Determining Acceptance of the 9:3:3:1 Ratio in Fruit Fly Crosses Using the Chi Squared Test

Abstract

In this experiment we set out to determine whether or not two different fruit fly crosses fit the 9:3:3:1 ratio, which is set up by the law of independent assortment. We did this by setting up a flask with first generation flies that gave rise to a second generation, which could be used to observe inheritance of phenotypes based on the parental phenotypes. We put the flies under a dissecting microscope to determine which phenotypes they exhibited, recorded the phenotypes in a table, used the data to determine the chi squared value, and compared our chi squared value to that of a table to determine if it actually fit the expected ratio. We found that in one cross this was true and that the other cross shouldn't have fit it because it didn't follow the law of independent assortment. The second cross shouldn't have fit the ratio because the traits were linked on one gene, which made it impossible for both phenotypes to be shown at the same time.

Introduction

Gregor Mendel succeeded where many had failed when it came to genetics. Two important rules Mendel came up with are the law of segregation and independent assortment. The law of segregation Mendel's first law states that allele pairs separate during gamete formation, and then randomly reform as pairs during the fusion of gametes at fertilization (Campbell and Reece, 2002). An allele is version of a gene. A gamete is a cell such as an egg or sperm, considered the sex cells. Mendel's second law, the law of independent assortment states that each allele pair segregates independently during gamete formation, applies when genes for two characteristics are located on different pairs of homologous chromosomes (Campbell and Reece). Homologous chromosomes have two identical alleles for any given trait.

There are two main types of crosses in genetics: dihybrid and monohybrid. A monohybrid cross uses a single trait that has two alleles, a single aspect of an organism is crossed. A dihybrid cross uses two traits with two alleles each, two different aspects of an organism are crossed each of which are often carried on the same gene. Each cross gives the genotypes of a parental generation's offspring. The given genotypes in turn show the organism's phenotype. Genotype is the genetic makeup of an organism and phenotype is the physical and physiological traits of an organism (Campbell and Reece, 2002). A gene is the inherited determinant of a trait (Dr. Pott, personal communication). A trait is a distinguishing feature of an organism phenotype. An example of a dihybrid cross is shown directly below.

Dihybrid Cross of Second Filial Generation

female \ male	vg^+, se^+	vg^+, se	vg, se^+	vg, se
vg^+, se^+	vg^+/vg^+, se^+/se^+	vg^+/vg^+, se^+/se	vg^+/vg, se^+/se^+	vg^+/vg, se^+/se
vg^+, se	vg^+/vg^+, se^+/se	vg^+/vg^+, se/se	vg^+/vg, se^+/se	vg^+/vg, se/se
vg, se^+	vg^+/vg, se^+/se^+	vg^+/vg, se^+/se	**vg/vg, se^+/se^+**	**vg/vg, se^+/se**
vg, se	vg^+/vg, se^+/se	vg^+/vg, se/se	**vg/vg, se^+/se**	vg/vg, se/se

Table 1 ~ In the above table normal eyes and normal wings show in normal text, sepia eyes and normal wings are underlined, normal eyes and vestigial wings are bold, and sepia eyes and vestigial wings are double underlined. These phenotypes are given in a 9:3:3:1 ratio (Laboratory Manual).

In this experiment the fruit fly, Drosophila melanogaster, was used. The fruit fly was used because they reproduce quickly, which allowed for us to see exactly what the outcomes of each cross were and the phenotypes were easily distinguishable. The objective of this lab was to determine whether or not the Drosophila crosses fit a 9:3:3:1 ratio using the Chi Squared Test. The 9:3:3:1 ratio simply means that nine are wild-type meaning they are normal; six exhibit one mutant and one normal character, three are normal for one trait the other three are normal for the opposite trait; one has both mutant phenotypes. Two different crosses were performed one between vestigial and sepia flies another between ebony and sepia flies. In the vestigial and sepia cross normal, wild-type, flies had normal eyes and wings; mutants which fit the second part of the ratio had vestigial wings and normal eyes while the other three had normal wings and sepia eyes; flies that fit that last part of the ratio had vestigial wings and sepia eyes. In the ebony and sepia cross wild-type flies had a normal body and normal eyes; mutants in the second part of the ratio had an ebony body and normal wings while the other three had a normal body and sepia eyes; flies that fit the last part of the ratio had an ebony body and sepia wings. We predicted that the Drosophila crosses would fit this ratio.

Materials and Methods

In order perform this experiment we first had to set up a cross between the first filial generation (F1) flies. To do this we obtained two culture flasks, we were sure to label them to know which were ours and what cross they contained. To each flask we added two scoops of Drosophila dry medium, which was blue, and added two scoops of water, we then added eight scoops of dried yeast to each flask. For each cross we obtained ten anesthetized flies from each parent generation. We put ten vestigial and ten sepia flies in the flask labeled vg x se. We put ten ebony and ten sepia flies in the flask labeled e x se. We were sure that the vg and e flies were females and the se flies were male.

Once the larva developed in the flasks we let the F1 generation out of the flask, this way we would be sure that only the second filial generation (F2) would be left in the flask when we tested the cross for a 9:3:3:1 ratio. After the flies hatched the first thing we did was anesthetized them using “FlyNap” being careful not too many escaped. We then observed the flies under the dissecting microscope to see what phenotypes they exhibited. We counted the flies in each category and kept track of each type in a table. We did the same thing with the second (e x se) cross.

We used the data from our tables to test our hypothesis using the Chi Squared Test. We accepted our hypothesis if our chi squared value was smaller than the chi squared amount at five percent probability. In order to calculate our chi squared value we had to first determine how many flies in each category were expected to exhibit the characteristic if it were to fit the 9:3:3:1 ratio. We did this by multiplying 9/16, 3/16, or 1/16 by the total number of flies we observed. You multiply by 9/16 if you are determining the number of flies that were normal. You multiply by 3/16 if you are determining the number of flies that were mutant for one characteristic. You multiply by 1/16 for determining the number of flies that were mutant for both characteristics. To calculate the chi squared value we then used this formula $(O-E)^2/E$ (Laboratory Manual), which is the observed number of flies in each category minus the expected number of flies in each category squared divided by the expected number of flies. We then calculated the degree of freedom in the experiment by using this formula df=z-1 (Laboratory Manual), df is the degree of freedom and z is the number of phenotypic classes in this experiment there were four different phenotypes possible. We then used a degree freedom of three at five percent probability on the Chi Squared Table (Laboratory Manual) to determine the value that our chi squared would have to be less than in order to accept it.

Results

The tables below represent the observed and expected number of flies as well as the chi squared value for each cross.

Vestigial and Sepia Cross

Phenotype	**O**	**E**	$\frac{(O-E)^2}{E}$
Normal Wings, Normal Eyes	35	39.375	.48611
Vestigial Wings, Normal Eyes	11	13.125	.344048
Normal Wings, Sepia Eyes	17	13.125	1.14405
Vestigial Wings, Sepia Eyes	7	4.375	1.575
Totals	70	70	3.54921

Table 2 ~ Calculation of chi squared value for a cross between vestigial and sepia flies.

Ebony and Sepia Cross

Phenotype	O	E	$\frac{(O-E)^2}{E}$
Normal Body, Normal Eyes	51	56.25	.49
Ebony Body, Normal Eyes	23	18.75	.963333
Normal Body, Sepia Eyes	22	18.75	.563333
Ebony Body, Sepia Eyes	4	6.25	.81
Total	100	100	2.82667

Table 3 ~ Calculation of chi squared value for a cross between ebony and sepia flies.

The chi squared value for degree of freedom of three at five percent probability is 7.815 based on the table given in the lab manual. I found the total chi squared value of each cross by adding the individual chi squared values from the characteristics together. The chi squared value for the vestigial and sepia cross was 3.54921. The chi squared value for the ebony and sepia cross was 2.82667. The chi squared values for each cross were less than 7.815 meaning that we could accept our hypothesis which was that the crosses would fit the 9:3:3:1 ratio.

Discussion

Our data fit the 9:3:3:1 ratio, however; after learning about gene linkage we were made aware of the fact that there is no possibility that flies which had both ebony bodies and sepia eyes could exist. We counted some flies as having ebony bodies and sepia eyes because of scientific bias, we expected a 9:3:3:1 ratio and we wanted to get one. Gene linkage proves that alleles that are together on the same chromosome tend to stay together, since they are physically connected (Dr. Pott, personal communication). Genes that are located on the same chromosome do not follow the Mendelian rule of independent assortment. Some genes that are linked can cross over, but crossing over is infrequent. Gene linkage only allows for three different phenotypes.

Phenotypes of F2 Generation in the Ebony and Sepia Cross

Female \ Male	**e,se^{+}**		**e^{+},se**	
e,se^{+}	$\frac{e,se^{+}}{e,se^{+}}$	Ebony	$\frac{e,se^{+}}{e^{+},se}$	Wild-type
e^{+},se	$\frac{e^{+},se}{e,se^{+}}$	Wild-type	$\frac{e^{+},se}{e^{+},se}$	Sepia
e^{+},se^{+}	$\frac{e^{+},se^{+}}{e,se^{+}}$	Wild-type	$\frac{e^{+},se^{+}}{e^{+},se}$	Wild-type
e,se	$\frac{e,se}{e,se^{+}}$	Ebony	$\frac{e,se}{e^{+},se}$	Sepia

Table 4 ~ Shows the phenotypic ratios of the ebony and sepia cross (Dr.Pott, personal communication).

Calculation of Chi Squared Value using Gene Linkage

Phenotype	**O**	**E**	$\frac{(O-E)^2}{E}$
Normal Body, Normal Eyes	51	50	.02
Ebony Body, Normal Eyes	23	25	.16
Normal Body, Sepia Eyes	26	25	.04
Totals	100	100	.22

Table 5 ~ Chi squared value of the ebony and sepia cross using the gene linkage ratio, which is 2:1:1.

The cross between the ebony and sepia obviously give us a different ratio since they are linked, this ratio is 2:1:1, the charts directly above prove this to be true. The chi squared value when using the gene linkage ratio is .22, which is much lower than the 2.82667 value from the 9:3:3:1 ratio. However, in order to determine whether or not to accept the new chi squared value a different degree of freedom had to be used. The value of .22 is an acceptable value because it less than 5.991, which is the value given by the chi squared table (Lab Manual). Since gene linkage occurred in this cross we would have to hypotheses that the ebony and sepia cross would give us a 2:1:1 ratio and that the vestigial and sepia cross would give us a 9:3:3:1 ratio.

There were a few places within the lab that allowed for error. An error could have been made when making the original F1 cross, anesthetizing the flies, or getting the flies out of the flask. When getting the flies out of the flask in order to observe them we were suppose to be careful not to get any of the blue medium into the “FlyNap” container, however; we did which meant our data was invalid. We essentially killed all of our flies and drowned them in the medium making it impossible to but them under the dissecting microscope for observation. We made this error when observing our flies from the ebony and sepia cross so we got data from another lab group. The conclusion that I have made from performing this experiment is that independent assortment allows for a 9:3:3:1 phenotypic ratio, while gene linkage allows for a phenotypic ratio of 2:1:1.

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