# GENETIC LAB
# REPORT

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# ABSTRACT

In this experiment, three phenotypic inheritance characteristics, as hypothesized using Mendelian laws, are used to perform three crucial crosses: the test cross, the monohybrid or dihybrid crosses, and test crosses to determine genetic linkage for the mapping of genes. All the tests were performed using fruit flies as simulated by Fly lab software. This experiment can be considered as highly successful, since most of the hypotheses generated from Mendelian ratios were recommended after Chi-square test. Finally, a genetic linkage map of two linked genes found in fruit flies was also devised as shown in figure 1.

***Key words:*** phenotype, phenotypic inheritance, Mendelian laws, monohybrid cross, dihybrid cross.

# INTRODUCTION

The Mendelian laws formulate the most basic principles of phenotypic inheritance and are used to predict the phenotypic ratio of $F_1$ and $F_2$ generations by using traits of P generation. Monohybrid and dihybrid ratios are different, as determined using the Punnett Square for each hybrid cross. However, these laws fail to explain incomplete dominance, epistasis, and co-dominance (Starr, 2009).

Furthermore, by using these laws, one can be able to predict the homozygous or heterozygous nature of an organism that has the dominant allele present. This is called a testcross and involves crossing an $F_1$ generation of true bred parents with the recessive homozygote. The percentage population determines the unknown phenotypic status of $F_1$ offspring.

Finally, by learning chromosomal recombination during gametogenesis using Mendelian laws and the development of pedigree generations, one can be able to draw a genetic linkage map that depicts the position and distance between two linked genes on the same chromosome (Hartl, 2014).

# METHODS

## Materials

The software fly lab was utilized to simulate mating and genetic inheritance in fruit flies. It was also used to generate and analyze the data. To do this, different fruits flies with unique and differing phenotypic traits such as body color, eye shape, and bristles were used.

## Experimental Procedure

The experiment was divided into three unique categories with the help of chromosomal recombination techniques: monohybrid and dihybrid crosses, test cross, and genetic mapping.

In the first part, two homozygous parents were true bred to produce an $F_1$ generation. Thereafter, two of the $F_1$ generation were then mated with each other to produce the $F_2$ generation. In both cases, the phenotypic characteristics of $F_1$ and $F_2$ were noted down and an analysis as to whether they fitted the predicted Mendelian phenotypic ratio was performed by using Chi-square test. Prediction of phenotypic ratios was done using the Punnett square. This prediction was then repeated for three other unique traits and their results were tabulated as shown in Appendix A.

In the second part, a test cross was done to determine the unknown phenotype of an $F_1$ generation. One of the $F_1$ generation offsprings was crossed with a fruit fly that possessed the recessive traits with respect to the P generation of $F_1$. The percentage number of the obtained

offspring was then used to determine whether the unknown phenotype was double homozygous or heterozygous.

Finally, the distance between the two linked genes was determined. This was done by first performing a test cross using the aforementioned method. The results of the test cross were then used to predict genetic linkage. Mapping was then done to depict the distance between the two genes. In all cases, results were observed and recorded in Appendix B.

# RESULTS AND DISCUSSION

## First Part (Assignment 1): **Monohybrid Crosses**

For part one, monohybrid crosses for true bred P generations were crossed to obtain the $F_1$ and $F_2$ generations as described in the experimental procedure above. The traits used were body color, wing angle and eye shape. The results of the monohybrid cross for the $F_1$ and $F_2$ generation were then tabulated as shown in tables 1, 3, 4, and 5 respectively. The dominant phenotype (allele) for each of the results was indicated in each of the tables. In order to test the phenotypic ratio, hypotheses were made using the Mendelian conventional ratios of monohybrid crosses for the $F_1$ generation (1:1) and $F_2$ generation (3:1) (Bailey, 2002). Chi-square test to determine the viability of each hypothesis was determined and tabulated in tables 2, 6, 7, and 8 respectively (for $F_1$ and $F_2$ generations). In most cases, the hypotheses were true except for those in table 7 that analyzed the wing angle trait. The accepted hypothesis was 1:1 (Table 7). The only viable explanation for this discrepancy from the conventional Mendelian ratios prediction is

that genes attributed to the trait may be highly linked but not segregated. This was the only anomaly. As a result, there are the following results for 1c:

**Question 1c.** The Mendelian ratios for monohybrid crossing for $F_1$ and $F_2$ crosses are 1:1 and 3:1 respectively. This is only applicable if the phenotypes adhere to the three basic Mendelian laws (Starr, 2009). The phenotype of each fly and dominance is indicated in Table 1. The hypotheses were tested and indicated in Table 2 and found to be true for this cross.

**Question 1d.** The $F_1$ phenotype, as predicted using Mendelian ratios (1:1), was found to be true. This is because both P generation mates were of pure bred type and were to produce offsprings with the dominant phenotype as indicated in Table 1.

**Question 1h.** The Chi-square test recommended the hypothesis as having a high probability of being true on a significance level of 0.4512, as shown in Table 2. Thus, the ratio was accepted.

**Question 1j.** The expected ratio for $F_2$ generation was predicted to be 3:1 by using Mendelian concepts employed on a Punnett square. The fact that this ratio was recommended, as shown in Table 2, makes the phenotypic ratio 4:1 untrue. The results thus showed the sepia allele (phenotype) to be recessive to the wild type allele indicated in Table 1.

**Question 2.** For each of the 3 results in Tables 3, 4, and 5, the number of expected offsprings was varied as in 10000, 1000, and 100 respectively. These variations had no effect on the ability of performing chi-square test. Thus, the number of offsprings does not affect phenotypic ratios.

**Question 3.** Using Mendelian ratios for dihybrid crossing, the $F_1$ and $F_2$ generations are to have 1:1:1:1 and 9:3:3:1 phenotypic ratios respectively (Bailey, 2002). Both ratios were accepted. Moreover, the second ratio (9:3:3:1) predetermines that each independent phenotype is inherited by the $F_2$ generation at ratios of 3:1. This are linked to the 1:1 ratio part of 1:1:1:1. Thus, the Mendelian laws are followed independently for each trait in a dihybrid cross.

## Second Part (Assignment 2): **Test Cross**

Results were recorded in Table 11. One of the $F_1$ generation members was crossed with recessive homozygous fly for both traits. The dominant traits where immediately visible in the $F_1$ generation before the test cross. The ratio of ebony bodied flies to wild type was 2:4 = 1:2, while that of brown to wild type eye color was 2:4 = 1:2. This was a 50% total, and thus $F_1$ must have been double heterozygous. If the test cross was done using a homozygous fly, all the flies would be expected to have a uniform color of the dominant trait (wild type color traits for both the eyes and body). The latter trait was not observed.

## Third Part (Assignment 6): **Genetic Linkage**

From Mendelian law of independence, any two genes that control two different traits are inherited independently. If more recombination occurs on both genes, such that the $F_2$ generations do not obey the 9:3:3:1 rule, then the genes are genetically linked. The genes for eyeless mutation (EY) and shaven bristles (SV) were found to be linked, and thus a linkage map was drawn as shown in Figure 1.

Calculation of gene distance in cM = $\dfrac{(48+37)}{10073} \times 100 = 8{,}4\% = 8{,}4\text{cM}$

# CONCLUSION

In conclusion, from the results obtained, most of Mendelian laws were followed. However, discrepancies such as the one obtained when using wing angle traits is a clear indication of the shortcomings of these laws. From the Figure 1, a distance of 8.4 cM for two linked genes is very probable and Mendelian laws lack these exceptions. By using the Fly Lab software to simulate mating generations of fruit flies, it became clear that traits that possess incomplete dominance are most likely to be rejected by the Chi-Square tests. It significantly limits the number of traits that can be effectively predicted under Fly Lab software. Consequently, the integration of incomplete dominance into simulations will improve the depth of experiment's results and similarities when compared to the actual natural mating of fruit flies.

# Appendix A

**1.** One table showing the result of cross from Assignment 1.1.

Table 1: Monohybrid Cross between Wild Type and Sepia Body Color for $F_1$ and $F_2$ Generations.

| Parents | (Female: +) x (Male: SE) | | |
|---|---|---|---|
| $F_1$ | Offspring   **wild type eye color trait dominant** | | |
| Phenotype | Number | Proportion | Ratio |
| Female: + | 4957 | 0.4945 | 1 |
| Male: + | 5067 | 0.5055 | 1.022 |
| Total | 10024 | | |
| $F_2$ generation (Offspring) | | | |
| Parents | (Female: +) x (Male: +) | | |
| Phenotype | Number | Proportion | Ratio |
| WT | 7525 | 0.7467 | 2.949 |
| SE | 2552 | 0.2532 | 1 |
| Total | 10077 | | |

**2.** One table showing Chi-square analysis of results from Assignment 1.1

Table 2: Chi-Square Analysis of $F_1$ generation and $F_2$ Generations Monohybrid Crosses

| Chi Square Analysis | | | | |
|---|---|---|---|---|
| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
| Female: + | 4957 | 1 | 5012 | 0.6036 |
| Male: + | 5067 | 1.0000 | 5012.0000 | 0.6036 |
| Total | 10024 | 2.0000 | 10024.0000 | 1.2071 |

Chi-Squared Test Statistic = 1.2071

Degrees of Freedom = 1

Level of Significance = 0.2719

Recommendation: **Do not reject your hypothesis**

$F_2$ generation

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|---|---|---|---|---|
| + | 7525 | 3.0000 | 7557.7500 | 0.1419 |
| SE | 2552 | 1.0000 | 2519.2500 | 0.4257 |
| Total | 10077 | 4.0000 | 10077.0000 | 0.5677 |

Chi-Squared Test Statistic = 0.5677

Degrees of Freedom = 1

Level of Significance = 0.4512

Recommendation: **Do not reject your hypothesis**

**3.** Three tables showing results of crosses from Assignment 1.2.

Table 3: Monohybrid Cross between Wild Type and Yellow Body Color Traits for $F_1$ and $F_2$ generations.

| Parents | Female: +) x (Male: Y) (traits Body color Female= wild type Male= yellow) | | |
|---|---|---|---|
| F₁ | Offspring | | |
| Phenotype | Number | Proportion | Ratio |
| Female: + | 4991 | 0.5041 | 1.016 |
| Male: + | 4910 | 0.4959 | 1.000 |
| Total | 10024 | | |
| F₂ generation | | | |
| Parents | (Female: +) x (Male: +) | | |
| Phenotype | Number | Proportion | Ratio |
| WT | 7543 | 0.7534 | 3.055 |
| SE | 2469 | 0.2466 | 1.000 |
| Total | 10077 | | |

Table 4: Monohybrid Cross between Wild Type and Dichaete Wing Angle Traits for F₁ and F₂ generations.

| Parents | (Female: +) x (Male: D) | | |
|---|---|---|---|
| F₁ | Offspring **Wild Type and Dichaete Wing Angle both expressed (incomplete dominance)** | | |
| Phenotype | Number | Proportion | Ratio |
| Female: + | 483 | 0.5084 | 1.034 |
| Male: + | 467 | 0.4916 | 1.000 |
| Total | 950 | | |
| F₂ generation | | | |
| Parents | (Female: +) x (Male: D) | | |

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| WT | 4927 | 0.4929 | 1.000 |
| D | 5068 | 0.5071 | 1.029 |
| Total | 9995 | | |

Table 5: Monohybrid Cross between Wild Type and Eyeless Eye Shape Traits for $F_1$ and $F_2$ generations.

| Parents | (Female: +) x (Male: EY) | | |
|---------|--------------------------|---|---|
| **F$_1$** | **all offspring had wild type eyes** | | |
| Phenotype | Number | Proportion | Ratio |
| Female: + | 58 | 0.5225 | 1.094 |
| Male: + | 53 | 0.4775 | 1.000 |
| Total | 111 | | |
| **F$_2$ generation** | | | |
| Parents | (Female: +) x (Male: +) | | |
| Phenotype | Number | Proportion | Ratio |
| WT | 750 | 0.7637 | 3.233 |
| EY | 232 | 0.2363 | 1.000 |
| Total | 982 | | |

**4.** Three tables showing Chi-square analysis of results from Assignment 1.2

Table 6: Chi-Square Analysis of $F_1$ generation and $F_2$ Generations

Monohybrid Crosses for Table 3.

| Chi Square Analysis | | | | |
|---|---|---|---|---|
| **Phenotype** | **Observed** | **Hypothesis** | **Expected** | **Chi-Square Term** |
| Female: + | 4991 | 1.0000 | 4950.5000 | 0.3313 |
| Male: + | 4910 | 1.0000 | 4950.5000 | 0.3313 |
| Total | 9901 | 2.0000 | 9901.0000 | 0.6627 |
| Chi-Squared Test Statistic = 0.6627 | | | | |
| Degrees of Freedom = 1 | | | | |
| Level of Significance = 0.4156 | | | | |
| Recommendation: **Do not reject your hypothesis** | | | | |
| $F_2$ generation | | | | |
| **Phenotype** | **Observed** | **Hypothesis** | **Expected** | **Chi-Square Term** |
| + | 7543 | 3.0000 | 7509.0000 | 0.1539 |
| Y | 2469 | 1.0000 | 2503.0000 | 0.4618 |
| Total | 10012 | 4.0000 | 10012.0000 | 0.6158 |
| Chi-Squared Test Statistic = 0.6158 | | | | |
| Degrees of Freedom = 1 | | | | |
| Level of Significance = 0.4326 | | | | |
| Recommendation: **Do not reject your hypothesis** | | | | |

Table 7: Chi-Square Analysis of $F_1$ generation and $F_2$ Generations Monohybrid Crosses for Table 4.

## Chi Square Analysis

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|----------|-----------------|
| Female: + | 483 | 1.0000 | 475.0000 | 0.1347 |
| Male: + | 5067 | 1.0000 | 475.0000 | 0.1347 |
| Total | 950 | 2.0000 | 950.0000 | 0.2695 |

Chi-Squared Test Statistic = 0.2695

Degrees of Freedom = 1

Level of Significance = 0.6037

Recommendation: **Do not reject your hypothesis**

## $F_2$ generation

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|----------|-----------------|
| + | 4927 | 3.0000 | 7496.2500 | 880.5797 |
| D | 5068 | 1.0000 | 2498.7500 | 2641.7391 |
| Total | 9995 | 4.0000 | 9995.0000 | 3522.3188 |

Chi-Squared Test Statistic = 3522.3188

Degrees of Freedom = 1

Level of Significance = 0.0000

Recommendation: **Reject your hypothesis**

## Using a ratio of 1:1

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|----------|-----------------|
| + | 4927 | 1.0000 | 4997.5000 | 0.9945 |
| D | 5068 | 1.0000 | 4997.5000 | 0.9945 |
| Total | 9995 | 4.0000 | 9995.0000 | 3522.3188 |

Chi-Squared Test Statistic = 1.9891

| Degrees of Freedom = 1 |
| Level of Significance = 0.1584 |
| Recommendation: **Do not reject your hypothesis** |

Table 8: Chi-Square Analysis of $F_1$ generation and $F_2$ Generations Monohybrid Crosses for Table 5.

| Chi Square Analysis | | | | |
|---|---|---|---|---|
| **Phenotype** | **Observed** | **Hypothesis** | **Expected** | **Chi-Square Term** |
| Female & Male | 111 | 1.0000 | 111.0000 | 0.0000 |
| Total | 111 | 1.0000 | 111.0000 | 0.0000 |
| Chi-Squared Test Statistic = 1.2071 | | | | |
| Degrees of Freedom = 1 | | | | |
| Level of Significance = 0.2719 | | | | |
| Recommendation: **Do not reject your hypothesis** | | | | |
| **$F_2$ generation** | | | | |
| **Phenotype** | **Observed** | **Hypothesis** | **Expected** | **Chi-Square Term** |
| + | 750 | 3.0000 | 736.5000 | 0.2475 |
| EY | 232 | 1.0000 | 245.5000 | 0.7424 |
| Total | 982 | 4.0000 | 982.0000 | 0.9898 |
| Chi-Squared Test Statistic = 0.9898 | | | | |
| Degrees of Freedom = 1 | | | | |
| Level of Significance = 0.3198 | | | | |
| Recommendation: **Do not reject your hypothesis** | | | | |

**5.** One table showing result of cross from Assignment 1.3

Table 9: Dihybrid cross between Body Color (ebony and wild type) and Wing Size (Vestigial and Wild Type for $F_1$ and $F_2$ Generations.

| Parents | (Female: E) x (Male: VG) | | |
|---|---|---|---|
| $F_1$ | Offspring with **wild type body color and wild type wings were obtained (complete dominance for each trait)** | | |
| **Phenotype** | **Number** | **Proportion** | **Ratio** |
| Female: + | 4977 | 0.4963 | 1.000 |
| Male: + | 5051 | 0.5037 | 1.015 |
| Total | 1002 | | |
| $F_2$ generation (predicted ratio 9:3:3:1) | | | |
| **Parents** | (Female: +) x (Male: +) | | |
| **Phenotype** | **Number** | **Proportion** | **Ratio** |
| WT | 5574 | 0.5592 | 9.093 |
| VG | 1905 | 0.1911 | 3.108 |
| E | 1876 | 0.1882 | 3.060 |
| VG;E | 613 | 0.0615 | 1.000 |
| Total | 9968 | | |

**6.** One table showing Chi-square analysis of results from Assignment 1.3

Table 10: Dihybrid Chi-Square Analysis of $F_1$ generation and $F_2$ Generations Monohybrid Crosses for Table 9 above.

| Chi Square Analysis (ignoring sex) | | | | |
|---|---|---|---|---|
| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
| E;VG | 10028 | 1.0000 | 10028.0000 | 0.0000 |
| Total | 10028 | 1.0000 | 10028.0000 | 0.0000 |

Chi-Squared Test Statistic = 0.0000

Degrees of Freedom = 0

Level of Significance = 1.0000

Recommendation: **Do not reject your hypothesis**

| $F_2$ generation | | | | |
|---|---|---|---|---|
| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
| + | 5574 | 9.0000 | 5607.0000 | 0.1942 |
| VG | 1905 | 3.0000 | 1869.0000 | 0.6934 |
| E | 1876 | 3.0000 | 1869.0000 | 0.0262 |
| VG;E | 9968 | 16.0000 | 9968.0000 | 1.0744 |
| Total | 10077 | 4.0000 | 10077.0000 | 0.5677 |

Chi-Squared Test Statistic = 1.0744

Degrees of Freedom = 3

Level of Significance = 0.7833

Recommendation: **Do not reject your hypothesis**

# Appendix B

**7.** One table showing the result of cross from Assignment 2

Table 11: Test Cross to Determine Phenotype for One Member of $F_1$ using Recessive Double Homozygous Mate (Traits used were Eye Color (Wild Type and Brown) and Body Color (Ebony and Wild Type).

| Parents | (Female: BW) x (Male: E) | | |
|---|---|---|---|
| $F_1$ | Offspring were **(50 percentage population had Dominant traits while the other lacked implying double heterozygous phenotypic character for $F_1$)** | | |
| Phenotype | Number | Proportion | Ratio |
| Female: + | 10003 | 1.0000 | 1.000 |
| Male: + | | | |
| Total | 10003 | | |
| Ratio of ebony bodied flies to wild type 2:4= 1: 2 Ratio of brown eyed color flied obtained after test cross 2:4 = 1:2 | | | |

**8.** One table showing the result of cross from Assignment 6

Table 12: Test Cross to Determine Genetic Linkage between Genes for Eye Shape (Wild Type) and Bristle (Wild Type) (dominant traits under $F_1$ genes)

| Parents | (Female: +) x (Male: SV;EY) | | |
|---------|------------------------------|---|---|
| F₂ after test cross | Offspring were **(wild type eyes and bristles dominant)** **Genes SV and EV involved** | | |

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 37 | 0.0037 | 1.000 |
| SV | 4948 | 0.4912 | 133.730 |
| EY | 5040 | 0.5003 | 136.216 |
| SV;EY | 48 | 0.0048 | 1.297 |
| Total | 10073 | | |
| **Total recombination** = **48 + 37= 85** | | | |

**9.** Linkage map constructed during Assignment 6

Figure 1: Illustrations of the Linkage Distance between Dominant Genes on the Same Chromosome as from Table 12.