Mode of Inheritance Fly Research Report

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Question

Our research study investigates the mode of inheritance for the traits of scarlet eye color, lobed eye shape, and scalloped wing shape in *Drosophila melanogaster*. Specifically, we want to find out if the traits are dominant or recessive, autosomal or sex-linked, and if the loci which contain genes that encode for the traits are linked or assort independently.

Mechanics

We use a Chi-squared goodness of fit test to establish the probability that the observed values from the experimental crosses are due to chance, maintaining $P \geq 0.05$ as the cutoff probability (P) value, below which a given hypothesis that chance produced the observed deviation ought to be rejected.

Research room temperature ranged from 20-25 degrees Celsius. Room temperature increased as the building's heating was turned up by the facilities department. Temperature of habitat jars changed with room temperature but were the same for each jar at any given time.

Preparing Jars

To start preparing jars, we decide which parental crosses to perform and prepare 1 fly habitat for each cross and their duplicate, if applicable. One fly habitat consists of 1 dry, 4-ounce glass jar, sterilized between each use. About 4 tablespoons of fly culture medium is added to the jar, a mixture of potato flakes and blue dye, which provides flies with carbohydrates and color contrast between medium and flies. To produce a flat, settled medium surface, the jar is tapped firmly against our leg to prevent indents or cave-ins in the medium surface that could trap flies in the medium. We slowly pour 1 ounce of distilled water down the sides of the jar at an angle to ensure the medium is evenly moistened. We transfer 4 to 6 granules of commercial baker's yeast to the jar using a scoopula to provide vitamins to flies. We label the jar on a small strip of lab tape placed about 1 inch below the jar shoulder. The label is written as follows:

♀ "Generation" (Parent, F1 or F2) "Female Phenotype" × ♂ "Generation" (Parent, F1 or F2) "Male Phenotype" [Red or Blue dot]

We notate number of females and males used for the cross and the date flies were added. We prepare 2 duplicate cross jars for each planned cross to ensure data can be obtained from a cross if one jar does not produce a large sample size and to safeguard against unsuccessful fly mating, parental generation death, or lethal bacterial growth leading to a small progeny sample size. One jar is labeled as the "red" cross, and the duplicate is labeled as the "blue" cross, notated with a red or blue dot. Jars are sealed with a foam plug, sterilized between each use.

Handling Flies

Over the course of the next two weeks the flies reproduced, and the females were inseminated by the male flies and laid eggs. The eggs then developed into larvae, the larvae fed on the medium provided and became pupae. Parents were removed before the first generation emerged. We record the date of first emergence to recognize the two-week time duration which the emerged population is useable because the progeny start reproducing after two weeks post emergence and the emerged flies are no longer purely the original generation. Throughout the study, we checked habitat jars for presence of harmful bacteria or signs of a flaky, dry medium. We move, flip and rotate individuals with a small paint brush to check their phenotypes.

Transferring Flies

To transfer emerged adult flies from the habitat jar to an empty collecting jar, we tap the habitat jar base firmly against our leg to knock flies to the bottom. We quickly remove the foam plug and place the mouth of a dry, sterilized, empty collecting jar to the mouth of the habitat jar before flies can escape. Care is taken to keep the jar mouths aligned during the transferring process. To coax emerged flies to crawl up the sides or fly into the collecting jar, we grasp the necks of both jars with one hand at an angle as the other hand rotates the habitat jar (this method sometimes encourages flies to move into the habitat jar more quickly). Once a majority of emerged flies are in the collecting jar, we slip two squares of paper between the jar mouths. With one hand, we grasp the paper square around the jar mouth to tightly seal it and set the top sealed collecting jar aside. We seal the habitat jar mouth, tap the jar base against our leg and replace the paper with the foam plug. We seal the collecting jar with a new, sterilized foam plug.

Napping Flies

To immobilize emerged flies for examination, we transfer emerged adult flies from the habitat cross jar to a new dry, sterilized collecting jar. We dip an anesthetic wand into triethylamine anesthesia (henceforth referred to as "nap"), used to anesthetize emerged flies (flies are exposed to nap for the shortest time needed to immobilize, which prevents the anesthesia from sterilizing or killing flies). Holding the dipped wand in one hand, we tap the collecting jar base against our leg to knock flies to the bottom. Anesthetic is administered by quickly creating an opening between the plug and jar neck, placing the dipped wand tip into the jar to suspend it without touching the jar sides, and releasing the plug to close the opening. The wand handle is held between the jar mouth and plug. We observe the plug to ensure it does not contain folds and flies are not between the plug and jar neck. We keep the wand in the jar until flies are immobilized, taking about 1 minute on average. Once treated with one wand of nap, flies will stay anesthetized for about 45 to 50 minutes, with nap effectiveness directly proportional to fly age and females being more resistant to nap than males.

Counting and Phenotyping Flies

To count and phenotype, we transfer emerged flies into a new collecting jar and nap them. We place flies on a plain white sheet of paper under a "Bausch & Lomb" stereo microscope. We separate flies by sex, then count and record their eye and wing phenotypes. Wild type wings are smooth, rounded wings while scalloped wings have small, scooped "nicks" on the edges. Wild type eyes are a dull red with brown dots in the center whereas scarlet eye color is a bright red without dots in the center. Wild type eye shape is a rounded oval while lobed eye shape involves eye size reduction, an "indent" in the eye due to a reduction in number of fascicles, or both. We observe and record the phenotype of each fly and then morgue them.

Distinguishing Fly Sexes

To distinguish between sexes of flies, we observe the physical traits of the male and female flies using a "Bausch & Lomb" stereo microscope. We observe that males have a rounded and dark black abdomen base, with the belly side possessing genital arches. Genital arches of males are 1 pair of black,

macaroni noodle-shaped structures. To compare, females have a pointed abdomen base, with stripes running down to the base of the abdomen and lack genital arches. When comparing adult body sizes, males are usually about 2/3 the size of females.

Removing Parent Flies

We remove all parents before emergence of the next generation. To successfully remove the parental generation from jars, we remove as many flies as possible using the same techniques as transferring, treat with nap, and place in the morgue. If flies are not removed using previous methods, we put the brush in front of the fly for it to crawl onto or pick it up with the brush and then place it in the morgue. Our last resort is to press the fly into the medium using the brush end so it is unable to breed. Once we remove and morgue all emerged flies, we replace the foam plug. All females who emerge within the next 12 hours will be virgin.

How To Get Virgin Females

To successfully remove virgin females, we empty a true breeding stock jar of all emerged flies and transfer them to a new prepared jar. After all emerged flies are removed, we consider newly emerged females pulled from the jar within 12 hours post-emergence as “virgin”, not inseminated, because *Drosophila melanogaster* are not sexually mature until after 12 hours post-emergence. We remove the virgins from the jar, treat them with nap, and sex them. We place the virgin females into a separate jar for later use.

Clean Up

Once data has been collected for a sample of flies, we dispose of them into a morgue, a beaker of a 25% dish soap solution. We fill the jar to the brim with warm water to drown the flies in a sink with a disposal. We rinse the flies down the drain, scrub the jar thoroughly using a bottle brush to remove all contents, rinse, and place the jar in the “dirty dishes” bin to be autoclaved by lab technicians. We scrub down the sink with a brush and rinse it to remove all contents, then turn on cool water and the disposal for 5 seconds.

Cross	Date Crossed	Date Parents Pulled	Date Progeny Started to Emerge	Date Counted & Phenotyped
Parent generation crosses: produce F1 progeny				
♀ Scalloped × ♂ Scarlet red	9.22	10.4	10.7	10.11
♀ Scalloped × ♂ Scarlet blue	9.23	10.4	10.7	10.11
♀ Scarlet × ♂ Scalloped red	9.23	10.4	10.9	10.11
♀ Scarlet × ♂ Scalloped blue	9.23	10.4	10.8	10.11
♀ Scarlet × ♂ Lobed red	9.22	10.4	10.7	10.11
♀ Scarlet × ♂ Lobed blue	9.22	10.4	10.7	10.7
♀ Lobed × ♂ Scarlet red & blue	9.22	10.4	10.7	10.7
♀ Scalloped × ♂ Lobed red	9.22	10.4	10.7	10.7 & 10.11
♀ Scalloped × ♂ Lobed blue	9.22	10.4	10.7	10.11
♀ Lobed × ♂ Scalloped red	9.22	10.4	10.7	10.11
♀ Lobed × ♂ Scalloped blue	9.22	10.4	10.7	10.7 & 10.11
F1 crosses: produce F2 progeny				
♀Scalloped × ♂Scarlet red	10.13	10.18	10.27	11.11
♀Scalloped × ♂Scarlet blue	10.13	10.18	10.24	11.2 & 11.13
♀Scarlet × ♂Lobed red	10.13	10.18	10.24	10.30, 11.4 & 11.14
♀Scarlet × ♂Lobed blue	10.13	10.18	10.24	11.2, 11.4, 11.15 & 11.20
♀Scalloped × ♂Lobed red	10.13	10.18	10.24	11.2 & 11.13
♀Scalloped × ♂Lobed blue	10.13	10.18	10.24	11.2 & 11.11
Linkage Study Crosses				
Parent Generation cross: produce F1 virgin females to use in test cross				
P ♀Scarlet × ♂Lobed red & blue	10.26	11.4	11.11	11.11
Test cross:				
♀ F1 Scarlet Lobed × ♂ F2 Scarlet Lobed linkage study red	11.11	11.20	11.26	12.2, 12.6
♀ F1 Scarlet Lobed × ♂ F2 Scarlet Lobed linkage study blue	11.11	11.20	11.26	11.30, 12.2, 12.6, 12.9

Parent generation crosses

On September 13th, we prepared and labeled 2 duplicate cross jars for each of the 6 planned parent generation crosses (12 total cross jars) and prepared 1 jar for each true breeding trait self cross, used as stock (3 stock jars), 15 jars overall. We treated true breeding stock with nap and added them to prepared jars to create the following parent generation and stock crosses and give rise to F1 progeny:

♀Lobed × ♂Scalloped red: (2 lobed females × 3 scalloped males)

♀Lobed × ♂Scalloped blue: (2 lobed females × 3 scalloped males)

♀Scalloped × ♂Lobed red: (3 scalloped females × 5 lobed males)

♀Scalloped × ♂Lobed blue: (2 scalloped females × 5 lobed males)

♀Scalloped × ♂Scarlet red: (2 scalloped females × 2 scarlet males)

♀Scalloped × ♂Scarlet blue: (3 scalloped females × 3 scarlet males)

♀Scarlet × ♂Scalloped red: (5 scarlet females × 5 scalloped males)

♀Scarlet × ♂Scalloped blue: (5 scarlet females × 5 scalloped males)

♀Lobed × ♂Scarlet red: (2 lobed females × 2 scarlet males)

♀Lobed × ♂Scarlet blue: (2 lobed females × 4 scarlet males)

♀Scarlet × ♂Lobed red: (5 scarlet females × 5 lobed males)

♀Scarlet × ♂Lobed blue: (2 scarlet females × 4 lobed males)

♀Scarlet × ♂Scarlet Stock: (4 scarlet females × 3 scarlet males)

♀Lobed × ♂Lobed Stock: (3 lobed females × 2 lobed males)

♀Scalloped × ♂Scalloped Stock: (3 scalloped females × 3 scalloped males)

F1 generation crosses

On October 13th, we prepared and labeled 2 duplicate cross jars for each of the 3 planned F1 crosses, 6 total cross jars. We selected and treated with nap a small sample of F1 flies with genotypes desired for the planned F1 crosses, using the fly's phenotype to infer their presumed genotype. We placed the selected F1 flies into labeled jars to create the following F1 generation crosses and produce F2 flies:

We treated selected F1 flies with nap and added them to prepared jars to create the following F1 generation crosses and give rise to F2 progeny:

♀Scalloped × ♂Scarlet red: (11 scalloped females × 7 scarlet males)

♀Scalloped × ♂Scarlet blue: (11 scalloped females × 10 scarlet males)

♀Scarlet × ♂Lobed red: (5 scarlet females × 5 lobed males)

♀Scarlet × ♂Lobed blue: (5 scarlet females × 5 lobed males)

♀Scalloped × ♂Lobed red: (5 scalloped females × 5 lobed males)

♀Scalloped × ♂Lobed blue: (7 scalloped females × 5 lobed males)

Linkage Study

We conducted a test cross to create a gene linkage study to determine if the loci containing genes that encode for lobed and scarlet traits are linked, close together on the same chromosome, or assort independent of each other. Loci that assort independently means the genes at the loci can cross over separate of each other during cell division. The linkage study is required to gain more information a possible linkage because we have not observed the expected ratios from our scarlet × lobed crosses. Also, taking data overtime could help us see the relative emergence of the flies and help us get an idea of their fitness levels. The progeny of the test cross are not expected to produce 1:1:1:1 if it is linked. If the ratio is 1:1:1:1 we can determine that they are independently assorting loci. Also, if the fitness levels are unequal, we would expect to see the ratio to be not 1:1 at either loci. We did not conduct a reciprocal cross of the test cross because meiotic crossing over does not occur in male *Drosophila melanogaster*, making a reciprocal cross useless in studying the presence of gene linkage, as map units can only be calculated using rates of crossover.

The test cross for the linkage study is: ♀ F1 SsLl × ♂ F2 ssll

(♀ F1 generation heterozygous scarlet lobed genotype, wild eyes & wild wings phenotype) ×

(♂ F2 generation homozygous recessive scarlet lobed genotype, scarlet lobed phenotype)

To create the F1 virgin heterozygous female parents for the test cross, we conduct a cross of true breeding female scarlet virgins with true breeding male lobed flies. This true breeding cross produces F1 generation female virgin progeny (heterozygous scarlet lobed genotype, wild eyes and wild wings phenotype), which we use as test cross parents. We prepare and label 2 jars for 2 duplicate crosses. We remove 2 male true breeding lobed stock from the stock jar and treat them with nap, transferring 1 male into each of the 2 duplicate P ♀ Scarlet × ♂ Lobed cross jars. We pull virgins from our true-breeding scarlet stock jar. We transfer the emerged female F1 generation scarlet virgins to the 2 P ♀ Scarlet × ♂ Lobed cross jars to be inseminated by the male lobed stock instead of the male scarlet stock, producing the desired true breeding cross.

For the F1 generation virgin females to be used as parents in the test cross, they must be virgin to know and retain the desired genotype in the female parents, purely heterozygous scarlet lobed. To do so, the test cross female virgin parents must not be inseminated by male true breeding scarlet stock. This allows the test cross to produce progeny with genotypes we can predict using the known genotypes of the parents. A female fly only has to be inseminated once in the course of her life to produce progeny with the inseminating male's genes for her entire life span. Therefore, the newly emerged virgin female scarlet stock are required to be removed from the scarlet stock jar before becoming sexually mature (12 hours post-emergence) and have the chance to be inseminated by male scarlet stock in the same jar.

Creating the Test Cross

We remove F1 female virgins (heterozygous scarlet lobed genotype, wild eyes and wild wings phenotype) from P ♀Scarlet × ♂Lobed red and blue jars and treat with nap. On November 11th, 2 emerged male F2 Scarlet Lobed genotype flies are selected from the F1 ♀Scarlet × ♂Lobed red and blue cross jars and treated with nap. To create the test cross, we transfer 4 F1 female virgins and 1 male F2 scarlet lobed to each of 2 duplicate prepared test cross jars, labeled "♀ F1 Scarlet Lobed × ♂ F2 Scarlet Lobed linkage study red" and duplicate "blue". We observe the progeny by treating them with nap, counting, phenotyping and distinguishing sex and analyzing the data to determine the gene linkage.

Logic

Initial Hypothesis

We initially hypothesize that all traits are autosomal. There are 2 alleles for each of the 3 eye color (scarlet), eye shape (lobed), and wing shape (scalloped) traits, with the wild type allele (dominant) and the trait allele (recessive). Wing shape, eye color and eye shape are traits which are all determined by genes at separate, non-interacting loci. We hypothesize there are 3 loci involved for the 3 traits, with one for eye color, one for eye shape and one for wing shape.

Expected Genotypes and Phenotypes for Crosses

Expected genotypic ratios and phenotypic ratios based on the initial hypothesis are depicted for each cross in the following cross flow charts. We hypothesize that the expected ratios are the same for

reciprocal crosses. Poor fit between observed and predicted progeny serves as a refutation to our hypothesis.

Note: The first individual of the cross is female and the second individual is male.

Key: P = Parent generation with true-breeding stock, F1= First generation progeny of parental generation

F2 = Second generation progeny of first generation, Phen= Expected phenotypic ratio

Wild = all wild type (wild type eyes and wings) phenotype for both loci

s = scarlet eye color allele, S = wild type eye color allele, l = lobed eye shape allele, L = wild type eye shape allele, c = scalloped wing shape allele, C=wild type wing shape allele

If our hypothesis is true, we expect to observe all flies to be wild in all of our F1 generation crosses. In the F2 generation, we expect the flies to form a 9:3:3:1 ratio in the next generation because of the recessive inheritance pattern.

Scarlet x Scalloped

P: ssCC (scarlet) x SScc(scalloped)

↓

F1: SsCc(wild)

↓

F2 1SSCC: 2SSCc: 2SsCC: 1ssCC 4: SsCc: 1SScc: 2Sscc: 2ssCc 1: sscc

Phen: 9 wild: 3 scarlet: 3 scalloped: 1 scarlet & scalloped

Scarlet x Lobed

P: ssLL (scarlet) x SSll (lobed)

↓

F1: SsLl (wild)

↓

F2 1SSLL: 2SSLl: 2SsLL: 1ssLL 4: SsLl: 1SSll: 2Ssll: 2ssLl 1: ssll

Phen: 9 wild: 3 Scarlet: 3 lobed: 1 scarlet & lobed

lobed x scalloped

P: ccLL (scalloped) x CCll (lobed)

↓

F1: CcLl (wild)

↓

F2 1CCLL: 2CCLl: 2CcLL: 1ccLL: 4: CcLl: 1CCll: 2Ccll: 2ccLl 1: ccll

Phen: 9 wild: 3 scalloped: 3 lobed: 1 scalloped & lobed

Second Revised Hypothesis for Scalloped Wings

Data shows that the scalloped wing trait is expressed in males, but not in females of the F1 generation, refuting our initial hypothesis that the scalloped wing trait is autosomal recessive. We therefore revise our hypothesize to identifying scalloped wings as an X-linked recessive trait, evidenced by its expression in F1 progeny males with one X chromosome from their true-breeding scalloped mother but not in F1 progeny females, who have two X chromosomes, with one from the mother with a scalloped wing genotype and one from the father with a wild type wing genotype.

Data supports our initial hypothesis of the scarlet and lobed eye traits as autosomal and recessive to the wild type because no lobed or scarlet F1 progeny are produced from any parent generation cross. All parent generation crosses produce F1 progeny with wild eyes, as predicted in our hypotheses. We brought forward ♀ Scalloped × ♂ Scarlet red and blue F1 progeny to be self-crossed because these were the only jars that expressed the scalloped wings trait in males only. We predict that the F1 progeny are heterozygous with respect to eye color or shape, in accordance with the parental genotype. We also conclude that the scarlet and lobed trait cannot be linked to the scalloped trait because the scalloped gene is located on the "X" sex chromosome.

Expected Genotypes and Phenotypes for Second Hypothesis Crosses

If our hypothesis is true, we expect to observe, in the F1 generation, all female flies to be wild and males to be scalloped. In the F2 generation, we expect the flies to form a 3:3:3:3:1:1:1:1 ratio with the scarlet × scalloped cross and the lobed × scalloped cross because of the recessive X-linked inheritance pattern.

Key: Y= male "Y" sex allele, X-linked alleles: X^s= scarlet eye color allele, X^S= wild type eye color allele, X^l = lobed eye shape allele, X^L = wild type eye shape allele,

X^c=scalloped wing shape allele, X^C=wild type wing shape allele

Scarlet x Scalloped

P: $X^cX^cSS \times X^CYss$

↓

F1: $X^cX^CSs \times X^cYSs$

↓

F2: $1X^cX^cSS$, $1X^CYSS$, $1X^cX^CSS$, $1X^cYSS$, $2X^cX^cSs$, $2X^CYSs$, $2X^cX^CSs$, $2X^cYSs$, $1X^cX^css$, $1X^CYss$, $1X^cX^css$, $1X^CYss$

Genotypic Ratio: 1:1:1:1:2:2:2:2:1:1:1:1

Phen: 3 ♀ Scalloped Wild, 3 ♀ Wild Wild, 3 ♂ Scalloped Wild, ♂ 3 Wild Wild, 1 ♀Scalloped Scarlet, 1 ♀ Wild Scarlet, 1 ♂ Scalloped Scarlet, ♂ 1|Wild Scarlet

Phenotypic Ratio: 3:3:3:3:1:1:1:1

Lobed x Scalloped

P: $X^cX^cLL \times X^CYll$

F1: $X^cX^CLl \times X^cYLl$

F2: $1X^cX^cLL, 1X^CYLL, 1X^cX^CLL, 1X^cYLL, 2X^cX^cLl, 2X^CYLl, 2X^cX^CLl, 2X^cYLl, 1X^cX^cll, 1X^CYll, 1X^cX^cll, 1X^CYll$

Genotypic Ratio: 1:1:1:1:2:2:2:2:1:1:1:1

Phen: 3 ♀ Scalloped Wild, 3 ♀ Wild Wild, 3 ♂ Scalloped Wild, ♂ 3 Wild Wild, 1 ♀Scalloped Lobed, 1 ♀ Wild Lobed, 1 ♂ Scalloped Lobed, ♂ 1 Wild Lobed

Phenotypic Ratio: 3:3:3:3:1:1:1:1

Third Revised Hypothesis

Given the F2 progeny did not fit the chi squared values we expected for the two traits of scarlet and lobed, we hypothesize that the scarlet eye color and lobed eye shape traits may have linked genes. We need to conduct a linkage study test cross of ♀ F1 Scarlet Lobed × ♂ F2 Scarlet Lobed and analyze the progeny to determine if the scarlet and lobed trait genes are located on loci that are linked or assort independently of each other.

Data

Parental generation true breeding stock (homozygous genotype) crosses produce F1 generation progeny (heterozygous genotype). F1 generation progeny (heterozygous genotype) are brought forward in the following crosses to produce F2 progeny (homozygous dominant, heterozygous, and homozygous recessive genotypes).

Parent Generation Cross Progeny Data Summary

F1 ♀ Scalloped × ♂ Scarlet [red]

Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
0	10	Wild	Wild
22	0	Wild	Scalloped
0	0	Scarlet	Wild
0	0	Scarlet	Scalloped
Total: 22	10		

F1 ♀ Scalloped × ♂ Scarlet [blue]

Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
0	31	Wild	Wild
32	0	Wild	Scalloped
0	0	Scarlet	Wild
0	0	Scarlet	Scalloped
Total: 32	31		

F1 ♀ Scarlet × ♂ Scalloped [red]

Total Observed	Phenotype Eyes	Phenotype Wings
35	Wild	Wild
0	Wild	Scalloped
0	Scarlet	Wild
0	Scarlet	Scalloped
Total: 35		

F1 ♀ Scarlet × ♂ Scalloped [blue]

Total Observed	Phenotype Eyes	Phenotype Wings
69	Wild	Wild
0	Wild	Scalloped
0	Scarlet	Wild
0	Scarlet	Scalloped
Total: 69		

F1 ♀ Scarlet × ♂ Lobed [red]

Total Observed	Phenotype Eyes	Phenotype Wings
101	Wild	Wild
0	Wild	Lobed
0	Scarlet	Wild
0	Scarlet	Lobed
Total: 101		

F1 ♀ Scarlet × ♂ Lobed [blue]

Total Observed	Phenotype Eyes	Phenotype Wings
15	Wild	Wild
0	Wild	Lobed
0	Scarlet	Wild
0	Scarlet	Lobed
Total: 15		

F1 ♀ Lobed × ♂ Scarlet [red]

Total Observed	Phenotype Eyes	Phenotype Wings
15	Wild	Wild
0	Wild	Lobed
0	Scarlet	Wild
0	Scarlet	Lobed
Total: 15		

F1 ♀ Lobed × ♂ Scarlet [blue]

Total Observed	Phenotype Eyes	Phenotype Wings
7	Wild	Wild
0	Wild	Lobed
0	Scarlet	Wild
0	Scarlet	Lobed
Total: 7		

F1 ♀ Scalloped × ♂ Lobed [red]

Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
0	37	Wild	Wild
26	0	Lobed	Wild
0	0	Wild	Scalloped
0	0	Lobed	Scalloped
Total: 26	37		

F1 ♀ Scalloped × ♂ Lobed [blue]

Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
0	2	Wild	Wild
0	0	Lobed	Wild
7	0	Wild	Scalloped
0	0	Lobed	Scalloped
Total: 7	2		

F1 ♀ Lobed × ♂ Scalloped [red]		
Total Observed	Phenotype Eyes	Wings
19	Wild	Wild
0	Lobed	Wild
0	Wild	Scalloped
0	Lobed	Scalloped
Total: 19		

F1 ♀ Lobed × ♂ Scalloped [blue]		
Total Observed	Phenotype Eyes	Wings
0	Wild	Wild
0	Lobed	Wild
11	Wild	Scalloped
0	Lobed	Scalloped
Total: 11		

F1 Generation Cross Progeny Data Summary

F2 ♀ Scalloped × ♂ Scarlet [red]			
Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Wings
95	109	Wild	Wild
47	50	Wild	Scalloped
3	2	Scarlet	Wild
0	1	Scarlet	Scalloped
Total: 145	162		

F2 ♀ Scalloped × ♂ Scarlet [blue]			
Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Wings
45	46	Wild	Wild
21	25	Wild	Scalloped
4	4	Scarlet	Wild
2	1	Scarlet	Scalloped
Total: 72	76		

F2 ♀ Scarlet × ♂ Lobed [red]			
Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Eyes
134	166	Wild	Wild
51	49	Wild	Lobed
20	37	Scarlet	Wild
6	7	Scarlet	Lobed
Total: 211	259		

F2 ♀ Scarlet × ♂ Lobed [blue]			
Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Eyes
180	184	Wild	Wild
48	29	Wild	Lobed
21	24	Scarlet	Wild
5	5	Scarlet	Lobed
Total: 254	242		

F2 ♀ Scalloped × ♂ Lobed [red]			
Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Wings
59	46	Wild	Wild
23	9	Lobed	Wild
36	36	Wild	Scalloped
13	7	Lobed	Scalloped
Total: 131	98		

F2 ♀ Scalloped × ♂ Lobed [blue]			
Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Wings
91	106	Wild	Wild
20	14	Lobed	Wild
96	70	Wild	Scalloped
13	13	Lobed	Scalloped
Total: 220	203		

Linkage Study Cross Progeny Data Summary

Linkage	♀ F1 Wild (AaBb) × ♂ F2 Scarlet, Lobed (aabb) [red]			
	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
	14	24	Wild	Wild
	3	5	Lobed	Wild
	8	8	Wild	Scarlet
	1	16	Lobed	Scarlet
Total:	26	53		

Linkage	♀ F1 Wild (AaBb) × ♂ F2 Scarlet, Lobed (aabb) [blue]			
	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
	72	82	Wild	Wild
	24	37	Lobed	Wild
	36	47	Wild	Scarlet
	13	20	Lobed	Scarlet
Total:	145	186		

Note: See Raw Data and F2 Progeny P-Value Summary in Appendix

Statistical Analysis

A Chi-squared test is used to analyze if the expected ratio of progeny fit the observed progeny data collected for each of the F2 generation crosses. Chi-squared test equation: $X^2 = \Sigma \frac{(O-E)^2}{E}$

Σ= summation of, O= Observed value, E= Expected value

♀ Scalloped × ♂ Lobed [Red]:

Testing for the hypothesis of expected ratio:

3 Wild Wild : 1 Lobed Wild : 3 Wild Scalloped : 1 Lobed Scalloped for males and females

For ♂ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(59-49.13)^2}{49.13} + \frac{(23-16.38)^2}{16.38} + \frac{(36-49.13)^2}{49.13} + \frac{(13-16.38)^2}{16.38}$ = 8.87

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the

expected ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(36-32.8)^2}{32.8} + \frac{(95-98.25)^2}{98.25} = 0.43$

Degrees of freedom = 1, 0.75 > P > 0.5

There is between a 50 and 75% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. This is no significant difference between observed values and expected values, meaning that the data are a good fit for our expected ratio.

For ♂ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(49-65.5)^2}{65.5} + \frac{(82-65.5)^2}{65.5} = 8.31$

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is significant difference between observed and expected values, which means that the data are not a good fit for the expected ratio.

For ♀ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(46-36.75)^2}{36.75} + \frac{(9-12.25)^2}{12.25} + \frac{(36-36.75)^2}{36.75} + \frac{(7-12.25)^2}{12.25} = 5.46$

Degrees of freedom = 3, 0.25 > P > 0.1

There is a between a 10 and 25% probability that random chance alone could produce this deviation from the expected hypothesized ratio. There is therefore no significant difference between observed values and expected values which means that the data are a good fit for the expected ratio.

For ♀ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(16-24.5)^2}{24.5} + \frac{(82-73.5)^2}{73.5} = 3.93$

Degrees of freedom = 1, 0.05 > P > 0.01

There is less than 1% probability that random chance alone could produce this deviation from the expected hypothesized ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(43-49)^2}{49} + \frac{(55-49)^2}{49} = 1.47$

Degrees of freedom = 1, 0.25 > P > 0.10

There is a between a 10 and 25% probability that discrepancies between expected and observed ratios can be caused by chance, so there is no significant difference between observed and expected values, meaning that the data are a good fit with our hypothesis.

♀ Scalloped × ♂ Lobed [Blue]:

Testing for the hypothesis of expected ratio:

3 Wild Wild : 1 Lobed Wild : 3 Wild Scalloped : 1 Lobed Scalloped for males and females

For ♂ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(91-82.5)^2}{82.5} + \frac{(20-27.5)^2}{27.5} + \frac{(96-82.5)^2}{82.5} + \frac{(13-27.5)^2}{27.5} = 12.78$

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(33-55)^2}{55} + \frac{(187-165)^2}{165} = 11.73$

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. This is a significant difference between observed values and expected values, meaning that the data are not a good fit for our expected ratio.

For ♂ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(109-110)^2}{110} + \frac{(111-110)^2}{110} = 0.018$

Degrees of freedom = 1, 0.9 > P > 0.75

There is between a 90 to 100% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is therefore no significant difference between observed and expected values, which means that the data are a good fit for the expected ratio.

For ♀ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(106-76.13)^2}{76.13} + \frac{(14-25.38)^2}{25.38} + \frac{(70-76.13)^2}{76.13} + \frac{(13-25.38)^2}{25.38}$ = 23.35

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this deviation from the expected hypothesized ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(27-50.75)^2}{50.75} + \frac{(176-152.25)^2}{152.25}$ = 14.82

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this deviation from the expected hypothesized ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(83-101.5)^2}{101.5} + \frac{(120-101.5)^2}{101.5} =$ 6.74

Degrees of freedom = 1, 0.01 > P

There is between a 0.5 and 1% probability that discrepancies between expected and observed ratios can be caused by chance, so there is a significant difference between observed and expected values, meaning that the data are not a good fit with our hypothesis.

♀ Scarlet × ♂ Lobed [Red]:

Testing for the hypothesis of expected ratio:

9 Wild Wild : 3 Wild Lobed : 3 Scarlet Wild : 1 Scarlet Lobed

For ♂ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(134-118.69)^2}{118.69} + \frac{(51-39.56)^2}{39.56} + \frac{(20-39.56)^2}{39.56} + \frac{(6-13.19)^2}{13.19}$ = 18.87

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(57-52.75)^2}{52.75} + \frac{(154-158.25)^2}{158.25}$ = 0.50

Degrees of freedom = 1, 0.5 > P > .25

There is between a 25 and 50% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. This is not a significant difference between observed values and expected values, meaning that the data are a good fit for our expected ratio.

For ♂ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(26-52.75)^2}{52.75} + \frac{(185-158.25)^2}{158.25} =$ 18.09

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is, therefore, a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(166-145.69)^2}{145.69} + \frac{(49-48.56)^2}{48.56} + \frac{(37-48.56)^2}{48.56} + \frac{(7-16.19)^2}{16.19}$ = 10.80

Degrees of freedom = 3, 0.05 > P > 0.01

There is between a 1 and 5% probability that random chance alone could produce this deviation from the expected hypothesized ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(56-64.75)^2}{64.75} + \frac{(203-194.25}{194.25}$= 1.58

Degrees of freedom = 1, 0.5 > P > 0.1

There is between a 10 and 50% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. This is not a significant difference between observed values and expected values, meaning that the data are a good fit for our expected ratio.

For ♀ Scarlet 3:1 ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(44-64.75)^2}{64.75} + \frac{(215-194.25)^2}{194.28} =$ 8.87

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

♀ **Scarlet × ♂ Lobed [Blue]:**

Testing for the hypothesis of expected ratio:

9 Wild Wild : 3 Wild Lobed : 3 Scarlet Wild : 1 Scarlet Lobed

For ♂ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(180-142.88)^2}{142.88} + \frac{(48-47.63)^2}{47.63} + \frac{(21-47.63)^2}{47.63} + \frac{(5-15.88)^2}{15.88}$ = 31.98

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected ratio. There is a significant difference of observed values from expected values which means the data are not a good fit for the expected ratio.

For ♂ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(53-63.5)^2}{63.5} + \frac{(201-190.5)^2}{190.5}$= 2.31

Degrees of freedom = 1, 0.5 > P > 0.25

There is between a 25 to 50% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. This is not a significant difference between observed values and expected values, meaning that the data are a good fit for the expected ratio.

For ♂ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(26-63.5)^2}{63.5} + \frac{(228-190.5)^2}{190.5} =$ 29.53

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is, therefore, a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(184-136.13}{136.13} + \frac{(29-45.38)^2}{45.38} + \frac{(24-45.38)^2}{45.38} + \frac{(5-15.13)^2}{15.13}$ = 39.59

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Lobed 3 Wild : 1 Lobed ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(34-60.5)^2}{60.5} + \frac{(213-181.5)^2}{181.5} = 21.87$

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(29-60.5)^2}{60.5} + \frac{(213-181.5)^2}{181.5} = 16.40$

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

♀ Scalloped × ♂ Scarlet [Red]:

Testing for the hypothesis of expected ratio:

3 Wild Wild : 3 Wild Scalloped : 1 Scarlet Wild : 1 Scarlet Scalloped

For ♂ Total Data

$$X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(95-54.375)^2}{54.375} + \frac{(47-54.375)^2}{54.375} + \frac{(3-54.375)^2}{18.125} + \frac{(0-54.375)^2}{18.125} = 62.10$$

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(47-72.5)^2}{72.5} + \frac{(98-72.5)^2}{72.5} = 17.94$

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(3-36.25)^2}{72.5} + \frac{(142-108.75)^2}{108.75}$ = 40.66

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(46-28.5)^2}{28.5} + \frac{(25-28.5)^2}{28.5} + \frac{(4-9.5)^2}{9.5} + \frac{(1-9.5)^2}{9.5}$ = 21.97

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(51-81)^2}{81} + \frac{(111-81)^2}{81}$ = 22.22

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(3-40.5)^2}{40.5} + \frac{(159-121.5)^2}{121.5} =$ 46.30

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

♀ **Scalloped × ♂ Scarlet [Blue]:**

Testing for the hypothesis of expected ratio:

3 Wild Wild : 3 Wild Scalloped : 1 Scarlet Wild : 1 Scarlet Scalloped

For ♂ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(45-27)^2}{27} + \frac{(21-27)^2}{27} + \frac{(4-9)^2}{9} + \frac{(2-9)^2}{9}$ = 21.56

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected ratio. There is therefore a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(23-36)^2}{36} + \frac{(49-36)^2}{36}$ = 9.39

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♂ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(6-18)^2}{18} + \frac{(66-54)^2}{54} =$ 10.67

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Total Data $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(46-28.5)^2}{28.5} + \frac{(25-28.5)^2}{28.5} + \frac{(4-9.5)^2}{9.5} + \frac{(1-9.5)^2}{9.5}$ = 21.97

Degrees of freedom = 3, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

For ♀ Scalloped 1 Wild : 1 Scalloped ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(26-38)^2}{38} + \frac{(50-38)^2}{38} = 7.58$

Degrees of freedom = 1, 0.01 > P

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is not a significant difference of observed values from expected values (since P > 0.05), which means that the data are a good fit for the expected ratio.

For ♀ Scarlet 3 Wild : 1 Scarlet ratio: $X^2 = \Sigma \frac{(O-E)^2}{E} = \frac{(5-19)^2}{19} + \frac{(71-57)^2}{57} = 13.75$

Degrees of freedom = 1, 0.01

There is less than 1% probability that random chance alone could produce this much deviation from the expected hypothesized ratio. There is a significant difference of observed values from expected values which means that the data are not a good fit for the expected ratio.

Conclusion

Inheritance of Lobed Eye Shape

Our hypothesis of the lobed eye trait in *Drosophila melanogaster* being autosomal recessive was supported by the data in our F1 cross. As predicted, the cross of true-breeding lobe-eyed flies with true-breeding wild type-eyed flies producing entirely wild F1 progeny, with wild type shape eyes. Similarly, this conclusion was not revised in the subsequent F2 progeny. The majority of red jar data yielded the expected 3 wild : 1 lobed progeny with acceptable (P > 0.5) probability that deviation was caused by chance alone. However, only the male flies in the ♀ Scarlet × ♂ Lobed [blue] were an acceptable fit with our hypothesis, while the rest of the blue cross were not. This being said, the general trend in our data was toward a higher than expected proportion of wild flies across both sexes, which does not rebut the conclusion that lobed eyes are both autosomal and recessive based on F1 data. It can be hypothesized that

the reason for discrepancy may involve a small reduction of fitness in flies with the lobed phenotype. Additionally, our team noticed that the lobed phenotype appears to be expressed with varying levels of expression, with some flies eyes having a varying reduction in the number of fascicles, varying size of nicks in the fascicles than others, or both (a reduction in fascicles and nick in the fascicles), or having one lobed eye and one normal wild type eye shape.

Inheritance of Scarlet Eye Color

Our hypothesis of scarlet eye color being autosomal recessive in *Drosophila melanogaster* was similarly supported by our F1 cross, as the cross of true-breeding scarlet-eyed flies with true-breeding wild-eyed flies producing all wild F1 progeny with wild type eye color. Despite this, all conducted F2 crosses yielded significantly fewer than expected scarlet-eyed progeny ($0.5 > P$), supporting the hypothesis that the trait is recessive, but also leading to the conclusion that the allele in question is not only a recessive cosmetic variant, but also generates a characteristic that causes a decrease in fitness for flies homozygous for the trait. A possible example is an increase in time taken to progress from embryo to adult stage. This would lead to fewer scarlet flies being counted in the emergent F2 generation, as they would emerge later, possibly after the emergence of the first F3 test cross progeny flies.

Inheritance of Scalloped Wing Shape

Our initial hypothesis of the scalloped wing trait being autosomal recessive compared to its wild counterpart in *Drosophila melanogaster* was disproved by the expression of all males with scalloped wings and all females with wild wings in the F1 progeny. Our revised hypothesis, that scalloping was X-linked recessive in inheritance, was in line with the composition of the F1 populations. The hypothesized 1 wild : 1 scalloped ratio was generally a poor fit with the F2 data, being only well fit in the males of ♀ Scalloped × ♂ Lobed [blue] and with the females of ♀ Scalloped × ♂ Lobed [red] and ♀ Scalloped × ♂ Scarlet [blue] crosses. In total, this represents a minority of our data on the scalloped wing trait. It is also notable that, as with the scarlet and lobed eyes, the trend in data is toward more flies with wild type than scalloped wings. From this, it can be concluded that expression of the scalloping phenotype may correlate with a drop in fly fitness, which may explain the greatly reduced number of scalloped flies that were

counted. Nonetheless, the conclusion suggested by our F1 crosses is inescapable, and scalloped scalloped wings are an X-linked recessive trait.

Test Cross

The test cross between heterozygous scarlet lobed females and homozygous scarlet lobed recessive males was completed. If independent assortment and both parents have equal fitness We expect to see a 1 scarlet:1 lobed and 1 wild:1 scarlet lobed. The test cross did not give us a 1:1:1:1 ratio and did not give us a 1:1 ratio at either loci. Not observing a 1:1 at either loci makes it hard to determine linkage. Looking at the results, we see that the best possible explanation is that fitness is involved, we see that we have considerably less scarlet, lobed and even less scarlet lobed. In the test cross, data was taken consistently over a period of time. We see that the wild progeny emerged first in greater numbers and then sharply declined in emergence rate, the other groups emerged in smaller numbers, but were consistent in rate. This leads us to believe that there was unequal fitness levels for the flies that have the lobed and scarlet alleles.

Discussion

Sources of Error and Disparities

The most prominent source of error is likely the small sample size of our research. The average sample size collected from a cross (with both duplicate crosses combined) was 761 flies, which does not represent a large enough sampling given the precise ratios, such as 9 : 3 : 3 : 1 or 3 : 1 : 3 : 1, that we were testing. A more desired sample size would be an average of over 1,000 flies per cross. Future crosses should begin with a larger number of parents, which will successfully increase the number of resultant progeny, leading to more reliable results.

We had difficulty in differentiating between the color of scarlet and wild eye phenotypes when counting fly phenotypes in each jar because different microscopes use different colors of light, making it difficult to establish a consistent method of distinguishing between eye colors. With not many scarlet progeny to begin with, even small errors of this kind may represent a statistically significant reduction of counted scarlet flies, possibly about 5-8 scarlet flies phenotyped as wild type instead of scarlet per jar.

Because of variation in the expression of the lobed trait phenotype, the lobed genotype could have been expressed as an unexpectedly small nick in the eye could easily go unnoticed, even with the aid of the microscope. In cases where only one eye is lobed, both eyes may not have been checked for the lobed trait expression for each sampled fly. Our failure to recognize expression such variation at the beginning of data collection causes lobed flies to be falsely categorized as wild type for an estimated 20% counted flies in a lobed cross jar, which produced a large reduction in observed lobed numbers for their trait jars.

The destruction of flies before their phenotypes can be recorded was another source of error. Flies get stuck in the medium on their own or due to tapping the jar too forcefully to drive flies away from the jar rim during transfer, which damages body structures and kills flies. The destruction of body structures and death, which darkens eye color and shrivels the bodies and wings, make it difficult or impossible to recognize phenotype and sex-determining body structures. Wings are sometimes stuck together from fecal matter, nap or pieces of medium. When wings are crumpled together or torn, we find it difficult to determine between scalloped and damaged wild type wings. This may have produced a large reduction in the number of observed flies, the data set size, because damaged, stuck, and dead flies that could not be included in the data. This reduction occurred for a large but varying numbers of flies per jar, about 15 to 20 flies on average.

The expiration date of jars causes us to not know with confidence which emerged flies were from the current or the next generation. After the jar's expiration date, flies could not be counted due to the uncertainty in their generation. We suspect this phenomenon may be partly responsible for the low number of observed F2 scarlet flies. This may have produced a large effect on the data set by greatly reducing the number of flies we could include in the data. It is unknown how many F2 flies were unable to be phenotyped.

Another source of error was flies not being included in the data set because they escaped or were squashed due to our handling when transferring flies. Fly bodies were squashed by catching flies against the foam plug and glass or paper and jar rim. Flies got stuck between glass and medium when the medium shifted when transferring files from one jar to the next. When pushed up against the glass jar in any of

these situations, the phenotypic defining structures, such as the wings and eyes, were squashed and the fly was killed, which also produced death-related challenges in phenotype determination. When dead, eye color is darker and the bodies and wings were shriveled which made the flies not countable or impossible to phenotypes. This may have produced a small effect on the quantity of flies included in the data set, where a total of about 10 flies per jar escaped or were squashed over the extent of time the jar was used.

Future Study Crosses

Future crosses should involve repetition of ♀ Scalloped × ♂ Scarlet, ♀ Scalloped × ♂ Lobed, and ♀ Scarlet × ♂ Lobed, with a greater number of heterozygous parents, producing a drastically higher number of F2 progeny to examine. These crosses offer a clearer indication of whether the observed progeny adhere to the expected ratios predicted by our hypothesis. Additionally, we should conduct an emergence study to most effectively determine whether lack of fitness is the reason for fewer appearances of the phenotypes in question. It would be best to cross flies that are heterozygous at 1 locus instead of 2 to verify that reduced fitness of one trait, such as scarlet eyes, does not affect the appearance of another trait, such as lobed eyes.

Appendix

Parent Generation Cross Progeny Raw Data

F1	Scalloped × Scarlet [red]						
		Male Progeny			Female Progeny		
		Observed	Phenotype		Observed	Phenotype	
			Eyes	Wings		Eyes	Wings
	Data from 10.11.2018	0	Wild	Wild	10	Wild	Wild
		22	Wild	Scalloped	0	Wild	Scalloped
		0	Scarlet	Wild	0	Scarlet	Wild
		0	Scarlet	Scalloped	0	Scarlet	Scalloped
F1	Scalloped × Scarlet [blue]						
		Male Progeny			Female Progeny		
		Observed	Phenotype		Observed	Phenotype	
			Eyes	Wings		Eyes	Wings
	Data from 10.11.2018	0	Wild	Wild	31	Wild	Wild
		32	Wild	Scalloped	0	Wild	Scalloped
		0	Scarlet	Wild	0	Scarlet	Wild
		0	Scarlet	Scalloped	0	Scarlet	Scalloped

F1 Scarlet × Scalloped [red]

	Total Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 10.11.2018	35	Wild	Wild
	0	Wild	Scalloped
	0	Scarlet	Wild
	0	Scarlet	Scalloped

F1 Scarlet × Scalloped [blue]

	Total Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 10.11.2018	69	Wild	Wild
	0	Wild	Scalloped
	0	Scarlet	Wild
	0	Scarlet	Scalloped

F1 ♀ Scarlet × ♂ Lobed [red]

	Total Progeny Observed	Phenotype Eyes	Phenotype Eyes
Data from 10.11.2018	101	Wild	Wild
	0	Wild	Lobed
	0	Scarlet	Wild
	0	Scarlet	Lobed

F1 ♀ Scarlet × ♂ Lobed [blue]

	Total Progeny Observed	Phenotype Eyes	Phenotype Eyes
Data from 10.7.2018	15	Wild	Wild
	0	Wild	Lobed
	0	Scarlet	Wild
	0	Scarlet	Lobed

F1 ♀ Lobed × ♂ Scarlet [red]

	Total Progeny		
	Observed	Phenotype	
		Eyes	Eyes
Data from 10.7.2018	15	Wild	Wild
	0	Wild	Lobed
	0	Scarlet	Wild
	0	Scarlet	Lobed

F1 ♀ Lobed × ♂ Scarlet [blue]

	Total Progeny		
	Observed	Phenotype	
		Eyes	Eyes
Data from 10.7.2018	7	Wild	Wild
	0	Wild	Lobed
	0	Scarlet	Wild
	0	Scarlet	Lobed

F1 ♀ Scalloped × ♂ Lobed [red]

	Male Progeny			Female Progeny		
	Observed	Phenotype		Observed	Phenotype	
		Eyes	Wings		Eyes	Wings
Data from 10.7.2018	0	Wild	Wild	6	Wild	Wild
	2	Lobed	Wild	0	Lobed	Wild
	0	Wild	Scalloped	0	Wild	Scalloped
	0	Lobed	Scalloped	0	Lobed	Scalloped
Data from 10.11.2018	0	Wild	Wild	31	Wild	Wild
	24	Lobed	Wild	0	Lobed	Wild
	0	Wild	Scalloped	0	Wild	Scalloped
	0	Lobed	Scalloped	0	Lobed	Scalloped

F1 ♀ Scalloped × ♂ Lobed [blue]

	Male Progeny			Female Progeny		
	Observed	Phenotype		Observed	Phenotype	
		Eyes	Wings		Eyes	Wings
Data from 10.11.2018	0	Wild	Wild	2	Wild	Wild
	0	Lobed	Wild	0	Lobed	Wild
	7	Wild	Scalloped	0	Wild	Scalloped
	0	Lobed	Scalloped	0	Lobed	Scalloped

F1 ♀ Lobed × ♂ Scalloped [red]

	Total Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 10.11.2018	19	Wild	Wild
	0	Wild	Lobed
	0	Scalloped	Wild
	0	Scalloped	Lobed

F1 ♀ Lobed × ♂ Scalloped [blue]

	Total Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 10.7.2018	3	Wild	Wild
	0	Wild	Lobed
	0	Scalloped	Wild
	0	Scalloped	Lobed
Data from 10.11.2018	8	Wild	Wild
	0	Wild	Lobed
	0	Scalloped	Wild
	0	Scalloped	Lobed

F1 Generation Cross Progeny Raw Data

F2 ♀ Scalloped × ♂ Scarlet [red]

	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 11.11.2018	95	109	Wild	Wild
	47	50	Wild	Scalloped
	3	2	Scarlet	Wild
	0	1	Scarlet	Scalloped

F2 ♀ Scalloped × ♂ Scarlet [blue]

	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 11.2.2018	8	3	Wild	Wild
	2	2	Wild	Scalloped
	1	1	Scarlet	Wild
	2	0	Scarlet	Scalloped
Data from 11.13.2018	37	43	Wild	Wild
	19	23	Wild	Scalloped
	3	3	Scarlet	Wild
	0	1	Scarlet	Scalloped

F2 ♀ Scarlet × ♂ Lobed [red]				
	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Eyes
Data from 10.30.2018	89	121	Wild	Wild
	37	30	Wild	Lobed
	13	28	Scarlet	Wild
	5	6	Scarlet	Lobed
Data from 11.4.2018	26	24	Wild	Wild
	4	5	Wild	Lobed
	7	3	Scarlet	Wild
	1	0	Scarlet	Lobed
Data from 11.14.2018	19	21	Wild	Wild
	10	14	Wild	Lobed
	0	6	Scarlet	Wild
	0	1	Scarlet	Lobed

F2 ♀ Scarlet × ♂ Lobed [blue]				
	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Eyes
Data from 11.2.2018	66	53	Wild	Wild
	21	8	Wild	Lobed
	6	9	Scarlet	Wild
	1	2	Scarlet	Lobed
Data from 11.4.2018	79	83	Wild	Wild
	17	11	Wild	Lobed
	5	5	Scarlet	Wild
	2	1	Scarlet	Lobed
Data from 11.15.2018	26	34	Wild	Wild
	8	8	Wild	Lobed
	6	7	Scarlet	Wild
	2	2	Scarlet	Lobed
Data from 11.20.2018	9	14	Wild	Wild
	2	2	Wild	Lobed
	4	3	Scarlet	Wild
	0	0	Scarlet	Lobed

F2 ♀ Scalloped × ♂ Lobed [red]

	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 11.2.2018	58	46	Wild	Wild
	20	7	Wild	Lobed
	35	36	Scalloped	Wild
	8	6	Scalloped	Lobed
Data from 11.13.2018	1	0	Wild	Wild
	3	2	Wild	Lobed
	1	0	Scalloped	Wild
	5	1	Scalloped	Lobed

F2 ♀ Scalloped × ♂ Lobed [blue]

	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 11.2.2018	65	78	Wild	Wild
	16	8	Lobed	Wild
	60	51	Wild	Scalloped
	12	8	Lobed	Scalloped
Data from 11.11.2018	26	28	Wild	Wild
	4	6	Lobed	Wild
	36	19	Wild	Scalloped
	1	5	Lobed	Scalloped

Linkage Study Cross Progeny Raw Data

Linkage ♀ F1 Wild (AaBb) × ♂ F2 Scarlet, Lobed (aabb) [red]

	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 12.2.18	13	22	Wild	Wild
	2	3	Lobed	Wild
	4	6	Wild	Scarlet
	0	4	Lobed	Scarlet
Data from 12.6.18	1	2	Wild	Wild
	1	2	Lobed	Wild
	4	2	Wild	Scarlet
	1	12	Lobed	Scarlet

Linkage ♀ F1 Wild (AaBb) × ♂ F2 Scarlet, Lobed (aabb) [blue]

	Male Progeny Observed	Female Progeny Observed	Phenotype Eyes	Phenotype Wings
Data from 11.30.18	24	34	Wild	Wild
	6	16	Lobed	Wild
	12	22	Wild	Scarlet
	6	14	Lobed	Scarlet
Data from 12.2.18	28	28	Wild	Wild
	9	9	Lobed	Wild
	14	14	Wild	Scarlet
	3	2	Lobed	Scarlet
Data from 12.6.18	13	12	Wild	Wild
	4	3	Lobed	Wild
	4	4	Wild	Scarlet
	2	2	Lobed	Scarlet
Data from 12.9.18	7	8	Wild	Wild
	5	9	Lobed	Wild
	6	7	Wild	Scarlet
	2	2	Lobed	Scarlet

F2 Progeny P-Value Summary

♀ Scalloped × ♂ Scarlet [red]			♀ Scarlet × ♂ Lobed [red]			♀ Scalloped × ♂ Lobed [red]		
145 ♂, 162 ♀			211 ♂, 259 ♀			131 ♂, 98 ♀		
Total P Value	♂	0.000	Total P Value	♂	0.000	Total P Value	♂	0.031
	♀	0.000		♀	0.013		♀	0.141
Scalloped P Value	♂	0.000	Lobed P Value	♂	0.499	Lobed P Value	♂	0.512
	♀	0.000		♀	0.209		♀	0.047
Scarlet P Value	♂	0.000	Scarlet P Value	♂	0.000	Scalloped P Value	♂	0.004
	♀	0.000		♀	0.003		♀	0.225
♀ Scalloped × ♂ Scarlet [blue]			♀ Scarlet × ♂ Lobed [blue]			♀ Scalloped × ♂ Lobed [blue]		
72 ♂, 76 ♀			254 ♂, 242 ♀			220 ♂, 203 ♀		
Total P Value	♂	0.000	Total P Value	♂	0.000	Total P Value	♂	0.005
	♀	0.000		♀	0.000		♀	0.000
Scalloped P Value	♂	0.002	Lobed P Value	♂	0.128	Lobed P Value	♂	0.001
	♀	0.006		♀	0.000		♀	0.000
Scarlet P Value	♂	0.001	Scarlet P Value	♂	0.000	Scalloped P Value	♂	0.893
	♀	0.000		♀	0.000		♀	0.009