A classic genetic study on mutant genes affecting eye shape and color in *Drosophila melanogaster*

Makenzie Kleinsteuber, Hussein Abujejah and Yusheng Wu*

[1]Department of Biological Sciences, University of the Southwest

*Corresponding author and email: ywu@usw.edu

Abstract

Drosophila melanogaster, the fruit fly, is an excellent organism for genetics studies because it has a short life span, produces large numbers of offspring, and has many types of hereditary variations that can be observed with low-power magnification. Mendelian traits studied were bar eye (*B*), and white eye (*w1*). A cross (bar × white) and its reciprocal cross (white × bar), the corresponding F1 and F2 generations were created according to the mating maps. The goodness of fit test for the observed data against the theoretical genotypic ratios was analyzed using $\chi 2$ statistical test. The results showed that the observed ratios for male and female in both crosses didn't fit the theoretical genotypic ratios since the $\chi 2$ values were much greater than the critical $\chi 2$ value (7.82) at 5% significant level. The causes of the disagreement could be the gene interaction and the role of modifier genes. For *Bar* gene, the penetrance was complete in male for both crosses. This was because of the hemizygote status of the gene. However, the incomplete penetrance and varied expressivities were observed in female for both crosses. In bar × white, when *Bar* was in homozygotic status, the penetrance was 54.7% under red eye (wild type) allele and 0.0% under *white* eye allele. In the heterozygotic status, the different expressivities were 0.4% of round eye, 27.7% of dent eye, and 17.2% of oval eye with red eye allele. There was no expressivity observed with *white* allele. In white × bar, the *Bar* gene in homozygotic status demonstrated 2.6% and 1.0% penetrance under the background of red eye allele and *white* allele in female. The various expressivities displayed 48.5% of round eye, 39.3% of dent eye, and 9.6% of oval eye with red eye allele, whereas 78.4% of round eye, 16.6% of dent eye, and 4.0% of oval eye with white allele, respectively. In male, the *Bar* gene demonstrated a 100% penetrance despite the background in both crosses. A genetic study is still an effective way to investigate the gene interaction.

Introduction

Drosophila melanogaster (fruit fly) is commonly used as a model organism because it has significant properties such as short life cycle, abundance in genetic variations, relative inexpensiveness, and small body size. The Bar mutant was first discovered in a single male by Tice (1914). Bar eyes were restricted to narrow vertical bar of about 90 facets in the male and 70 facets in the female, as contrasted with normal numbers of about 740 for males and 780 for females (Sturtevant, 1925). The nature of the screening pigments of insect affects the color of its eyes. These pigments function to isolate optically each facet of the compound eye from its neighbors, thus improve contrast sensitivity and visual acuity (Summers et al., 1982). The structures of 19 mutant alleles at the *white* locus were analyzed. The results strongly suggested that the insertions were responsible for their associated mutant alleles. Most or all of the insertions were transposons (Zachar et al., 1982). Mackenzie et al. (1999) identified the DNA sequences of five mutant *white* alleles and figured out point mutations which alter amino acids in the encoded White protein.

The Mendelian traits including eye shape, eye color, wing shape, wing presence and body color were chosen to explore whether the segregation ratios followed the traditional genetic laws (Wu et al., 2020; Stock et al., 2021). Those studies showed that most of the traits didn't segregate in accordance with the classic genetic laws. The penetrance and expressivity were the good explanations for the results. Penetrance is the percentage of individuals in each genotype that express the phenotype associated with that underlying genotype. Expressivity refers to the degree that a particular genotype is expressed as a phenotype within a population. Retinoblastoma (Rb) is a hereditary malignant tumor of the retina and occurs in 1 of every 15,000 births. Rb is transmitted as an autosomal dominant trait with almost complete penetrance. However, unaffected carriers with low penetrance were observed. The variable expressivity displayed a range of symptoms such as carriers developing bilateral, unilateral Rb or even benign retinomas (Eloy et al., 2016). In zebrafish, the hypersensitive *houdini* mutant showed the variable phenotypic penetrance. On one hand, the most sensitive 15% of the clutch contained 59% *houdini* homozygotes and 41% heterozygous or wildtype siblings. On the other hand, the least sensitive 15% of the clutch only contained 4% *houdini* homozygotes and 96% heterozygous or wildtype siblings (Jain et al., 2011).

Both *Bar* and *white* genes are located on chromosome 1 or sex chromosome, but at least 50 cM apart. The objectives of the study were to examine whether two-gene segregation in each cross followed the second Mendelian genetic law by comparing the observed ratios with theoretical ratios using $\chi 2$ statistical test, and to determine the penetrance and expressivity for the mutant genes.

Materials and Methods

Fruit Fly Strain

The mutant strains, bar eye and white eye were purchased from Carolina Biological Supply Company (Carolina, 2020) and maintained in the biology lab at the institution.

Sexing flies

It is easy to tell males from females. The coloration of the abdomen is the easiest way to recognize. Males are generally smaller and have a dark and rounded abdomen. On the other hand, females are larger and have a light and tipped abdomen. In addition, males have tarsal sex combs on their first pair of legs. These features are black and very distinctive but can only be seen in males under relatively high magnification.

Collecting virgin females

Females remained virgins for only 8-10 hours after eclosure and needed to be collected within this time frame. Females had the ability to store sperm after a single mating, so if the female for a cross was not a virgin, you were not sure what kind of the genotype of the female used for your cross. It was strongly suggested that you obtained extra virgins in case the fly died before mating. Although females were able to lay eggs as virgins, they would be sterile and no larvae would be produced.

Removal method for selecting virgins

All flies were removed 8-10 hours before collecting (generally this was done first thing in the morning). The surface of food was visually inspected to ensure complete removal of flies. After 8-10 hours (usually before you left work) all females that emerged were collected. All would be virgins. They were placed in a fresh culture vial and waited 2-3 days looking for larvae. Virgin females could lay eggs, but they would be sterile. Since they were photoperiod-sensitive, females tended to eclose early in the morning. Therefore, early collections would ensure the greatest number of virgins for experimentation. However, collection was possible later in the day. When you used CO2 as an anesthetic agent, please place an ice pack on the microscope platform, wipe the moist on the surface by a paper towel and put a piece of white filter on top of the ice pack. After you transferred the flies that had been anesthetized to the paper, those files would not be mobile due to the cold ice pack underneath. Now they were ready to be sorted out.

Culture medium

Instant Drosophila medium from Carolina Biological Company needed to neither cook nor sterilize. It contained a blue coloring agent to facilitate observation of larvae. Equal volume of instant Drosophila medium (one inch of culture medium) and 0.8% propionic acid solution were added to a vial. Then a few grains of dry viable yeast were sprinkled on top. After one minute, flies could be introduced and the vial plugged. In addition, to make the 0.8% propionic acid solution, please add 0.8 ml of propionic acid to 100 ml dH2O (autoclaved).

Fruit Fly Handling

To cross the flies, FlyNap (an anesthesia agent) was soaked on the end of a wand. The wand was then inserted into the vial containing the F1 generation of flies, 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 minutes. Caution was taken to avoid overexposure to FlyNap which was lethal to the flies in excessive dosage.

Creating Crosses

After the flies were fully anesthetized, the cap of the vial was removed and the flies were transferred on to a white surface. They were then placed under a dissecting microscope to identify sexual features. Once the sex of each fly was identified, 5 males and 5 virgin females were placed into a vial containing culture media. This selection occurred three times and a total of fifteen males and fifteen females were selected and placed in three separate vials. The flies had to be placed in their respective vials while the vials were lying on the side to ensure the flies did not get stuck to the culture medium in the new vials. After the flies recuperated from the FlyNap, the vials were placed upright in an incubator at 20°C.

Scoring Fruit Flies

After four days, the F1 generation of flies was removed from the vials. Upon the removal of the F1 generation, larvae developed into mature fruit flies within 10-20 days. Upon the emergence of the F2 generation, mature fruit flies were counted and scored under a dissecting microscope according to their inherited traits.

Mating maps

For the gene on sex chromosome, male and female flies were scored separately. The genotype, phenotype and segregation ratios could be found below. The Mendelian traits chosen were eye shape and eye color (B represents *Bar* gene, and w represents *white* gene).

Cross 1 (P1 × P2)

P1 WWXBXB (red eye, bar eye) ♀ × P2 wwXbY (white eye, round eye) ♂

↓

F1 WwXBXb (red eye, bar eye) ♀ × WwXBY (red eye, bar eye) ♂

↓

F2 generation

Gamete genotype	WXB	wXB	WY	wY
WXB	WWXBXB	WwXBXB	WWXBY	WwXBY
WXb	WWXBXb	WwXBXb	WWXbY	WwXbY
wXB	WwXBXB	wwXBXB	WwXBY	wwXBY
wXb	WwXBXb	wwXBXb	WwXbY	wwXbY

Expected ratio: 3 red, bar (W_XBXB) : 3 red, bar (W_XBXb) : 1 white, bar (wwXBXB) : 1 white, bar (wwXBXb) in female, 3 red, bar (W_XBY) : 3 red, round (W_XbY) : 1 white, bar (wwXBY) : 1 white, round (wwXbY) in male.

Reciprocal cross (P2 × P1)

P2 wwXbXb (white, round eye) ♀ × P1 WWXBY (red, bar eye) ♂

↓

F1 WwXBXb (red eye, bar eye) ♀ × WwXBY (red eye, bar eye) ♂

↓

F2 generation

Gamete genotype	WXB	wXB	WY	wY
WXB	WWXBXB	WwXBXB	WWXBY	WwXBY
WXb	WWXBXb	WwXBXb	WWXbY	WwXbY
wXB	WwXBXB	wwXBXB	WwXBY	wwXBY
wXb	WwXBXb	wwXBXb	WwXbY	wwXbY

Expected ratio: 3 red, bar (W_XBXB) : 3 red, bar (W_XBXb) : 1 white, bar (wwXBXB) : 1 white, bar (wwXBXb) in female, 3 red, bar (W_XBY) : 3 red, round (W_XbY) : 1 white, bar (wwXBY) : 1 white, round (wwXbY) in male.

Statistical analysis

The χ2 statistical test was defined as follows:

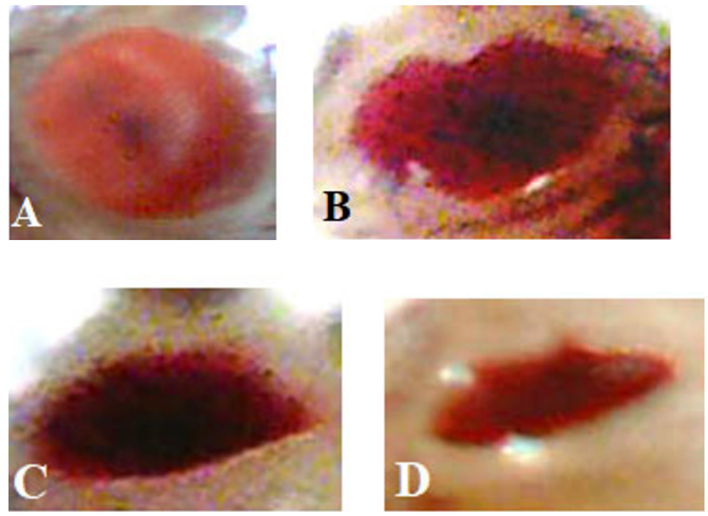
$\chi^2 = \sum \frac{(O-E)^2}{E}$ where O stands for observed number, and E expected number (Klug et al., 2010). It was conducted to detect the fitness of the segregation ratios in Excel.

Results

Eye Shape Phenotypes in The Crosses

Figure 1

The phenotypes of eye shape in two crosses



In Figure 1. A-D displayed the four different eye shapes including round, dent, oval and bar in bar × white and white × bar. A: round eye, B: dent eye, C: oval eye, D: bar eye.

χ2 Goodness of Fit Test

Table 1

The result of χ2 test for 3:3:1:1 genotypic ratio of male in bar × white

Phenotype	Obs	Exp	χ2
Red, bar (hemizygote)	79	103.1	5.64
Red, round (hemizygote)	70	103.1	10.64
White, bar (hemizygote)	54	34.4	11.20
White, round (hemizygote)	72	34.4	41.18
Total	275	275.0	68.67

The ratios of four genotypes (four phenotypes) in male and four genotypes (two phenotypes) in female were derived from the mating map of bar eye strain crossed by white eye strain. In Table 1, it was obvious that the observed number of male files was different from the expected values. The χ2 test result showed the χ2 value (68.67) was much higher than 7.82 (χ2 value at 5% significant level with degrees of freedom of three) indicating that the observed genotypic ratio didn't fit the expected 3:3:1:1 ratio.

Table 2

The result of χ2 test for 3:3:1:1 genotypic ratio of female in bar × white

Phenotype	Obs	Exp	χ2
Red, bar (homozygote)	150	102.8	21.73
Red, variable (heterozygote)	124	102.8	4.39
White, bar (homozygote)	0	34.3	34.25
White, variable (heterozygote)	0	34.3	34.25
Total	274	274.0	94.62

For female, the similar trend appeared in Table 2. The observed number of female flies were much different from the expected number. Again, the χ2 test result demonstrated that the χ2 value (94.62) was much larger than the critical value (7.82) meaning that the observed genotypic ratio didn't follow the expected 3:3:1:1 ratio either. It was noticeable that there was not any white eye flies observed in the cross.

Table 3

The result of χ2 test for 3:3:1:1 genotypic ratio in male of white × bar

Phenotype	Obs	Exp	χ2
Red, bar (hemizygote)	70	109.5	14.25
Red, round (hemizygote)	76	109.5	10.25
White, bar (hemizygote)	45	36.5	1.98
White, round (hemizygote)	101	36.5	113.98
Total	292	292	140.46

In the reciprocal cross (white × bar), the ratios of four genotypes (four phenotypes) in male and four genotypes (two phenotypes) in female were obtained according to the mating map between white eye and bar eye strains. In Table 3, the observed number of male flies were much larger than the expected number in each genotype. The χ2 test revealed that χ2 value (140.46) was much greater than the critical value (7.82). The probability was much lower than 5% demonstrating that the observed genotypic ratio didn't agree with the expected 3:3:1:1 ratio.

Table 4

The result of χ2 test for 3:3:1:1 genotypic ratio in female of white × bar

Phenotype	Obs	Exp	χ2
Red, bar (homozygote)	6	160.5	148.72
Red, variable (heterozygote)	223	160.5	24.34
White, bar (homozygote)	2	53.5	49.57
White, variable (heterozygote)	197	53.5	384.90
Total	428	428	607.54

For female, the similar tendency showed in Table 4. The observed number of female flies were much different from the expected number. Once again, the χ2 test result elucidated that the χ2 value (640.54) was much bigger than the critical value (7.82) illustrating that the observed genotypic ratio didn't conform to the expected 3:3:1:1 ratio either.

Penetrance and Expressivity

Table 5

The penetrance and expressivity of *Bar* gene for female in bar × white

Phenotype	No. of flies	Penetrance (%)	Expressivity (%)
Red, bar (homozygote)	150	54.7	54.7
Red, round (heterozygote)	1		0.4
Red, dent (heterozygote)	76		27.7
Red, oval (heterozygote)	47		17.2
White, bar (homozygote)	0	0.0	0.0
White, round (heterozygote)	0		0.0
White, dent (heterozygote)	0		0.0
White, oval (heterozygote)	0		0.0

In cross of bar × white (Table 5), the *Bar* gene had 54.7% and 0.0% penetrance under the background of red eye allele (wild type) and *white* allele in female. The distinct expressivities were 0.4% of round eye, 27.7% of dent eye, and 17.2% of oval eye with red eye (wild type) allele, respectively. No expressivity was found with *white* allele. In male, the *Bar* gene had a 100% penetrance regardless of the background. (The data was not shown here.)

Table 6

The penetrance and expressivity of *Bar* gene for female in white × bar

Phenotype	No. of flies	Penetrance (%)	Expressivity (%)
Red, bar (homozygote)	6	2.6	2.6
Red, round (heterozygote)	111		48.5
Red, dent (heterozygote)	90		39.3
Red, oval (heterozygote)	22		9.6
White, bar (homozygote)	2	1.0	1.0
White, round (heterozygote)	156		78.4
White, dent (heterozygote)	33		16.6
White, oval (heterozygote)	8		4.0

In the reciprocal cross white × bar (Table 6), the *Bar* gene showed 2.6% and 1.0% penetrance under the background of red eye allele and *white* allele in female. The varied expressivities exhibited 48.5% of round eye, 39.3% of dent eye, and 9.6% of oval eye with red eye allele, whereas 78.4% of round eye, 16.6% of dent eye, and 4.0% of oval eye with *white* allele, respectively. In male, the *Bar* gene demonstrated a 100% penetrance despite the background. (The data was not shown here.)

Conclusion

In this study, the $\chi 2$ goodness of fit test showed that the segregation ratios for the cross and its reciprocal cross didn't agree with second Mendelian genetic law. The incomplete penetrance and distinct expressivities for *Bar* gene were observed in the female of those two crosses. However, 100% penetrance for *Bar* gene was present in the male of the same crosses. It became interesting that bar eye shape inherited in a dominate fashion had the phenotypes that differ in subtle ways.

Discussion

The results are likely caused by the interaction between the *Bar* gene and the *white* gene. Other researchers provided intriguing results in gene interaction, temperature, and modifier genes. Zeleny (1922) created a white bar-eye strain to study the variation in eye facet number over the selection process. The effect of *vestigial* on facet number in *Bar* showed a decrease by slowing down the rate of these general developmental processes (Margolis, 1934). Beam et al. (2010) identified a novel complementation group in *Drosophila melanogaster* named *gang of four (gfr)* that displayed effects on multiple cell biological processes, including tissue growth and retinal patterning. They found that *gfr* alleles behaved as gain-of-function lesions and overexpressed the gene, *bruno-3*, which suppressed *gfr* mutant phenotypes. Singh et al. (2005) reported that the dorsal selector gene *pannier(pnr)* and *Lobe (L)* mutually counteracted each other during second instar of larval development to restrict their functional domains in the eye. They suggested that the mutual antagonism between *L* and dorsal genes was crucial for balanced eye growth. Steinberg (1941) pointed out that an increase in temperature led to a decrease in facet number in *Bar* flies and that temperature was effective during only a limited period of the larval stage. Modifier of *Bar* increased the mean facet number of each of the *Bar* "alleles" but did not affect that of eyeless or wild type (Steinberg, 1941). In sum, the traditional genetic studies played an important role in finding the gene interactions in *Drosophila melanogaster*.

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