# Analysis of a Mutant Strain of *Drosophila Melanogaster*

**Abstract:**

*Drosophila melanogaster*, known as the common fruit fly, is often used in genetic research. An unknown fruit fly, U-5309, was found to have several characteristics that deviate from the Oregon-R wild type strain. These characteristics are white eyes, shortened longitudinal veins, and dark body. It was determined that the mode of inheritance for the white eye color mutation is sex-linked, recessive, and controlled through epistatic gene interaction between two genes white (a) and orange (b); the gene for white is epistatic over the gene for orange. The mode of inheritance for both the dark body color (d) and shortened longitudinal veins (sv) is autosomal recessive and each controlled by a single gene. Both mutant alleles were discovered to be located on chromosome 3. The determined location of each of the examined genes aside the known marker mutations used in this experiment is found below. (Figure 1)

**Figure 1**

A. Chromosome 1 (X Chromosome)

B. Chromosome 2

C. Chromosome 3

## Introduction:

The fly *Drosophila melanogaster* is one of the most studied organisms used in biological research in genetics and is considered a model system for several reasons: they contain only 3 pairs of autosomal chromosomes and one pair of sex chromosomes; the entire genome has been sequenced; they are small and easy to grow in the laboratory; they have a short generation and high productivity; and the males do not show recombination.

The purpose of this experiment was to determine the number of genes involved in each mutant phenotype (white eye color, dark body color, short veined wings veins), the mode of inheritance for these mutations, and the genetic loci of each of these genes. The experiment was carried out in three main steps. Unknown flies with wild type flies underwent two reciprocal crosses to determine the mode of inheritance for each mutation; the analysis of the F1 and F2 generations revealed whether the mutant phenotypes were dominant or recessive and autosomal or sex linked. In the next step, U-5309 virgin females were separately crossed with males from M2 and M3 marker stocks that contained known mutations on autosomal chromosomes 2 and 3 respectively. The F1 males of these crosses were back crossed to U-5309 virgin female flies in order to ascertain the genetic loci of the autosomal mutations. Finally, a cross between U-5309 female flies with male flies of known marker mutations on the X chromosome (M1) was carried out to the F2 generation in hopes of establishing the genetic loci of possible sex linked mutations.

## Materials and Methods:

The flies were prepared for observation and handling by anesthetization using $CO_2$. The flies were then transferred to $CO_2$ pad and examined and counted under a microscope. (See pg. 1-6 of lab manual for more details).

Several different strains of flies were used for this experiment: unknown mutant strain U-5309, wild type Oregon R strain, and three marker mutation strains with known mutations. The genotypes and phenotypes for each strain are listed in Table 1.

**Table 1**. Genotypes and phenotypes of fly strains

| Name | Genotype | Phenotype |
|---|---|---|
| U-5309 | unknown | White eyes, shortened longitudinal wing venation, dark body color |
| Oregon-R | + type | Red eyes, normal longitudinal wing venation, light body color |
| Marker 1 (M1) | cv f (x chromosome | Wings lacking cross veins, bent bristles |
| Marker 2 (M2) | Bl L/Cy (chromosome 2) | Short thin bristles, small eyes, curly wings |
| Marker 3 (M3) | Gl Sb/ LVM (chromosome 3) | Small smooth eye, short blunt bristles |

In this experiment, 5 different crosses were used. In Cross A, Oregon-R males and U-5309 virgin females were crossed to produce an F1 generation. The males and females of the F1 generation were then crossed with each other to produce an F2 generation. Likewise, U-5309 males and Oregon R virgin females were crossed to produce an F1 and F2 generation in Cross B. In a separate cross, Cross I, U-5309 virgin females were crossed with M1 males also to produce an F1 and F2 generation. In Cross II and Cross III respectively, U-5309 virgin females and M2 and M3 males were carried out to the F1 generation, and then the F1 males were backcrossed with U-5309 virgin females. (See pp 19-25 of lab manual for more details)

**Results and Discussion:**

The F1 data of crosses A and B show the wild type phenotype for body color in both males and females (Tables 2 and 3). This signifies that the wild type phenotype is dominant over the mutant dark body phenotype. The F2 results of crosses A and B show two phenotypes, light and dark body, in both males and females. The presence of both wild type and mutant body color in the F1 and F2 generations indicate that the gene for body color is autosomal. In the F2 generation, there is a sizable majority of light body color over dark body color, which further supports that dark body color is a recessive mutation and

**Table 2**. F1 of cross A (U-5309 virgin ♀x Oregon R ♂)

| Trait | Wild type | | Mutant Type | |
|---|---|---|---|---|
| | F | M | F | M |
| Body Color | 62 | 38 | 0 | 0 |
| Eye Color | 62 | 0 | 0 | 38 |
| Wing Venation | 62 | 38 | 0 | 0 |

**Table 3**. F1 of cross B (U-5309 ♂ x Oregon R virgin ♀)

| Trait | Wild type | | Mutant Type | |
|---|---|---|---|---|
| | F | M | F | M |
| Body Color | 65 | 61 | 0 | 0 |
| Eye Color | 65 | 61 | 0 | 0 |
| Wing Venation | 65 | 61 | 0 | 0 |

independently assorts (Table 4). Chi square analysis was used to determine whether body color is a single gene trait that independently assorts properly to produce a 3:1 phenotypic ratio in progeny. The p value was calculated to be $1.86 \times 10^{-3}$, which indicates that our data body color is not segregating properly. This either implies that there may be another factor that influences the segregation of body color alleles so that they do not independently assort, or that a larger sample of F2 progeny should be examined to produce more accurate results.

**Table 4**. F2 body color and wing venation data of crosses A and B

| Trait | F2 of A | | F2 of B | |
|---|---|---|---|---|
| | + type | mutant | + type | mutant |
| Body color | 227 | 55 | 270 | 66 |
| Wing venation | 219 | 63 | 284 | 52 |

The F1 data of Crosses A and B also show wild type phenotype for wing venation in both males and females. This suggests that the mutant wing venation is recessive and autosomal. Furthermore, the F2 generation shows males and females both containing both wild type and mutant longitudinal wing venation, and also resulted in significantly more wild type longitudinal wing venation (503) than the mutant phenotype (115). This suggests that the gene for wing venation undergoes independent assortment. However, chi square analysis to test if wing venation is a single gene trait that undergoes independent assortment produced a p value of $2.43 \times 10^{-4}$, which indicates that wing venation is not undergoing independent segregation properly. This result, similar to body color, indicates that there also may be another factor influencing body color segregation or that a larger sample of F2 progeny should be examined to produce more accurate results.

Chi square analysis was also used to see if longitudinal wing venation and body color genes assorted independently from each other. Using the observed ratios obtained from body color data and wing venation data separately, chi square analysis produce a p value of 0, which led to the conclusion that body color and longitudinal wing venation did not assort independently from each other. Recombination analysis produced a genetic map distance of 42.1 map units between the two genes. (See Appendix for all chi square calculations)

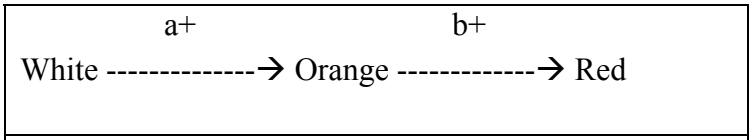
The Cross A F1 results for eye color produced all females with red eyes and all males with white eye color. (Table 2) The females of the F1 generation must all be heterozygous, which shows that the mutant eye color is a recessive mutation. However, since the F1 generation only contained males with white eyes, the gene for eye color was determined to be sex linked; all the males in the F1 generation receive one X chromosome from the mutant female parent, and a Y chromosome from the wild type male parent which does not contain any corresponding wild type alleles. The Cross B F1 results for eye color show both male and female flies with red eyes (Table 3) This is because the males inherit their single x chromosome from their wild type female parent.

**Table 5**. F2 eye color data from crosses A and B

| Eye Color | A | | B | |
|---|---|---|---|---|
| | F | M | F | M |
| Red | 69 | 35 | 145 | 87 |
| White | 76 | 51 | 0 | 84 |
| Orange | 26 | 25 | 0 | 20 |

The F2 generation of Cross B produced several different eye color phenotype: red, white, and orange (Table 5). This suggests that there are two genes involved in determining eye color. The appearance of specifically three different eye colors suggests that eye color is controlled by two genes showing epistatic interaction (Figure 2). The high number of mutant phenotype observed indicates that the gene coding for white eyes (a) is epistatic over the gene coding for orange eyes (b). Also in the B Cross F2 generation, only the red eye phenotype was found in females. If the two genes had undergone independently assortment, the females in F2 would contain more than one phenotype. However, females only showed wild type phenotype for eyes, indicating that these two genes are linked. Moreover, the unequal appearance of different eye colors between the male and female flies of the F2 generation in cross B further supports the previous conclusion that the genes for eye color located on the x chromosome. Using recombination analysis, the genetic map distance between the two genes controlling eye color was determined to be 30.0 map units for cross A and cross B combined.

**Figure 2**: Proposed Pathway for eye color inheritance




| **Phenotype** | **Genotype** |
|---|---|
| Red eye | a+_b+_ |
| Orange eye | a+_bb |
| White eye | aab+_ |
| White eye | aabb |

In order to determine the genetic loci of the two linked autosomal recessive mutations of U-5309, body color and wing venation, males from the M2 and M3 balanced marker stocks were crossed with U-5309 virgin females in Crosses 2 and 3 respectively. The crosses produced an F1 generation that showed wild type phenotypes for both wing venation and body color, as expected. Balanced marker stocks are true breeding heterozygous strains that maintain the same genotype through a homozygous lethal system. 50 flies of each cross were scored to confirm that the marker mutations, body color, and wing venation were all assorting properly.

Continuing Cross 2, male F1 flies with lobed eyes were back crossed with virgin U-5309 females. There were not enough progeny from this cross for allow for statistical significance. However, from the few flies that were scored, the back cross progeny showed three distinct phenotypes: light body, normal wing venation, and bristle lobed; light body normal wing venation, and curly wing; dark body, mutant wing, and curly wing. Since dark body color and mutant longitudinal wing appeared with the marker mutations, it indicates that the body color gene and wing venation gene are not located on the same chromosome as the marker mutations, the 2$^{nd}$ chromosome (Figure 1B). The white eye phenotype also appeared with the marker 2 mutations in male backcross progeny, confirming that the genes for eye color are not located on the 2$^{nd}$ chromosome, as expected. The less stable homozygous lethal system of the marker genes along with the exposure of $CO_2$ to the F1 males may account for the extremely low backcross progeny count.

Cross 3 was continued by back crossing male F1 flies with virgin U-5309 females to see if the body color gene and wing venation gene are located on the 3$^{rd}$ chromosome. There were only two different phenotypes observed in the progeny: light body, normal wing venation, glued eyes, and stubble bristles; dark body, mutant wing venation. Only wild type phenotypes for body color and wing venation appeared with the marker mutations glued eye and stubble bristles in the male backcross progeny, indicating that both wing venation and body color genes are both on the 3$^{rd}$ chromosome. It was known that the wing venation mutation is located at 0 on the chromosome

3. Therefore, according to the distance between the two genes previously determined through recombination analysis, the location of the body color gene is at 42.1 map units on the third chromosome, between the genes for glued eye and stubble bristles (Figure 1C).

To discover the genetic loci on the x chromosome of the two genes controlling eye color, virgin U-5301 females were crossed with marker 1 males (Cross I). The F1 generation contained red eyed females and white eyed males, as expected. Among the 50 female progeny of the F2 generation, the phenotype found in the least number was orange eyes. This further supports our conclusion of an epistatic relationship between the two genes. A total of 104 male progeny were scored and 6 phenotypes were observed with the possibility of 8 different genotypes between the three genes: cv, a, b. (Table 6). The phenotypes that occurred twice were corrected to the number of its corresponding recombinant allele in order to account for having two possible genotypes. Since the location of gene a already was already known to be at position 1.5, the distance between gene a and gene cv was used to determine the correction factor. The corrected distances showed that the distance between cv-b was the largest, thus placing gene a between cv and b (Table 7). However, this would place gene b in a location entirely off of the chromosome. Therefore it was predicted that the data collected was not representative of the actual genetic loci of gene b.

| Genes | Calculated map distance (m.u.) | Corrected map distance (m.u) (c.f.=12.2/52) |
|---|---|---|
| cv---a | 52 | 12.20 |
| cv---b | 61 | 14.31 |
| a---b | 47 | 11.03 |

**Table 6**. 3-point cross between cv-a-b

| cv-a-b | | |
|---|---|---|
| Phenotypes | 8 Genotypes | #'s |
| White eye | cv+ a b | 36 (8) |
| Crossveinless | cv a+ b+ | 8 |
| White eye | cv+ a b+ | 36 (12) |
| Crossveinless, orange eye | cv a+ b | 12 |
| Wild type | cv+ a+ b+ | 12 |
| Crossveinless, white eye | cv a b | 26 (12) |
| Orange eye | cv+ a+ b | 8 |
| Crossveinless white eye | cv a b+ | 26 (8) |

Another 3 point cross was calculated to determine the location of the two eye color genes in relation to the known forked bristle gene of the marker mutation. (Table 8). Similar to the cv-a-b cross, the phenotypes that occurred twice were corrected to the number of its corresponding recombinant allele in order to account for having two possible genotypes. The

**Table 8**. 3-point cross between f-a-b

| cv-a-b | | |
|---|---|---|
| Phenotypes | 8 Genotypes | #'s |
| White eye | f+ a b | 38 (10) |
| Forked bristles | f a+ b+ | 10 |
| White eye | f+ a b+ | 38 (12) |
| Forked bristles, orange eye | f a+ b | 12 |
| Wild type | f+ a+ b+ | 12 |
| Forked bristles, white eye | f a b | 24 (12) |
| Orange eye | f+ a+ b | 8 |
| Forked bristles, white eye | f a b+ | 24 (8) |

distance between f-b was calculated to be the largest, therefore supporting the placement of gene a between genes b and f. Since the locations of gene a and gene f were both given, the distance between them was used in the correction factor. (Table 9).

One possible explanation for the inconsistent results in the 3 point crosses could be attributed to the difficulty in distinguishing between orange and red eyes of the back cross progeny in Cross I. Also, if more Cross 1 F2 progeny had been scored, perhaps more accurate results could be obtained.

**Table 9**. f-a-b 3-point distance calculations

| Genes | Calculated map distance | Corrected map distance (c.f.= 55.2/47) |
|---|---|---|
| f---a | 47 m.u. | 55.2 |
| f---b | 57 m.u. | 66.9 |
| a---b | 47 m.u. | 55.2 |

A 2-point cross was also determined between cv-f. (Table 10) Based on knowing the actual gene loci of genes cv and f, it was expected that would be more crossveinless and forked bristled progeny than actually observed. The calculated map distance between the two genes based on our observed results was 3.8, which is significantly smaller than the known 43 m.u. It is not reasonable to use the correction factor of 43/3.8 to correct the values derived from the 3-point crosses because the values calculated would be much larger than the actual chromosome. Scoring more than progeny may have resulted in relatively more recombinant progeny, therefore producing a more accurate distance between the two genes.

**Table 10**. cv-f 2-point cross

| cv-f | |
|---|---|
| 4 phenotypes | #'s |
| Crossveinless, forked bristles | 44 |
| Wild type | 56 |
| Crossveinless | 2 |
| Forked bristles | 2 |

Based on previously knowing the locations of genes a, cv, and f, and only looking at the relative positions of the genes to each other, the gene order should be b-a-cv-f. However, the map distances between the four genes given in the point crosses are significantly inconsistent with each other. All three point crosses placed gene b in a location off of the actual x chromosome. Because of these unfeasible results, the genetic distance determined through recombination analysis of Crosses A and B was ultimately used to determine the location of gene b. Since the location of gene a at 1.5 is known, gene b must be located at position 31.5. This would place gene b between gene cv and gene f at positions 13.7 and 56.7 respectively (see Figure 1).

**Summary:**

In our experiment, it was determined through crosses A and B that the genes for wing venation and body color are autosomal, recessive, and linked. From the F2 generation of both crosses, the genetic distance between the two genes was calculated to be 42.1. Crosses II and III located gene d and gene sv on chromosome 3. Although Cross II did not produce enough back cross progeny for significance, the data obtained still suggested that genes sv and d were both located on the 3rd chromosome. Therefore, given that the location of gene sv is at 0 on chromosome 3, it was determined that the location of gene d is at position 42.1 of the same chromosome.

Through Cross A and Cross B, it was revealed that our mutant eye color is controlled by two genes that exhibit epistatic interaction. The gene for white eyes is epistatic over the gene for orange eyes. Based on the phenotypes of the F1 and F2 generations, it was found that the genes for eye color are also recessive and sex linked. The two 3-point crosses and one 2-point cross analysis of Cross I resulted in a location for the orange eye gene that was unfeasible. Since the genetic location of gene a on the x chromosome was previously known, the location of gene b was determined to be at position 30.0 based on the recombinant frequencies calculated from crosses A and B. This would place gene b between the marker genes cv and f at 13.7 and 56.7 m.u. respectively.

**Appendix:**

1. Skeletal Report
2. Chi square calculations:

| | +type: mutant |
|---|---|

| | |
|---|---|
| Observed # | 497:121 |
| Observed ratio | 4.107:1 |
| Expected ratio | 3:1 |
| Expected # | 463.5:154.5 |

$p=1.86x10^{-3}$

Body Color:
hypothesis: body color is a single gene trait that segregates properly to produce a 3:1 phenotypic ratio in progeny

$X^2=9.63$
d.f.=1

Wing Venation:

| | +type: mutant |
|---|---|
| Observed # | 497:121 |
| Observed ratio | 4.107:1 |
| Expected ratio | 3:1 |
| Expected # | 463.5:154.5 |

hypothesis: longitudinal wing venation is a single gene trait that segregates properly to produce a 3:1 phenotypic ratio in progeny

$X^2=13.47$
d.f.=1
$p=2.43x10^{-4}$

Body Color and Wing Venation

| | Observed | | Expected | |
|---|---|---|---|---|
| | Actual # | Ratio | Actual # | Ratio |
| wt body color wt wing venation | 435 | 8.2 | 476.5 | 17.63 |
| m body color wt wing venation | 68 | 1.17 | 110.8 | 4.1 |
| wt body color m wing venation | 62 | 1.17 | 116.3 | 4.3 |
| m body color m wing venation | 53 | 1 | 27.0 | 1 |

Hypothesis: body color and longitudinal wing venation assort independently

$X^2= 70.2$
d.f.=3
p=0

**References:**

1. MCDB 306 Genetics Laboratory Manual, Fall 2005