Phenotypic segregation, penetrance, and expressivity of eye shape and wing shape in *Drosophila melanogaster*

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Abstract

Drosophila melanogaster (fruit fly) is commonly used as a model organism because it has significant properties such as short life cycles, abundance in genetic variations, relative inexpensiveness, and small body size. Mendelian traits studied were bar eye (*B*), apterous body (*ap*), and vestigial body (*vg*). Two crosses, (Bar × apterous) and (Bar × vestigial), the corresponding F_1 and F_2 generations were created according to the mating map. The goodness of fit test for the observed data against the theoretical segregation ratios was analyzed using the χ^2 statistical test. The results showed that the observed segregation ratios for males and females 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 a 5% significant level. The causes of the disagreement could be the gene interaction and the role of modifier genes. For the *Bar* gene, the male penetrance was complete for both crosses. The phenomenon was due to the hemizygote status of the gene. However, incomplete penetrance and variable expressivities were observed in females for both crosses. In Bar × apterous, when *Bar* was in homozygotic status, the penetrance was 12.5% under wild type background of wing shape and 0.0% under *apterous* body background. In the heterozygotic status, the variable expressivities were 41.8% of a round eye, 15.2% of a dent eye, and 30.5% of an oval eye with wild type wing allele, whereas 33.3% of a round eye and 66.7% of an oval with *apterous* allele respectively. There was no dent eye fly. In Bar × vestigial, the *Bar* gene in homozygotic status showed 17.8% and 7.7% penetrance under the background of wild-type and vestigial alleles in females. The variable expressivities exhibited 39.8% of a round eye, 17.5% of a dent eye, and 24.9% of an oval eye with wild type winged allele, whereas 30.8% of a round eye, 42.3% of dent eye, and 19.2% of an oval eye with *vestigial* allele respectively. The genetic study is still an effective way to investigate the gene interaction.

Introduction

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. Bar eyes were restricted to a narrow vertical Bar of about 90 facets in males and 70 facets in females, as contrasted with normal numbers of about 740 for males and 780 for females (Sturtevant, 1925). Mutations at the apterous (*ap*) locus in *Drosophila melanogaster* give rise to three distinct phenotypes: aberrant wings, female sterility, and precocious adult death. The wing phenotype includes five types of abnormality: blistering, deficiencies, duplications, high-order repetitions, and transformation of structures (Stevens & Bryant, 1985). The vestigial locus seems to be mainly involved in developing the wing margin. The mutants are recessive viable (with or without a visible phenotype), recessive lethal, or dominant (with a visible phenotype over the wild type or a *vg* allele); some alleles complement each other; others show pleotropic effects or homeosis (Bownes & Roberts, 1981).

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 traits didn't segregate according to the classic genetic laws. The penetrance and expressivity were reasonable 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 to which a particular genotype is expressed as a phenotype within a population. Erica et al. (2006) created a mouse null for one of the murine homologs, *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 a stable penetrance of more than 85% for axillary branching and exhibited variable expressivity.

The project's objective was to study gene segregation ratios for eye shape and wing shape 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, apterous body, and vestigial body,

were purchased from Carolina Biological Supply Company (Carolina, 2022) and maintained in a 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 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 can 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-laying can occur. Although females can 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 the surface of the food to ensure complete removal of flies. After 8-10 hours (usually before you leave work), collect all females who are present. All will be virgins. Place in a fresh culture vial and wait 2-3 days to 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 most significant 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. To cross the flies, the researchers soak is soaked the end of a wand in FlyNap (an anesthesia agent). The wand is then inserted into the vial containing the F_1 generation of flies, which prevents the flies from escaping. 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 to avoid overexposure to FlyNap, which is lethal to the flies in excessive dosage.

Generation of Crosses

After the flies are fully anesthetized, the vial cap is removed, and the flies are transferred onto a white surface. They are then placed under a dissecting microscope to identify sexual features. Once the sex of each fly is identified, five males and five females are placed into a vial containing culture media. This selection occurred four times, and twenty males and twenty females were selected and placed in four 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. 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 four days, the F_1 generation of flies is removed from the vials. After removing 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 the sex chromosome, male and female flies are scored separately. The genotype, phenotype, and segregation ratios can be found below.

Mendelian traits: apterous wing and eye shape (w represents *apterous* gene, B represents *Bar* gene)

($P_1 \times P_2$) cross 1

P_1: WWX^BX^B (wing, bar eye) ♀ × P_2: wwX^bY (apterous, round eye) ♂

↓

F_1 WwX^BX^b (winged, bar eye) ♀ × WwX^BY (winged, bar eye) ♂

↓

F_2 generation

Gamete genotype	WX^B	wx^B	WY	wY
WX^B	WWX^BX^B	WwX^BX^B	WWX^BY	WwX^BY
WX^b	WWX^BX^b	WwX^BX^b	WWX^bY	WwX^bY
wX^B	WwX^BX^B	wwX^BX^B	WwX^BY	wwX^BY
wX^b	WwX^BX^b	wwX^BX^b	WwX^bY	wwX^bY

Expected ratio: 3 winged, bar ($W\_X^BX^B$) : 3 winged, bar ($W\_X^BX^b$): 1 apterous, Bar (wwX^BX^B): 1 apterous, Bar (wwX^BX^b) in female, three winged, bar (W_X^BY) : 3 winged, round (W_X^bY): 1

apterous, bar (wwX^{B}Y): 1 apterous, round (wwX^{b}Y) in male.

Mendelian traits: vestigial wing and eye shape (v represents *vestigial* gene, B represents *Bar* gene)

(P_1 x P_2) cross 2

P_1: VVX^{B}X^B (wing, bar eye) ♀ × P_2: vvX^{b}Y (vestigial, round eye) ♂

↓

F_1 VvX^{B}X^b (winged, bar eye) ♀ × VvX^{B}Y (winged, bar eye) ♂

↓

F_2 generation

Gamete genotype	VX^B	vx^B	VY	vY
VX^B	VVX^{B}X^B	VvX^{B}X^B	VVX^{B}Y	VvX^{B}Y
VX^b	VVX^{B}X^b	VvX^{B}X^b	VVX^{b}Y	VvX^{b}Y
vX^B	VvX^{B}X^B	vvX^{B}X^B	VvX^{B}Y	vvX^{B}Y
vX^b	VvX^{B}X^b	VvX^{B}X^b	VvX^{b}Y	vvX^{b}Y

Expected ratio: 3 winged, bar (V_X^{B}X^B) : 3 winged, bar (V_X^{B}X^b): 1 vestigial, Bar (vvX^{B}X^B): 1 vestigial, Bar (vvX^{B}X^b) in female, three winged, bar (V_X^{B}Y) : 3 winged, round (V_X^{b}Y): 1 vestigial, bar (vvX^{B}Y): 1 vestigial, round (vvX^{b}Y) in male.

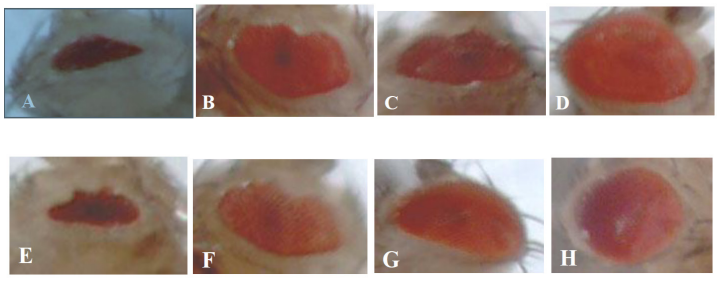
Statistical analysis The χ^2 statistical test is chosen to detect the fitness of the segregation ratios. Microsoft Excel is used to analyze the data.

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 × apterous; E bar eye, F dent eye, G oval eye, H round eye in Bar × vestigial



The eye shapes of two crosses, Bar × apterous and Bar × vestigial, are shown in Figure 1. A-D and E-H displayed the four different eye shapes, including Bar, dent, oval, and round in those crosses, respectively.

χ2 Goodness of Fit Test

Table 1

The result of the χ^2 test for the 3:3:1:1 ratio of males in Bar × apterous

Phenotype	Obs	Exp	χ^2
Winged, bar (hemizygote)	47	71.3	8.25
Winged, round (hemizygote)	143	71.3	72.25
Apterous, bar (hemizygote)	0	23.8	23.75
Apterous, round (hemizygote)	0	23.8	23.75
Total	190	190.0	128.01

The ratios of four male and female phenotypes were derived from the cross which Bar eye strain was crossed by apterous body strain. In Table 1, it was evident that the observed number of male files in each phenotype differed from the expected values. The χ^2 test result showed the χ^2 value (128.01) 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 the χ^2 test for the 3:3:1:1 ratio of females in Bar × apterous

Phenotype	Obs	Exp	χ^2
Winged, bar (homozygote)	37	112.5	50.67
Winged, variable (heterozygote)	260	112.5	193.39
Apterous, bar (homozygote)	0	37.5	37.50
Apterous, variable (heterozygote)	3	37.5	31.74
Total	300	300.0	313.30

For females, a similar trend appeared in Table 2. The observed female flies differed significantly from the expected number in each phenotype. Again, the χ^2 test result demonstrated that the χ^2 value (313.30) 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 the χ^2 test for the 3:3:1:1 ratio in a male of Bar × vestigial

Phenotype	Obs	Exp	χ^2
Winged, bar (hemizygote)	63	73.5	1.50
Winged, round (hemizygote)	122	73.5	32.00
Vestigial, bar (hemizygote)	3	24.5	18.87
Vestigial, round (hemizygote)	8	24.5	11.11
Total	196	196	63.48

The ratios of four male and female phenotypes were obtained according to the mating map between bar eye and vestigial body

strains. In Table 3, the observed male flies were much larger than the expected number in each phenotype. The χ^2 test revealed that the χ^2 value (63.48) was much more significant than 7.82. The probability was much lower than 5%, demonstrating that the observed segregation ratio didn't agree with the expected 3:3:1:1 ratio.

Table 4

The result of the χ^2 test for the 3:3:1:1 ratio in a female of Bar × vestigial

Phenotype	Obs	Exp	χ^2
Winged, bar (homozygote)	43	100.1	32.59
Winged, variable (heterozygote)	198	100.1	95.68
Vestigial, bar (homozygote)	2	33.4	29.49
Vestigial, variable (heterozygote)	24	33.4	2.63
Total	267	267	160.40

For females, a similar tendency is shown in Table 4. The observed female flies differed significantly from the expected number in each phenotype. Once again, the χ^2 test result elucidated that the χ^2 value (160.40) was much bigger than 7.82, illustrating that the observed segregation ratio didn't conform to the expected 3:3:1:1 ratio.

Penetrance and Expressivity

Table 5

The penetrance and expressivity of the *Bar* gene for females in Bar × apterous

Phenotype	No. of flies	Penetrance (%)	Expressivity (%)
Winged, bar (homozygote)	37	12.5	12.5
Winged, round (heterozygote)	124	–	41.8
Winged, dent (heterozygote)	45	–	15.2
Winged, oval (heterozygote)	91	–	30.5
Apterous, bar (homozygote)	0	0.0	0.0
Apterous, round (heterozygote)	1	–	33.3
Apterous, dent (heterozygote)	0	–	0.0
Apterous, oval (heterozygote)	2	–	66.7

In the cross of Bar × apterous (Table 5), the *Bar* gene had 12.5% and 0.0% penetrance under the background of wild-type and apterous alleles in females. The variable expressivities were 41.8% of a round eye, 15.2% of a dent eye, and 30.5% of an oval eye with wild-type wing allele, whereas 33.3% of a round eye and 66.7% of an oval with *apterous* allele, respectively. There was no dent eye fly. For males, the *Bar* gene had a 100% penetrance (data not shown here).

Table 6

The penetrance and expressivity of the *Bar* gene for females in Bar × vestigial

Phenotype	No. of flies	Penetrance (%)	Expressivity (%)
Winged, bar (homozygote)	43	17.8	17.8
Winged, round (heterozygote)	96	–	39.8
Winged, dent (heterozygote)	42	–	17.5
Winged, oval (heterozygote)	60	–	24.9
Vestigial, bar (homozygote)	2	7.7	7.7
Vestigial, round (heterozygote)	8	–	30.8
Vestigial, dent (heterozygote)	11	–	42.3
Vestigial, oval (heterozygote)	5	–	19.2

In the cross of Bar × vestigial (Table 6), the *Bar* gene showed 17.8% and 7.7% penetrance under the background of wild-type and vestigial alleles in females. The variable expressivities exhibited 39.8% of a round eye, 17.5% of a dent eye, and 24.9% of an oval eye with wild type winged allele, whereas 30.8% of a round eye, 42.3% of dent eye, and 19.2% of an oval eye with *vestigial* allele respectively. For males, 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 the classic Mendelian second genetic law. The incomplete penetrance and variable expressivities for the *Bar* gene were observed in the females of those two crosses. However, 100% penetrance for the *Bar* gene was present in the males of the same crosses. It became interesting that bar eye

shapes inherited in a simple Mendelian fashion can have phenotypes that differ subtly. Genetic background played a role in guiding the phenotypic consequences of the trait.

Among the causes of variable phenotypes for Mendelian traits are alternative alleles, environmental factors, and modifier genes. Scriver & Waters (1999) and Davis & Justice (1998) characterized allelic and environmental variability examples. 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 examples of modifier genes and their phenotypic effects in mice alleles. The first example was the phenotype of mice with the disorganization (*Ds*) mutation, 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 short tails, 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 occurs when one gene's expression alters another gene's expression. Among the methods used to study the modification are genomic sequencing, direct interactions between proteins, mass spectroscopy, and other related methods. However, genetic studies remain one of the most powerful ways to find indirect and direct interactions.

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