A Study on Inheritance Patterns on Wing Shape, Body Color, and Eye Color in *Drosophila melanogaster*

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Unique Stock

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ABSTRACT

Due to its small size, short life cycle, abundance of genetic variability, and relative inexpensiveness, *Drosophila melanogaster*, the fruit fly, is used as the model organism. Mendelian traits studied in the project were eye color on chromosome 1 (sex-linked trait), vestigial wing (on chromosome 2), and ebony-colored body (on chromosome 3). For one-gene segregation, the flies with red eye were wild phenotype while these with white eye were mutant phenotype. For two-gene segregation, the flies being full wing and normal body color were wild phenotype while these being vestigial wing and ebony body were mutant phenotype. The traits for two-gene segregation were wing and body color. The crosses and reciprocal crosses were produced for one-gene and two-gene segregations. The expected overall phenotypic ratios for one-gene segregation were 3 red eye : 1 white eye in the cross and 1 red eye female : 1 white eye female : 1 red eye male : 1 white eye male in the reciprocal cross. The expected overall phenotypic ratios for two-gene segregation were the same, 9:3:3:1. The results of X^2 statistical test for one-gene segregation didn't fit 3:1 ratio but fit 1:1:1:1 ratio. The results of X^2 statistical test for two-gene segregation didn't fit 9:3:3:1 ratio in both crosses. The reasons that caused the distortion appear to include the purity of the commercial strains and the Meiotic Drive Elements which are the complex nuclear genetic loci found in the natural population of fruit fly and distort the fundamental laws of inheritance. Future investigations at the molecular level seems likely to provide the insightful explanations and the potential areas to study further.

KEYWORDS: *Drosophila melanogaster*, Eye Color, Body Color, X^2 Test

INTRODUCTION

Klug thoroughly described two Mendelian genetic laws and the modification of Mendelian ratios in the book titled Essentials of Genetics (Klug et al., 2010). The genes present on homologous chromosomes segregate from each other and assort independently with other segregating chromosomes during gamete formation. These two genetic laws illustrate the basic principles of gene transmission from parent to offspring. However, when gene expression does not adhere to a simple dominant/recessive mode, or when more than one pair of genes influences the expression of a single character, or when the genes are present on the X chromosome, namely X-linkage, the classic 3:1 and 9:3:3:1 ratios are usually modified. Despite the greater complexity of these exceptions, the fundamental principles of classical Mendelian genetics still hold.

Fruit fly, *Drosophila melanogaster*, has long been useful for demonstrating the principles in the classroom and has also helped students understand biochemical and behavioral genetics. This model organism possesses many attributes which have contributed to its popularity. The fruit fly is easily cultured and its generation time is only two weeks at 21 ºC. Each individual fly is large enough for clear notation of mutant phenotypes. Carolina Biological Supply Company has a large inventory of the strains displaying the various mutant traits such as white eye, vestigial wing, etc. (Carolina Biological Supply Company, 2020). Transmission genetics plays a critical role in comprehending the many branches in modern genetics although not many such experiments are conducted nowadays. The objective of the study was to investigate gene segregation ratios in the classical, genetic patterns for eye color, wing presence and body color using the commercial strains and to verify the validation of these strains in genetic research.

METHODS

Four mutant strains were purchased from Carolina Biological Supply Company in 2019. They were wild type on chromosome 1 (X^RY, X^RX^R), white eye on chromosome 1 (X^rY, X^rX^r), vestigial-winged with red-eye, normal-colored body on chromosome 2 (vvEE), and winged with red-eye, ebony-colored body on chromosome 3 (VVee)

For the gene segregation in the dihybrid cross, vestigial-winged with red-eye, normal-colored body strain (bbAA) was mated by winged with red-eye, ebony-colored body strain. The expected segregation ratio in F_2 is 9 winged, normal (V_E_), 3 winged, ebony (V_ee), 3 vestigial, normal (vvE_), 1 vestigial, ebony (vvee). The reciprocal cross was created with the same expected segregation ratio. Male and female flies are scored separately.

For the X-link cross, wild type strain (X^RY, X^RX^R) was crossed by white eye strain (X^rY, X^rX^r). The expected female phenotypic ratio is all red eye female and the expected male phenotypic ratio is 1 red eye male : 1 white eye male. In the reciprocal X-link cross, the expected female phenotypic ratio: 1 red eye female : 1 white eye female and the expected male phenotypic ratio: 1 red eye male : 1 white eye male. Male and female flies are scored separately.

The mating maps were given as follows.

Mendelian traits: wing and body color

($P_1 \times P_2$) cross

P_1: vvEE (vestigial, normal-colored body) ♀ × P_2: VVee (winged, ebony-colored body) ♂

↓

F_1 VvEe (winged, normal) ♀ × VvEe (winged, normal) ♂

↓

F_2

Gamete genotype	VE	Ve	vE	ve
VE	VVEE	VVEe	VvEE	VvEe
Ve	VVEe	VVee	VvEe	Vvee
vE	VvEE	VvEe	vvEE	vvEe
ve	VeEe	Vvee	vvVe	vvee

($P_2 \times P_1$) reciprocal cross

P_2: VVee (winged, ebony-colored body) ♀ × P_1: vvEE (vestigial, normal-colored body) ♂

↓

F_1 VvEe (winged, normal) ♀ × VvEe (winged, normal) ♂

↓

F_2

Gamete genotype	VE	Ve	vE	ve
VE	VVEE	VVEe	VvEE	VvEe
Ve	VVEe	VVee	VvEe	Vvee
vE	VvEE	VvEe	vvEE	vvEe
ve	VeEe	Vvee	vvVe	vvee

Eye color on X chromosome

($P_1 \times P_2$) cross

P generation X^RX^R (Red) ♀ × X^rY (White) ♂

↓

F_1 X^RX^r (Red) ♀ × X^RY (Red) ♂

↓

F_2

Gamete genotype	X^R	Y
X^R	X^RX^R (Red) ♀	X^RY(Red) ♂
X^r	X^RX^r (Red) ♀	X^rY (White) ♂

($P_2 \times P_1$) reciprocal cross

P reciprocal X^rX^r (White) ♀ × X^RY (Red) ♂

↓

F_1 X^RX^r (Red) ♀ × X^rY (White) ♂

↓

F_2

Gamete genotype	X^r	Y
X^R	X^RX^r (Red) ♀	X^RY (Red) ♂
X^r	X^rX^r (White) ♀	X^rY (White) ♂

In order to cross the flies, FlyNap, an anesthesia agent available commercially through Carolina Biological Supply Company, was soaked on the end of a wand. The wand was then inserted into the vial in a manner which allowed none of the flies to escape. The flies were monitored to determine when the FlyNap should be removed from the vial once fully anesthetized. The process of anesthetizing the flies took around 2-5 minutes. Caution is taken to avoid overexposure to FlyNap which is lethal to the flies in excessive dosage.

After the flies were fully anesthetized, the cap of the vial was removed and placed under a dissecting microscope to identify sexual features. Once the sex of each fly was identified, five males and five females were placed into a vial containing culture media. A total of twenty males and twenty females were selected in four separate vials. The vials were laid on the side to ensure the flies did not get stuck to the culture medium. After the flies recuperated from the FlyNap, the vials were placed upright.

In four days, the parent flies from the previous generation was removed from the vials. The larvae were developed into mature flies within 14-20 days. In F_2 generation, mature flies were scored under a dissecting microscope according to their traits.

The flies were maintained in sponge-capped plastic vials containing roughly one inch of culture media and yeast cells. The whole culturing process took place at room temperature.

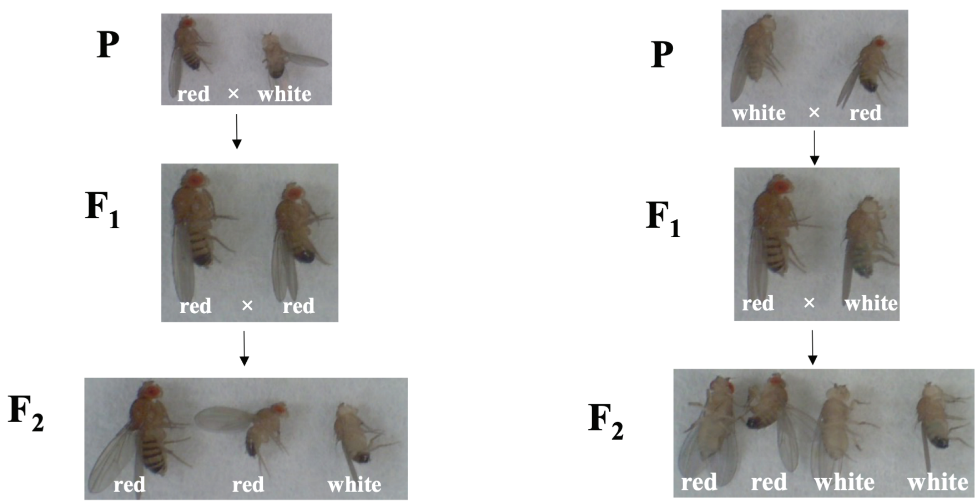
The X^2 statistical test was chosen to detect the fitness of the segregation ratios (Klug, 2010).

RESULTS

Eye color gene on X chromosome in monohybrid crosses

From a cross between red eye female and white eye male in (a) of Figure 1, the phenotypes in F_2 were present as red eye female, white eye male and white eye female, whereas from a reciprocal cross between white eye female and red eye male the phenotypes in F_2 were shown as red and white eye in both female and male.

Table 1 shows the results of the X^2 tests performed on the total number of flies in two crosses related to eye color. The results for the cross between red eye and white eye strain



(a) red × white cross (b) white × red reciprocal cross

Figure 1. A display of the phenotypes of fruit flies in two crosses.

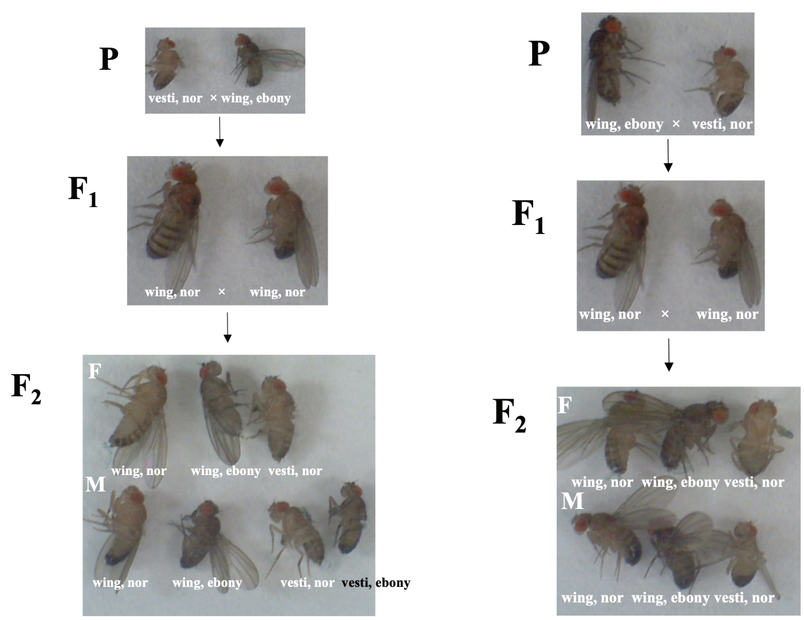
showed that X^2 value was higher than 3.84 (X^2 value at 5% significant level with degree of freedom of one). The probability was smaller than 5% indicating that the observed segregation ratio didn't fit the expected 3:1 ratio. However, the result for the reciprocal cross between white eye and red eye strain revealed that X^2 value was lower than $X^2_{0.05,3}$ = 7.82. The probability was greater than 5% demonstrating that the observed segregation ratio followed the expected 1:1:1:1 ratio.

Red × White				White × Red			
Phenotype	Obs	Exp	X^2	Phenotype	Obs	Exp	X^2
Red eye (female and male)	154	185.3	5.27	Red eye (female)	186	166.3	2.35
White eye (male)	93	61.8	15.81	White eye (male)	157	166.3	0.51
Total	247	247	21.09	Red eye (male)	179	166.3	0.98
				White eye (male)	143	166.3	3.25
				Total	665	665	7.09

Table 1. The results of X^2 tests for two crosses.

Wing shape and body color in dihybrid crosses

In a dihybrid cross between the strain of vestigial wing with red-eye, normal-colored body and the strain of wing with red-eye, ebony-colored body shown in (a) of Figure 2, the phenotypes for female and male in F_2 were present as winged with normal-colored body, winged with ebony-colored body, and vestigial with normal-colored body, but the vestigial with ebony-colored body was absent in female and present in male.



(a) vesti, nor × wing, ebony cross (b) wing, ebony × vesti, nor reciprocal cross

Figure 2. A display of the phenotypes of fruit flies in two dihybrid crosses.

In the dihybrid reciprocal cross between the strain of wing with red-eye, ebony-colored body and the strain of vestigial wing with red-eye, normal-colored body shown as (b) in Figure 2, the phenotypes for female and male in F_2 were present as winged with normal-colored body, winged with ebony-colored body, and vestigial with normal-colored body, but the vestigial with ebony-colored body was absent.

The X^2 tests were conducted for two dihybrid crosses on the female, male and the total number of flies in relation to wing presence and body shape.

	Female			Male			Female + Male		
Phenotype	Obs	Exp	X^2	Obs	Exp	X^2	Obs	Exp	X^2
Winged, normal (V_E_)	223	154.7	30.17	214	147.4	30.12	437	302.1	60.28
Winged, ebony (V_ee)	36	51.6	4.70	35	49.1	4.06	71	100.7	8.75
Vestigial, normal (eeV_)	15	51.6	25.93	12	49.1	28.06	27	100.7	53.93
Vestigial, ebony (vvee)	1	17.2	15.25	1	16.4	14.44	2	33.6	29.68
Total	275	275	76.04	262	262	76.67	537	537	152.64

Table 2. The results of X^2 tests for the dihybrid cross (vvEE × VVee).

In the dihybrid cross to observe two-gene segregation in Table 2, a total of 537 flies were counted. Of these, there were 262 female and 275 male flies. All X^2 test results indicated that probabilities were smaller than 5% because all X^2 values were greater than $X^2_{0.05,3} = 7.82$. It meant that the segregations of the genes controlling wing presence and body color didn't obey second Mendelian genetic law. The number of vestigial wing, ebony body color flies was less than expected number.

	Female			Male			Female + Male		
Phenotype	Obs	Exp	X^2	Obs	Exp	X^2	Obs	Exp	X^2
Winged, normal (V_E_)	259	202.5	15.76	194	149.1	13.55	453	351.6	29.27
Winged, ebony (V_ee)	83	67.5	3.56	67	49.7	6.03	150	117.2	9.19
Vestigial, normal (eeV_)	18	67.5	36.30	4	49.7	42.01	22	117.2	77.32
Vestigial, ebony (vvee)	0	22.5	22.50	0	16.6	16.56	0	39.1	39.06
Total	360	360	78.12	265	265	78.15	625	625	154.84

Table 3. The results of X^2 tests for the dihybrid reciprocal cross (VVee × vvEE).

As shown in Table 3, the dihybrid reciprocal cross was for the observation of two-gene segregation. A total of 625 flies were recorded. Of these, there were 360 female and 265 male flies. All X^2 test results indicated that probabilities were smaller than 5% because all X^2 values were greater than $X^2_{0.05,3} = 7.82$. It meant that the segregations of the genes controlling wing presence and body color didn't conform to second Mendelian genetic law. The similar test results were found from the dihybrid cross above. Unfortunately, vestigial wing, ebony body color flies were not even seen in the cross.

DISCUSSION

Thomas Hunt Morgan discovered his first *Drosophila* mutant, a white-eyed male more than a century ago (Morgan, 1910). The mutant gene was named as white and resided on the X chromosome. Since then, the gene has been used to explore fundamental questions in genetics (Green, 2010). Morgan's skepticism about Mendelian genetics insired him to do research on Mendelism by searching for heritable phenotypic changes in the vinegar fly, *Drosophila melanogaster*. His research experiment related to the genes on X-chromosome was the classic example to interpret the segregating ratio. In our study, the goodness of fit test in one of the crosses for eye color gene on sex chromosome didn't conform with Morgan's early discovery indicating that the purity of parental strains from Carolina might not contain the highest quality.

The wing shape and body color didn't observe the second Mendelian genetic law, either. The possible reasons might be found in other studies. Meiotic Drive Elements (MDs) are the complex nuclear genetic loci found in various eukaryotic genomes and distorting segregation in their favor. Mendel laws of inheritance can be altered by them (Grognet et al., 2014). MDs skew the expected 1:1 ratio in their favor and are thus overrepresented in the progeny after meiosis. They have been observed in metazoans, plants, and fungi (Pennisi, 2003). They may play a critical role in population behavior, leading to sex ratio distortion and thus decreasing population size. Additionally, fitness can also be altered by MD factors if they are genetically linked to alleles that confer deleterious traits (Saupe, 2012). The results of the goodness of fit tests for the traits on X-chromosome and other autosomes in our experiment indicated that the cause of distorted ratios might come from MDs in the genes of the commercial strains used.

Investigation of "Segregation Distorter" in *Drosophila* has showed that MDs are composed of at least two linked genes, the distorter that acts as a toxin by disrupting the formation of gametes, and the responder that acts as an antitoxin that protects from the deleterious distorter effects (Larracuente and Presgraves, 2012) and (Sandler et al., 1959). Anderson et al. (2009) characterized patterns of polymorphism and divergence in the protein-coding regions of 33 genes across of *Drosophila melanogaster*. Along the *D. melanogaster* lineage several loci exhibited patterns consistent with the maintenance of protein variation (Anderson et al., 2009). Our findings in the study could provide the clue to examine whether these two linked genes in MDs and corresponding protein sequence diversity could be detected and how the genes function in the commercial strains.

It is attractive to continue investigations on the factors that cause the deviation of classical genetic ratios in these fruit fly strains, e.g., find the different interest of other gene and cross the parental strains to identify the segregation ratios, or look into the deviation at a molecular level using DNA markers.

CONCLUSION

For the eye color gene on X chromosome, the segregation in the reciprocal cross (White × Red) followed 1:1:1:1 ratio, but that in a cross didn't observe 3:1. The smaller population size in the cross (Red × White) might cause the observed ratio deviated from the expected one. The appropriate population size in the reciprocal cross resulted in the compliance with 1:1:1:1 ratio.

In two dihybrid crosses, the results the segregations in female, male, and overall didn't fit 9:3:3:1 ratio. The prospective causes can be examined through several approaches mentioned in the discussion.

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