			$X^2 = \frac{2}{4}$

$$4.00 > 3.84$$

Conclusion: The hypothesis is rejected.

## Chromosome maps

When the linkage relationship between a large number of genes has been determined, detailed genetic maps of the chromosomes can be developed. See Figure 6.14 in your textbook for a diagram of the chromosomal map of *D. melanogaster*. As new genes are identified they can be included on the map by establishing a linkage relationship between two genes already on the map.

## Sex Linkage

Although this should not come as a surprise, males and females are not identical. In many higher eukaryotes (you, me and flies), males and females differ in one pair of chromosomes called the sex chromosomes. All the other chromosomes which are identical in both sexes are known as autosomes.

In humans and *Drosophila*, females have two identical sex chromosomes called the X chromosomes. Therefore, females have an XX genotype. Males have only one copy of the X chromosome. Instead of a second X chromosome males have a second sex chromosome called the Y chromosome. Males have an XY genotype. The X chromosome contains many important genes. Genes located on the X chromosome are said to be sex linked. The Y chromosome lacks the genes found on the X chromosome. In fact, the Y chromosome seems to possess very little genetic information at all.

The upshot of all this is that inheritance of sex-linked genes is a little different from inheritance of autosomal genes. Females will have two alleles for each sex-linked trait, but males will have just one allele. The condition of having just one copy of a gene is called hemizygous.

The test for sex-linkage is simply to conduct reciprocal crosses between two different homozygous strains. For example, use strain A as the female parent in one cross and strain B as the male parent. In a second cross, use strain B as the female parent and strain A as the male parent. If the gene is autosomal, then the outcome of the two reciprocal crosses will be identical progeny in the two crosses. If the gene

## Two Point Linkage Mapping

When two genes are on the same chromosome the rate of chiasmata formation between them is related to the distance between the two genes. In general, the closer two genes are on a chromosome then the lower the frequency of chiasmata forming between the two genes. Conversely, the farther apart two genes are on the chromosome the greater the frequency of chiasmata formation. (There are exceptions to this generality, because some regions of the chromosome appear to have chiasmata formation more frequently than other regions.)

Using this relationship between chromosomal distance and frequency of chiasmata formation it is possible to generate genetic maps of chromosomes. The genetic distance between gene loci is equal to the frequency of recombination ( $R_f$ ) between loci.  $R_f$  is measured in map units or centimorgan which both correspond to a recombinant frequency of 1%. Map distance is easy to calculate from the test cross data.

$$R_f = \# \text{ of recombinant} / \text{total progeny}$$

For the previous example with the B and D genes:

is sex-linked, then the two reciprocal crosses will generate different phenotypes in the progeny. This phenomenon is illustrated in the following examples.

Example 6, autosomal genes

Cross #1 female AA X male aa  
Expected progeny: both females and males will have the A phenotype.

Cross #2 female aa X male AA  
Expected progeny: both females and males will have the A phenotype.

Example 7, sex-linked genes

Cross #1 female BB X male bY  
Expected progeny: both females and males will have the B phenotype

Cross #2 female bb X male BY  
  
Expected progeny: all the males will be phenotypically b and all the females will be phenotypically B.

With sex-linked genes, the reciprocal crosses the phenotype of the male and female progeny will differ in one of the crosses. (Note if the phenotypes of the males and females differ in both of the crosses that might be a case of sex influenced trait instead of sex-linked.)

## Procedure:

1. Obtain two strains of flies from the instructor. One strain will be wildtype. The second strain will be homozygous recessive mutant for two genes. Identify the two mutant phenotypes of your mutant strain.
2. Conduct appropriate crosses to determine the following.
  - A. Determine whether the genes controlling each of your traits obey random segregation.

- B. Determine whether either of the genes are sex linked or autosomal.
- C. Determine if the genes controlling your traits are linked or assort independently.

## Report:

1. Turn-in two recombinant flies (one of each type) as evidence you successfully completed the crosses. You will receive 10pts credit for the flies.
2. Answer the questions at the end of this exercise and submit them before the end of the lab session (5pts).
3. Write a Results section of a formal laboratory report describing the results of this laboratory project. This results section should include both a narrative and tables. Address the three issues above (2, A-C) in your results section. This results section is due at the beginning of the lab session on April 10,11. (35 pts)

Be sure to conduct the following analyses in your report.

- I. Use Chi – Square to test the hypothesis that alleles of each of your genes randomly segregate.
- II. Use Chi Square to test whether the alleles of your two genes independently assort.
- III. Is your wing gene sex linked or autosomal? What data support your conclusion.
- IV. If the alleles do not assort independently, calculate the linkage distance.

## Example Chi Square Table

Table 2: Chi-Square Goodness of Fit Analysis of Valine Codon Usage in Porifera

Codon	Observed (O)	Expected (E)	(O-E) <sup>2</sup> /E
GTC	28	39.75	3.47
GTG	63	39.75	13.60
GTT	43	39.75	0.27
GTA	25	39.75	5.47
			<b>X<sup>2</sup> = 22.81<sup>1</sup></b>

<sup>1</sup>The null hypothesis that the 4 valine codons were used with equal frequency in Porifera was rejected (X<sup>2</sup> = 22.81, d.f. = 3, P < 0.0001).

Due at the End of Class

1. Identity your two mutant traits

Trait I: \_\_\_\_\_

Trait II: \_\_\_\_\_

2. What results do you expect from your reciprocal crosses, if both traits are autosomal?

3. What results do you expect from your reciprocal crosses if both traits are sex linked?

4. What results do you expect from your reciprocal crosses if trait I is sex linked and Trait II is autosomal?

5. What ratio in your testcross progeny would you use to test whether the gene controlling trait I is segregating randomly?

6. What ratio in your testcross progeny would you use to test whether the genes controlling your two traits independently assort.

7. What phenotypic ratio would you expect in your testcross progeny if the traits are unlinked?

8. What phenotypic ratio would you expect in your testcross progeny if the traits are completely linked?

9. What phenotypic ratio would you expect in your testcross progeny if the two genes are 10 map units apart?

10. In your testcross progeny, what phenotypic combinations would correspond with the parental types and what phenotypes would correspond with recombinant types.