A genetic study on eye shape, body color and eye color in *Drosophila melanogaster*

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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*), scarlet eye color (*st*), and yellow body color (*y*). Two crosses, (bar × yellow) and (bar × scarlet), the corresponding F_1 and F_2 generations were created according to the mating maps. The goodness of fit test for the observed data against the theoretical segregation ratios was analyzed using χ^2 statistical test. The results showed that the observed segregation ratios for male and female in both crosses didn't fit the theoretical ratios because the χ^2 values were much greater than the critical χ^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 due to the hemizygote status of the gene. However, the incomplete penetrance and variable expressivities were observed in female for both crosses. In bar × yellow, when *Bar* was in homozygotic status, the penetrance was 46.5% under wild type background of body color and 55.6% under yellow body color background. In the heterozygotic status, the variable expressivities were 47.1% dent eyes and 6.4% oval eyes with wild type allele, whereas 38.9% and 5.6% with *yellow* mutant. In bar × scarlet, the *Bar* gene in homozygotic status showed 64.2% and 10.5% penetrance under wild type allele and *yellow* allele. In heterozygotic status, the variable expressivities appeared 22.0% dent eye and 13.8% oval eye with wild type background, whereas 79.0% and 10.5% with *scarlet* allele respectively. The genetic study is still an effective way to investigate the gene interaction.

Introduction

Fruit fly, *Drosophila melanogaster*, is commonly used as a model organism because it has significant properties such as short life cycle, abundance in genetic variations, relative inexpensiveness, small body size, etc. 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 yellow locus controlled the melanotic pigment pattern of the cuticle of the adult fly showed a mosaic pigment pattern, some regions of the cuticle being wild type and others yellow in color (Nash & Yarkin, 1974). Scarlet eyes were bright vermilion, darkening with age. It was a reliable trait for classification (Beadle & Ephrussi, 1936).

The Mendelian traits such as wing shape, body color, eye color, and wing presence 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 who 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) did a study on penetrance and expressivity of axillary branching in Sorghum and revealed the stable penetrance of more than 85% for axillary branching and exhibited variable expressivity.

The objectives were to study gene segregation ratios for eye shape, body color and eye color using the commercial strains and to evaluate the penetrance and expressivity of *Bar* gene under different genetic backgrounds.

Materials and Methods

Fruit Fly Strain

The mutant strains, bar eye, yellow body, and scarlet eye, were purchased from Carolina Biological Supply Company (Carolina, 2022) and maintained in biology lab.

Sexing flies

It is quite easy to tell males from females. Males are generally smaller and have a darker and more rounded abdomen. The coloration of the abdomen is the easiest to recognize. In addition, males have tarsal sex combs on their first pair of legs. These are black and very distinctive but can only be seen under relatively high magnification.

Collecting virgin females

Females remain virgins for only 8-10 hours after enclosure and must be collected within this time frame. Females have the ability to store sperm after a single mating, so if the female for a cross is not a virgin, you will not know the genotype of the male used for your cross. It is strongly suggested that you obtain extra virgins in case a mistake is made in identification or the fly dies before mating and egg lying can occur. Although females are able to lay eggs as virgins, they will be sterile, and no larvae will be produced.

Removal method for selecting virgins

Remove all flies 8-10 hours before collecting (generally this is done first thing in the morning). Visually inspect surface of food to ensure complete removal of flies. After 8-10 hours (usually before you leave work) collect all females that are present. All will be virgins. Place in a fresh culture vial and wait 2-3 days look for larvae. Virgin females can lay eggs, but they will be sterile. Since they are photoperiod-sensitive, females tend to enclose early in the morning. Therefore, early collections will ensure the greatest number of virgins for experimentation. However, collection is possible later in the day.

Fruit Fly Handling

Flies are maintained in spongecaped plastic vials containing roughly one inch of culture media and yeast cells. In order to cross the flies, FlyNap (an anesthesia agent) is soaked on the end of a wand. The wand is then inserted into the vial containing the F_1 generation of flies, in a manner which allowed none of the flies to escape. The flies are 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 is taken in order to avoid overexposure to FlyNap which is lethal to the flies in excessive dosage.

Generation of Crosses

After the flies are fully anesthetized, the cap of the vial is removed and the flies are transferred on to a white surface. They are then placed under a dissecting microscope to identify sexual features. Once the sex of each fly is identified, 5 males and 5 females are placed into a vial containing culture media. This selection occurred four times and a total of twenty males and twenty females are selected and placed in four separate vials. The flies had to be placed in their respective vials while the vials are 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 are placed upright. The same procedure is followed in setting up the crosses for the monohybrid flies which are only heterozygous for the sepia eye mutation.

Scoring Fruit Flies

After 4 days, the F_1 generation of flies is removed from the vials. Upon the removal of the F_1 generation, larvae developed into mature fruit flies within 10-20 days. Upon the emergence of the F_2 generation, mature fruit flies are counted and scored under a dissecting microscope according to their inherited traits.

Mating maps

For the gene on sex chromosome, male and female flies are scored separately. The genotype, phenotype and segregation ratios can be found below.

Mendelian traits: yellow body and eye shape (g represents *yellow* gene, B represents *Bar* gene)

($P_1 \times P_2$) cross 1

P_1: GGX^BX^B(gray, bar eye) ♀ × P_2: ggX^bY (yellow, round eye) ♂

↓

F_1 GgX^BX^b (gray, bar eye) ♀ × GgX^BY (gray, bar eye) ♂

↓

F_2 generation

Gamete genotype	GX^B	gX^B	GY	gY
GX^B	GGX^BX^B	GgX^BX^B	GGX^BY	GgX^BY
GX^b	GGX^BX^b	GgX^BX^b	GGX^bY	GgX^bY
gX^B	GgX^BX^B	ggX^BX^B	GgX^BY	ggX^BY
gX^b	GgX^BX^b	ggX^BX^b	GgX^bY	ggX^bY

Expected ratio: 3 gray, bar ($G\_X^BX^B$) : 3 gray, bar ($G\_X^BX^b$) : 1 yellow, bar (ggX^BX^B) : 1 yellow, bar (ggX^BX^b) in female, 3 gray, bar (G_X^BY) : 3 gray, round (G_X^bY) : 1 yellow, bar (ggX^BY) : 1 yellow, round (ggX^bY) in male.

Mendelian traits: scarlet eye and eye shape (r represents *scarlet* gene, B represents *Bar* gene)

($P_1 \times P_2$) cross 2

P_1: RRX^BX^B (red, bar eye) ♀ × P_2: rrX^bY (scarlet, round eye) ♂

↓

F_1 RrX^BX^b (red, bar eye) ♀ × RrX^BY (red, bar eye) ♂

↓

F_2 generation

Gamete genotype	RX^B	rX^b	RY	rY
RX^B	RRX^BX^B	RrX^BX^B	RRX^BY	RrX^BY
RX^b	RRX^BX^b	RrX^BX^b	RRX^bY	RrX^bY
rX^B	RrX^BX^B	rrX^BX^B	RrX^BY	rrX^BY
rX^b	RrX^BX^b	rrX^BX^b	RrX^bY	rrX^bY

Expected ratio: 3 red, bar ($R\_X^BX^B$) : 3 red, bar ($R\_X^BX^b$) : 1 scarlet, bar (rrX^BX^b) : 1 scarlet, bar (rrX^BX^b) in female, 3 red, bar (R_X^BY) : 3 red, round (R_X^bY) : 1 scarlet, bar (rrX^BY) : 1 scarlet, round (rrX^bY) in male.

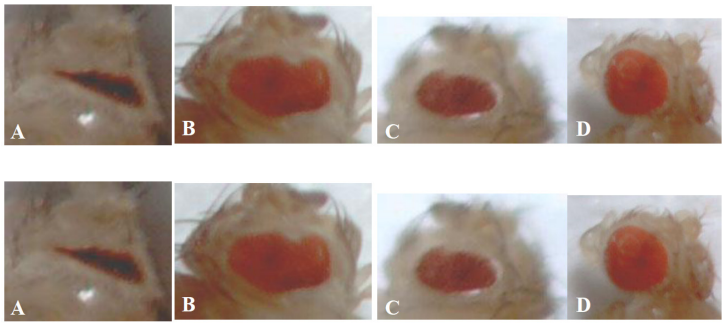
Statistical analysis The χ^2 statistical test is chosen to detect the fitness of the segregation ratios. Microsoft Excel is used to analyze the data.

Results

Eye Shape Phenotypes in The Crosses

Figure 1

The phenotypes of eye shape in two crosses. A bar eye, B dent eye, C oval eye, D round eye in bar × yellow; E bar eye, F dent eye, G oval eye, H round eye in bar × scarlet



The eye shapes of two crosses, bar × yellow and bar × scarlet, were shown as in Figure 1. A-D and E-H displayed the four different eye shapes, bar, dent, oval and round, in those two crosses respectively.

χ^2 Goodness of Fit Test

Table 1

The result of χ^2 test for 3:3:1:1 ratio of male in bar × yellow

Phenotype	Obs	Exp	χ^2
Gray, bar (hemizygote)	77	115.9	13.04
Gray, round (hemizygote)	68	115.9	19.78
Yellow, bar (hemizygote)	55	38.6	6.94
Yellow, round (hemizygote)	109	38.6	128.22
Total	309	309.0	167.99

The ratios of four phenotypes in both male and female was derived from the mating map of bar eye strain crossed by yellow body strain. In Table 1, it was clear that the observed number of male files in each phenotype was different from the expected values. The χ^2 test result showed the χ^2 value was much higher than 7.82 (χ^2 value at 5% significant level with degrees of freedom of three) indicating that the observed segregation ratio didn't fit the expected 3:3:1:1 ratio.

Table 2

The result of χ^2 test for 3:3:1:1 ratio of female in bar × yellow

Phenotype	Obs	Exp	χ^2
Gray, bar (homozygote)	152	129.4	3.96
Gray, variable (heterozygote)	175	129.4	16.09
Yellow, bar (homozygote)	10	43.1	25.44
Yellow, variable (heterozygote)	8	43.1	28.61
Total	345	345.0	74.10

For female, the similar trend appeared in Table 2. The observed number of female flies were much different from the expected number in each phenotype. Again, the χ^2 test result demonstrated that the χ^2 value was much larger than 7.82 meaning that the observed segregation ratio didn't follow the expected 3:3:1:1 ratio either.

Table 3

The result of χ^2 test for 3:3:1:1 ratio in male of bar × scarlet

Phenotype	Obs	Exp	χ^2
Red, bar (hemizygote)	126	117.0	0.69
Red, round (hemizygote)	74	117.0	15.80
Scarlet, bar (hemizygote)	7	39.0	26.26
Scarlet, round (hemizygote)	105	39.0	111.69
Total	312	312.0	154.44

The ratios of four phenotypes for both male and female were obtained according to the mating map between bar eye and scarlet eye strains. In Table 3, the observed number of male flies were much larger than the expected number in each phenotype. The χ^2 test

revealed that χ^2 value was much greater than 7.82. The probability was much lower than 5% demonstrating that the observed segregation ratio didn't fit the expected 3:3:1:1 ratio.

Table 4

The result of χ^2 test for 3:3:1:1 ratio in female of bar × scarlet

Phenotype	Obs	Exp	χ^2
Red, bar(homozygote)	140	103.1	13.19
Red, bar(heterozygote)	78	103.1	6.12
Scarlet, bar(homozygote)	6	34.4	23.42
Scarlet, bar(heterozygote)	51	34.4	8.04
Total	275	275.0	50.77

For female, the similar tendency showed in Table 4. The observed number of female flies were much different from the expected number in each phenotype. Once again, the χ^2 test result elucidated that the χ^2 value was much bigger than 7.82 illustrating that the observed segregation ratio didn't follow the expected 3:3:1:1 ratio either.

Penetrance and Expressivity

Table 5

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

Phenotype	No. of flies	Penetrance (%)	Expressivity (%)
Gray, bar (homozygote)	152	46.5	46.5
Gray, dent (heterozygote)	154	–	47.1
Gray, oval (heterozygote)	21	–	6.4
Yellow, bar (homozygote)	10	55.6	55.6
Yellow, dent (heterozygote)	7	–	38.9
Yellow, oval (heterozygote)	1	–	5.5

In cross of bar × yellow (Table 5), the *Bar* gene had 46.5% and 55.6% penetrance under the background of wild type allele and *yellow* allele in female. The variable expressivities were 47.1% of dent eye and 6.4% of oval eye with wild type body color allele, whereas 38.9% and 5.6% with *yellow* allele respectively. For male, the *Bar* gene had a 100% penetrance (data not shown here).

Table 6

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

Phenotype	No. of flies	Penetrance (%)	Expressivity (%)
Red, bar (homozygote)	140	64.2	64.2
Red, dent (heterozygote)	48	–	22.0
Red, oval (heterozygote)	30	–	13.8
Scarlet, bar (homozygote)	6	10.5	10.5
Scarlet, dent (heterozygote)	45	–	79.0
Scarlet, oval (heterozygote)	6	–	10.5

In cross of bar × scarlet (Table 6), the *Bar* gene showed 64.2% and 10.5% penetrance under the background of wild type allele and *yellow* allele in female. The variable expressivities revealed 22.0% of dent eye and 13.8% of oval eye with wild type eye color allele, whereas 79.0% and 10.5% with *scarlet* allele respectively. For male, the *Bar* gene demonstrated a 100% penetrance (data not shown here).

Discussion

In this study, the χ^2 goodness of fit test showed that the segregation ratios for both crosses didn't agree with classic Mendelian second genetic law. The incomplete penetrance and variable 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 interested that bar eye shape inherited in a simple Mendelian fashion can have phenotypes that differ in subtle ways. Genetic background played a role in guiding the phenotypic consequences of the trait.

Among the reasons of variable 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. Nadeau (2001) provided the examples of modifier genes and their phenotypic effects in mice alleles. The first example was the phenotype of mice with the disorganization (*Ds*) mutation is an example of a trait in which modifiers affect penetrance but not other aspects of the phenotype. The second example was mice that are heterozygous for the *T* mutation in the brachyury gene have

a short tail and homozygotes die during embryonic development. Although *T/+* heterozygotes usually have a short tail, the extent of tail-shortening varies considerably among genetic backgrounds.

Phenotypic modification happens when the expression of one gene alters the expression of another gene. Among the methods to study the modification are the genomic sequence, direct interactions between proteins, mass spectroscopy and other related methods. But genetic studies remain one of the most powerful ways to find both indirect and direct interactions.

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