# A genetic study on penetrance and expressivity for *Bar* gene in *Drosophila melanogaster*

**Elena Moya, Yusheng Wu***

Department of Biological Sciences, University of the Southwest

*Corresponding author and email E-mail: ywu@usw.edu

**Abstract**

*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 Mendelian trait, eye shape, was chosen in the study. The bar eye (*Bar*) shape was compared to the round eye shape. A cross (bar × round) and its reciprocal cross (round × 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 values for *Bar* gene in male and female of both crosses fit the theoretical genotypic ratios because the $\chi 2$ values were much smaller than the critical $\chi 2$ value (3.84) at 5% significant level. For *Bar* gene, the penetrance was complete in male of both crosses. This was due to the hemizygous status. However, the incomplete penetrance and variable expressivities were observed in female of both crosses. In bar × round, when *Bar* was in homozygous status, the penetrance was 53.0%. In the heterozygotic status, the varied expressivities were 53.0% of bar eye and 47.0% of dent eye, respectively. In round × bar, the *Bar* gene in homozygous status demonstrated 0.0% penetrance. The expressivities displayed 0.0% of bar eye and 100.0% of dent eye, correspondingly. The allelic interaction was the cause for the incomplete penetrance and different expressivities of the gene. The finding provided a foundation for studying the interaction between *Bar* gene and other genes.

## Introduction

*Drosophila melanogaster*, the fruit fly, is an excellent organism for genetics studies because it has short life span, produces large numbers of offspring, and has many types of hereditary variations that can be observed with low-power magnification. Tice (1914) originally discovered *Bar* gene in *Drosophila*. In the mutation of a single male, the eye facets were restricted to a vertical band or bar of irregular outline due to a reduced number of ommatidia. This gene, located at 57.0 cM on Chromosome 1 (sex chromosome), was defined as a semi-dominant mutant. In males and homozygous females, the eyes were small and slit-like. But heterozygous females had dent-shaped eyes. It seemed that the Bar mutation was inconsistent. 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 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 a given 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. Erica et al. (2006) created a mouse null for one of the murine homologues, *Bbs4*, to assess the contribution of one gene to the pleiotropic murine Bbs phenotype and uncovered phenotypic features with age-dependent penetrance and variable expressivity. Immadi et al. (2014) conducted a study on penetrance and expressivity of axillary branching in sorghum. They revealed the stable penetrance of more than 85% for axillary branching and the deviated expressivity.

The objectives of the project were to examine whether *Bar* gene segregation in the crosses followed the first Mendelian genetic law by comparing the observed values with theoretical ratios using $\chi 2$ statistical test, and to determine the penetrance and expressivity for this mutant gene.

## 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 trait selected was eye shape (B represents *Bar* gene on X chromosome).

Cross 1 (bar × round)

P1 XBXB (bar eye) ♀ × P2 XbY (round eye) ♂

↓

F1 XBXb (bar eye) ♀ × XBY (bar eye) ♂

↓

F2

| Gamete genotype | XB | Y |
|---|---|---|
| XB | XBXB (bar) ♀ | XBY (bar) ♂ |
| Xb | XBXb (bar) ♀ | XbY (round) ♂ |

Expected female phenotypic ratio: All bar eye

Expected male phenotypic ratio: 1 bar eye : 1 round eye

Expected overall phenotypic ration: 3 bar eye : 1 round eye

Reciprocal cross (round × bar)

P2 XbXb (round eye) ♀ × P1 XBY (bar eye) ♂

↓

F1 XBXb (bar eye) ♀ × XbY (round) ♂

↓

F2

| Gamete genotype | Xb | Y |
|---|---|---|
| XB | XBXb (bar) ♀ | XBY (bar) ♂ |
| Xb | XbXb (round) ♀ | XbY (round) ♂ |

Expected female phenotypic ratio: 1 bar eye : 1 round eye

Expected male phenotypic ratio: 1 bar eye : 1 round eye

Expected overall phenotypic ration: 1 bar eye : 1 round eye

**Statistical analysis**

The χ2 statistical test was defined as follows:

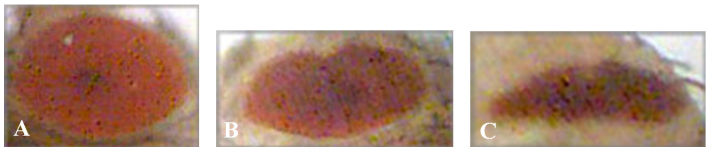
$\chi^2 = \sum \frac{(O-E)^2}{E}$ where O stands for observed number, and E expected number (Klug et al., 2010). It was conducted to detect the fitness of the segregation ratios in Excel.

**Results**

Eye Shape Phenotypes in Two Crosses

Figure 1

The phenotypes of eye shape in bar × round and round × bar




In Figure 1, A-C displayed three different eye shapes including round, dent, and bar in bar × round and round × bar. A: round eye, B: dent eye, C: bar eye.

**χ2 Goodness of Fit Test**

**Table 1**

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

| Category | O | E | 0-E | (O-E)² | (O-E)²/E |
|---|---|---|---|---|---|
| Round | 62 | 63.5 | -1.5 | 2.3 | 0.04 |
| Bar | 65 | 63.5 | 1.5 | 2.3 | 0.04 |
| Total | 127 | 127 | 0 | | 0.07 |

The ratios of two genotypes (two phenotypes) in male and two genotypes (only one phenotype) in female were derived from the mating map of bar eye strain crossed by round eye strain. In Table 1, the observed numbers of round and bar eye male files were not significantly different from the expected values. The χ2 test result showed the χ2 value (0.07) was much smaller than 3.84 (χ2 value at 5% significant level with degrees of freedom of one) indicating that the observed genotypic ratio followed the expected 1:1 ratio.

**Table 2**

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

| Category | O | E | 0-E | (O-E)² | (O-E)²/E |
|---|---|---|---|---|---|
| Dent | 55 | 58.5 | -3.5 | 12.3 | 0.21 |
| Bar | 62 | 58.5 | 3.5 | 12.3 | 0.21 |
| Total | 117 | 117 | 0 | | 0.42 |

For female, the similar trend was showed in Table 2. The observed numbers of dent and bar eye female flies were not much different from the expected values. Again, the χ2 test result demonstrated that the χ2 value (0.42) was much less than 3.84 meaning that the observed genotypic ratio fit the expected 1:1 ratio. It was noticeable that no round eye female flies were observed in the cross.

**Table 3**

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

| Category | O | E | 0-E | (O-E)² | (O-E)²/E |
|---|---|---|---|---|---|
| Round | 75 | 71.0 | 4.0 | 16.0 | 0.23 |
| Bar | 67 | 71.0 | -4.0 | 16.0 | 0.23 |
| Total | 142 | 142 | 0 | | 0.45 |

In the reciprocal cross (round × bar), the ratios of two genotypes (two phenotypes) in male and two genotypes (two phenotypes) in female were obtained according to the mating map between round and bar eye strains. In Table 3, the observed numbers of round and bar eye male flies were not much deviated from the expected numbers. The χ2 test revealed that χ2 value (0.45) was much less than 3.84. The probability was much higher than 5% demonstrating that the observed genotypic ratio agreed with the expected 1:1 ratio.

TABLE 4

THE RESult of χ2 test for 1:1 genotypic ratio in female of round × bar

| Category | O | E | 0-E | (O-E)² | (O-E)²/E |
|---|---|---|---|---|---|
| Round | 84 | 89.0 | -5.0 | 25.0 | 0.28 |
| Dent | 94 | 89.0 | 5.0 | 25.0 | 0.28 |
| Total | 178 | 178 | 0 | | 0.56 |

For female, the similar tendency was present in Table 4. The ob-

served numbers of round and bar eye female flies were not deviated from the expected numbers. Once again, the $\chi 2$ test result elucidated that the $\chi 2$ value (0.56) was much smaller than 3.84 illustrating that the observed genotypic ratio conformed to the expected 1:1 ratio.

**Penetrance and Expressivity**

**Table 5**

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

| Phenotype | No. of flies | Penetrance (%) | Expressivity (%) |
|---|---|---|---|
| Bar (homozygote) | 62 | 53.0 | 53.0 |
| Dent (heterozygote) | 55 | | 47.0 |

In cross of bar × round (Table 5), the *Bar* gene had 53.0% penetrance in homozygous status in female. The expressivities were 53.0% of bar eye and 47.0% of dent eye, respectively. In male, the *Bar* gene had a 100% penetrance in hemizygous status. (The data was not shown here.)

**Table 6**

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

| Phenotype | No. of flies | Penetrance (%) | Expressivity (%) |
|---|---|---|---|
| Bar (homozygote) | 0 | 0.0 | 0.0 |
| Dent (heterozygote) | 94 | | 100.0 |

In the reciprocal cross round × bar (Table 6), the *Bar* gene showed 0.0% penetrance in female. In other words, there was no penetrance in homozygous status in female. The expressivity exhibited 0.0% of bar eye and 100.0% of dent eye, respectively. In male, the *Bar* gene demonstrated a 100% penetrance in hemizygous status. (The data was not shown here.)

## Conclusion

In this study, the $\chi 2$ goodness of fit test showed that the segregation ratios for *Bar* gene in the bar × round and round × bar crosses agreed with first Mendelian genetic law. The incomplete penetrance and disparate expressivities 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 dominant fashion had phenotypes that differ from the theoretical predictions in female only. Homozygotes and heterozygotes for *Bar* gene demonstrated bar eye and dent eye shapes, respectively.

## Discussion

Among the causes of the different phenotypes for Mendelian traits are alternative alleles, environmental factors and modifier genes. Scriver & Waters (1999) and Davis & Justice (1998) well characterized the examples of allelic and environmental variability. Bridges (1919) reported that an eye color gene (eosin) in *Drosophila melanogaster* demonstrated the scale from a deep pink darker than eosin to a pure white. The modifications of eosin produced by these several modifier genes. Allelic interaction between *Bar* gene and round gene might cause the incomplete penetrance. Classic genetic studies remain one of the most powerful ways to find gene and allele interactions.

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