

Inheritance Patterns in Monohybrid and Dihybrid Crosses for *sepia* eye color and apterous (wingless) Mutations in *Drosophila melanogaster*

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INTRODUCTION

The purpose of this project is to develop an understanding for the inheritance patterns observed in a fruit fly. Due to its small size, short life cycle, abundance of genetic variability, and relative inexpensiveness, *Drosophila melanogaster*, the fruit fly, will be used as the model organism. Traits studied were eye color and presence or absence of wings. The wild-type phenotypes were winged flies and red eyes, while the mutant phenotypes were *apterous* (wingless) and *sepia* (brown) eyes. To build an adequate profile of inheritance patterns both monohybrid and dihybrid crosses were performed.

The monohybrid fruit flies that were crossed possessed a wild-type phenotype for all traits, but were heterozygous for the *sepia* eye mutation, meaning their genotype was Ss for eye color. The parent generation of the monohybrid flies consisted of a fly homozygous dominant for wild-type or red eye color and a fly homozygous recessive for the *sepia* mutation. The genotypes previously described were SS crossed with ss.

The dihybrid flies exhibited the wild-type phenotype for both traits; however, the genotypes for these flies were heterozygous for both the *sepia* eye mutation and the *apterous* mutation. Due to the flies' heterozygosity, the genotype of the F1 generation was Ss for eye color and Aa for the *apterous* mutation. The parent generation of the dihybrid flies consisted of one fly homozygous dominant for both the eye color and the wing presence (SSAA), and the other fly was homozygous recessive for both genes (ssaa).

If the inheritance patterns followed Mendel's hypothesis demonstrating segregation and independent assortment, the F2 generation flies for monohybrid crosses (Ss x Ss) will exhibit a 3:1 phenotypic ratio of wild-type or red eyes to *sepia* eyes. On the other hand, the phenotypic ratio resulting from the dihybrid cross (SsAa x SsAa) will be a 9:3:3:1 of red eyed and winged, red eyed and wingless, *sepia* eyed and winged, *sepia* eyed and wingless, respectively.

METHODS

Fruit Fly Handling Flies were maintained in sponge-capped plastic vials containing roughly one inch of culture media and yeast cells. In order 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 in order to avoid overexposure to FlyNap which is lethal to the flies in excessive dosage.

Generation of 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 females were placed into a vial containing culture media. This selection occurred four times and a total of 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 was followed in setting up the crosses for the monohybrid flies which were only heterozygous for the *sepia* eye mutation.

Scoring Fruit Flies After 4 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. For the monohybrid cross, the eye color was the only phenotype taken into consideration. Male and female flies were initially divided and counted separately. The number of male and female flies was recorded based on the expression of the eye color gene. The two phenotypic categories were red eyed or *sepia* eyed. For the dihybrid cross, male and female flies were also scored separately. For this group both the eye color and the presence or absence of wings was accounted for. The categories in which the male and female flies were counted are as follows: red eye and winged, red eye and wingless, *sepia* eye and winged or *sepia* eye and wingless

RESULTS

For monohybrid crosses, a total of 232 flies were counted. Of these flies, 105 were males and 127 were female. Of the males, 87 expressed the dominant phenotype (red eyes) and 18 expressed the recessive phenotype (*sepia* eyes). Of the female population, 108 had red eyes and 19 had *sepia* eyes. Out of the total amount of flies 195 had red eyes and 37 had *sepia* eyes.

After the monohybrid cross of fruit flies were scored, a chi-square test was performed on the male flies, female flies, and the total number of monohybrid flies that were accounted for. A chart of Chi-square

values and corresponding probability values was used to interpret results (Fig 1.) The male monohybrid flies had a chi-square value of 3.457, with one degree of freedom, resulting in a p value of $0.2 > p > 0.05$ (Table 1). A chi-square analysis for the female monohybrid fruit flies resulted in a chi-square value of 6.8627, with one degree of freedom and a p value of $0.01 > p > 0.001$ (Table 2). Overall, the monohybrid cross had a chi-square value of 10.1378, with one degree of freedom, resulting in a p value of $0.01 > p > 0.001$ (Table 3).

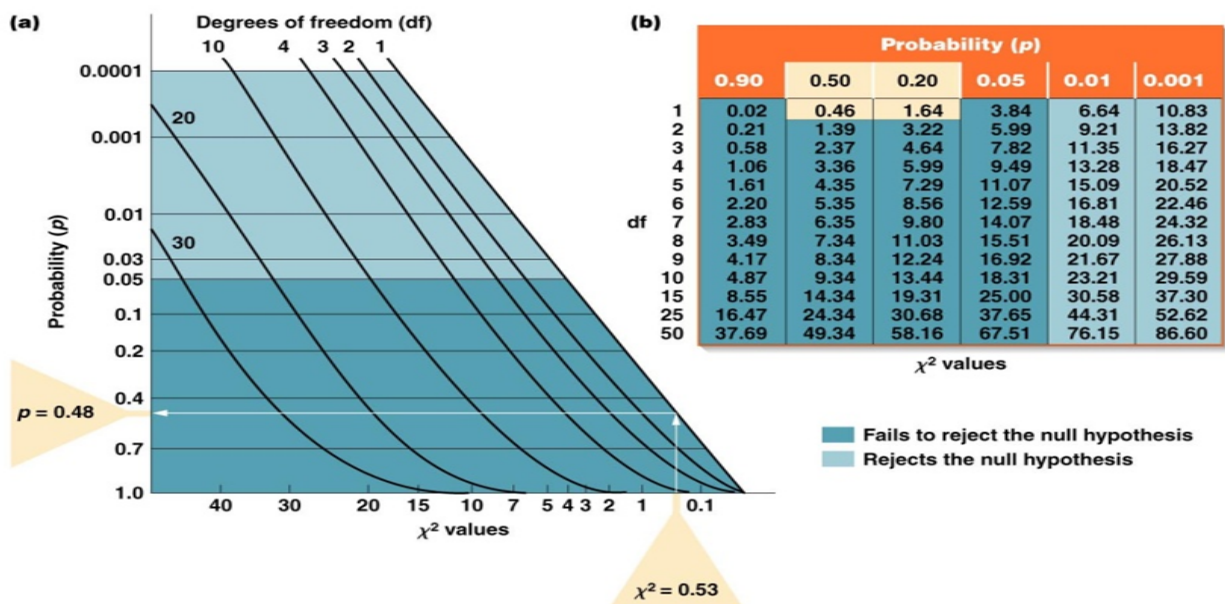


Fig 1. Chart for interpretation of Chi-square values in terms of a corresponding probability value using degree of freedoms exhibited in different types of crosses.

Table 1. Monohybrid Chi-square analysis for male fruit flies

Phenotype	Observed	Expected	Observed-Expected	(Observed-Expected) ²	(Observed-Expected) ² /Expected
Red Eye	87	78.75	8.25	68.065	0.8642
Sepia Eye	18	26.25	-8.25	68.0625	2.5928
					Chi-square = 3.457 P value = 0.2 > p > 0.05

Table 2. Monohybrid Chi-square analysis for female fruit flies

Phenotype	Observed	Expected	Observed-Expected	(Observed-Expected) ²	(Observed-Expected) ² /Expected
Red Eye	108	95.25	12.75	162.5625	1.7067
Sepia Eye	19	31.75	-12.75	162.5625	5.12
					Chi-square = 6.8267 P value = 0.01 > p > 0.001

Table 3. Overall Monohybrid Chi-square analysis

Phenotype	Observed	Expected	Observed-Expected	(Observed-Expected) ²	(Observed-Expected) ² /Expected
Red Eye	195	174	21	441	2.5344
Sepia Eye	37	58	-21	441	7.6034
					Chi-square = 10.1378 P value = 0.01 > p > 0.001

For the dihybrid crosses, a total of 364 flies were scored. Of these, 158 were male and 206 were females. Of the males, 95 exhibited red eyes and wings (double wild-type), 30 exhibited the phenotype for red eyes and wingless, 25 exhibited the phenotype of *sepia* eyes and wings, and 8 exhibited the phenotype *sepia* eyes and wingless (double recessive). Of the females, 117 expressed red eyes and wings, 42 expressed red eyes and wingless, 34 expressed *sepia* eyes and wings, and 13 expressed *sepia* eyes and wingless. For the total, 212 showed red eyes and wings, 72 showed red eyes and wingless, 59 showed *sepia* eyes and wings, and 21 were *sepia* eyed and wingless.

After scoring the flies, a chi-square analysis was conducted on the males, females, and the entire group. Because there were four possible phenotypic outcomes in the dihybrid cross, three degrees of freedom were used to determine the p value of each analysis. The male dihybrid crosses had a chi-square value of 1.4995, with three degrees of freedom, resulting in a P value of 0.9 > p > 0.5 (Table 4). The female dihybrids had a chi-square value of 0.87032, with three degrees of freedom, resulting in a P value of 0.9 > p > 0.5 (Table 5). Overall, the dihybrid cross resulted in a chi-square value of 1.851, with three degrees of freedom and a p value of 0.9 > p > 0.5 (Table 6).

Table 4. Dihybrid Chi-squared analysis for male fruit flies

Phenotype	Observed	Expected	Observed-Expected	(Observed-Expected) ²	(Observed-Expected) ² /Expected
Red Eye, Winged	95	88.875	6.125	37.515625	0.42211
Red Eye, Wingless	30	29.625	0.375	0.140625	0.00474
Sepia Eyed, Winged	25	29.625	-4.625	21.3906	0.72204
Sepia Eyed, Wingless	8	9.875	-1.875	3.5156	0.35601

					Chi-Square=1.4995 P value =0.9>p>0.5
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Table 5. Dihybrid Chi-squared analysis for female fruit flies

Phenotype	Observed	Expected	Observed-Expected	(Observed-Expected) ²	(Observed-Expected) ² /Expected
Red Eye, Winged	117	115.3125	1.6875	2.8476	0.02513
Red Eye, Wingless	42	38.4375	3.5625	12.6914	0.3302
Sepia Eyed, Winged	34	38.4375	-4.4375	19.6914	0.5123
Sepia Eyed, Wingless	13	12.8125	0.1875	0.03515	0.0027
					Chi-square = 0.87032 P value = 0.9>p>0.5

Table 6. Overall Dihybrid Chi-Square Analysis

Phenotype	Observed	Expected	Observed-Expected	(Observed-Expected) ²	(Observed-Expected) ² /Expected
Red Eye, Winged	212	204.75	7.25	52.5625	0.2567
Red Eye, Wingless	72	68.25	3.75	14.0625	0.2061
Sepia Eyed, Winged	59	68.25	-9.25	85.5625	1.2536
Sepia Eyed, Wingless	21	22.75	-1.75	3.0625	0.1346
					Chi-square = 1.851 P value = 0.9>p>0.5

DISCUSSION

The null hypothesis stated that the deviation in observed results from the expected ratio is due to random chance alone. In order to evaluate the null hypothesis, the p values from the chi-square tests were taken into consideration. If the p value was less than .05, this indicated that there was less than 5% chance that deviation was due to random chance, and therefore the null hypothesis would be rejected.

For the monohybrid cross experiments, the p value for the male flies was greater than 0.05, so we failed to reject the null hypothesis. On the other hand, the p value for the female monohybrids and the total amount of monohybrids was less than 0.05 and, therefore, the null hypothesis was rejected. This indicates that something other than random chance caused the variation in the observed results from the

expected results. The variation could be the result of environmental factors, such as the flies and larvae were stored on a cold window seal, which prolonged their life cycle. Another possible factor could be that the F1 generation that was crossed may not have been fully heterozygous for the eye color gene. If both parents were not fully heterozygous, this would alter the expected 3:1 phenotypic ratio of inheritance. For the dihybrid cross, all p values (the male flies, female flies, and total flies) were greater than 0.05. Therefore we failed to reject the null hypothesis, meaning that the deviation of the observed results to the expected results could be explained by random chance.

For future references a larger sample size should be considered. A larger sample size would

give a better projection of inheritance patterns exhibited in the monohybrid and dihybrid crosses.