Drosophila Lab Report

Abstract

Introduction

Drosophila melanogaster is an ideal genetic model organism in several respects. Because of its small size it is easily manipulated, its short life cycle allows for genetic analysis over several generations in a short period of time, its few chromosomes simplify genetic analysis, and previous researchers have already identified and mapped many mutations. The overall purpose for this experiment was to map unknown heritable mutations in *Drosophila* to their chromosomal locations. The main hypothesis was that the three mutant traits, dark body color, white eye, and short longitudinal veins, were being inherited in a normal autosomal recessive pattern. Specifically, our aims were to cross unknown *Drosophila* stock to Oregon-R and to Marker flies, with previously mapped chromosomal mutations, to determine for each mutant trait the method of inheritance and the chromosomal location. Specific hypotheses are: that genes for body color are segregating normally, genes for wing venation are segregating normally, body color and wing venation genes are independently assorting, two genes determine eye color, the two eye color genes are independently assorting, and the eye color genes are linked to the body color gene.

Results

Crosses A&B

Cross A
P: U-4033 female x Ore-R male

Table 1 – F_1 Results: Organized Data

TRAIT	WILD TYPE		MUTANT TYPE	
	F	M	F	M
Body Color	47	53	---	---
Eye Color	47	53	---	---
Wing Venation	47	53	---	---

F_1 were wild type for all three mutant traits, with no difference between male and female phenotype.

Table 2 – F_2 Results: Raw Data
(Note: M=male, F=female, wt=wild type, m=mutant)

		EYE COLOR								
		Red		White		Orange		Brown		Total
Body Color	Wing Venation	M	F	M	F	M	F	M	F	
Dark	wt	18	14	3	2	1	2	--	4	44
	m	1	3	2	2	1	1	--	--	10
Light	wt	48	56	6	7	3	3	6	13	142
	m	4	3	10	5	7	6	1	7	43

Total		71	76	21	16	12	12	7	24	239

F_2 progeny had four distinct eye colors, with red the most frequent, but white, orange, and brown appearing with similar frequencies. Light body occurred more frequently than dark body.

Cross B

P: Ore-R female x U-4033 male

Table 3 – F_1 Results: Organized Data

TRAIT	WILD TYPE		MUTANT TYPE	
	F	M	F	M
Body Color	49	51	---	---
Eye Color	49	51	---	---
Wing Venation	49	51	---	---

F_1 were wild type for all three mutant traits, with no difference between male and female phenotype.

Table 4 – F_2 Results: Raw Data
(Note: M=male, F=female, wt=wild type, m=mutant)

		EYE COLOR								
		Red		White		Orange		Brown		Total
Body Color	Wing Venation	M	F	M	F	M	F	M	F	
Dark	wt	17	14	1	1	1	1	1	4	40
	m	--	--	--	3	4	1	1	2	11
Light	wt	51	57	1	6	5	7	6	2	135
	m	6	9	3	5	2	4	4	5	38
Total		74	80	5	15	12	13	12	13	224

F_2 progeny had four distinct eye colors, with red the most frequent, but white, orange, and brown appearing with similar frequencies. Light body occurred more frequently than dark body.

Table 5 – F_2 Results of Crosses A & B: Organized Data

TRAIT	F_2 OF A		F_2 OF B	
	Wild type	Mutant	Wild type	Mutant
Body Color	185	54	173	51
Wing Venation	186	53	175	49

More wild type phenotypes appear for both body color and wing venation.

Table 6 – Eye Color Data from F_2 of Crosses A & B

	A		B		
	Females	Males	Females	Males	Total
Red	76	71	80	74	301
White	16	21	15	5	57
Orange	12	12	13	12	49
Brown	24	7	13	12	56
Total	128	111	121	103	463

Red eye color is the most frequent, and white, orange, and brown appear with similar frequencies.

Crosses II & III

Table 7 – Cross II – F_1 Male backcross progeny
(wt=wild type, m=mutant)

		Male Backcross of			
		F_1 Male Bl L		F_1 Male Cy	
Body Color	Wing Venation	Bl L	Bl^+ L^+	Cy	Cy^+
Dark	wt	5	0	9	0
	m	0	5	0	19
Light	wt	4	4	6	3
	m	0	4	0	15

Dark body color shows up with Bristle Lobed; mutant wing venation never shows up with Bristle Lobed.

Table 8 – Cross III – F_1 Male backcross progeny
(wt=wild type, m=mutant)

Body Color	Wing Venation	Gl Sb	Gl^+ Sb^+
Dark	wt	1	28
	m	0	37
Light	wt	39	1
	m	26	1

With one exception, dark body color does not show up with Glued Stubble. Mutant wing venation does show up with Glued Stubble.

Crosses A, B, & I

Table 9 – Cross A, B, & I: F_2

(wt=wild type, m=mutant)

		Eye Color				
		Red	White	Orange	Brown	Total
Body Color	Wing Venation					
Dark	wt	75	7	5	9	96
	m	19	13	13	7	43
Light	wt	326	28	28	35	417
	m	34	39	29	25	127
Total		445	87	75	76	683

Red eye color appears most frequently, and white, orange, and brown appear with similar frequencies. Light body color is more frequent than dark body color. Wild type wing venation is more frequent than mutant wing venation.

Discussion

Crosses A&B

Crosses A and B were set up as reciprocal crosses in order to determine whether the unknown mutations were autosomal or sex-linked and dominant or recessive. From the Cross A – F_1 results (Table 1), we conclude that the pattern of inheritance for all three mutations is recessive, because they do not appear in the F_1 offspring, and autosomal, because the F_1 males appear wild type. If any of the mutations had been sex-linked, F_1 males would have received a mutant gene from the P female and a Y chromosome from the P male and would have presented a mutant phenotype. From the Cross B – F_1 results (Table 3), we also conclude that the pattern of inheritance for all three mutations is recessive because they do not appear in the F_1 offspring, but we cannot determine whether the mutations are autosomal. If all the mutations were autosomal, F_1 progeny would have one mutant and one wild type copy of each gene and a wild type phenotype. If any of the mutations were sex-linked, they still would not show up in the F_1 progeny because the females would have one X chromosome with a wild type copy of the genes, giving it a wild type phenotype, and the males would only have a wild type copy of the mutant genes on their X chromosome from the P female. However, since the reciprocal crosses produced the same results, we can conclude that the mutations are autosomal.

If genes for body color are segregating normally as hypothesized, we expect a F_2 ratio of wild type body color to mutant body color of 3:1. Based on the combined F_2 results of A and B, we observed a ratio of 3.4:1 (Table 10). The X^2 value for these results is 1.39, which corresponds to a p value greater than 0.05, and we can deduce that the mutant body color gene is segregating normally. A 3:1 phenotypic ratio corresponds to an autosomal recessive trait so we conclude that the body color gene is autosomal recessive. With the null hypothesis that genes for wing venation are segregating normally, we expect a F_2 ratio of wild type wing venation to mutant wing venation of 3:1. From the combined F_2 results of A and B (Table 11), we observed a ratio of 3.5:1. The X^2 value for these results is 2.25, which corresponds to a p value greater than 0.05, and by the same reasoning as above, we deduce that the wing venation gene is autosomal recessive. We next hypothesized that body color gene and wing venation gene were assorting independently. With this hypothesis we expect a ratio for wild type body color and wild type wing venation: mutant body color and wild type wing venation: wild type body color and mutant

wing venation: mutant body color and mutant wing venation of 11.9:3.5:3.4:1 (Table 12). We observed a ratio of 13.2:4.0:3.9:1.0; the X^2 value for this set of data is 0.24, corresponding to a p value greater than 0.05, and we can deduce with that body color and wing venation are assorting independently.

We observed red, brown, orange, and white eyes in a ratio close to 6:1:1:1 in the F_2 progeny (Table 6). Based on this we propose that two genes (orange eyes, a, and brown eyes, b) are involved in the expression of eye color, because with the simplifying assumption that each mutant gene has two alleles, at least two genes are needed to produce four phenotypes. Our hypothesis of interaction is a parallel pathway where at least one wild type copy of each gene is needed to produce the wild type red eye (Fig. 1). Our null hypothesis for their association is that they are independently assorting. This gives a X^2 value of 61, which corresponds to a p value less than 0.05 and a strong rejection of the hypothesis. Therefore the two genes for eye color are linked. F_1 progeny were all a^+b^+/ab (ab received from the unknown parent and a^+b^+ received from the wild type parent). Because crossing-over during meiosis only occurs in female gametes, all male gametes were either ab or a^+b^+, and recombinant female gametes were a^+b or ab^+, producing the following recombinant genotypes and phenotypes: a^+b/ab (brown), a^+b^+/ a^+b^+ (red), ab^+/ab (orange), and ab^+/a^+b^+ (red). Based on the frequency of these recombinant genotypes within the F_2 progeny, the two eye color genes are located 45.4 map units apart.

Overall the data from F_1 and F_2 of Crosses A and B did not present any problems and from it we concluded that the three mutant traits have a high probability of being autosomal, body color and wing venation have a high probability of assorting independently, and it is highly probable that two linked genes control eye color.

Male Parent Backcross: Crosses II and III

Crosses II and III were set up with unknown flies crossed with Marker II and Marker III stocks respectively. Marker II and Marker III stocks serve as chromosomal markers because they carry dominant homozygous lethal mutations. Marker II has mutant alleles of the Bristle and Lobed genes and a wild type allele of the Curly gene on one copy of chromosome II and wild type alleles of the Bristle and Lobed genes and a mutant allele of the Curly gene on the other copy. Because these three mutations are dominant, these flies should present short bristles, curly wings, and small lobed eyes but the bristle phenotype does not show up in the Marker II flies we have. Marker III has mutant alleles of the Glued and Stubble genes and a wild type allele of the chromosomal inversion LVM on one copy of chromosome III and wild type alleles of the Glued and Stubble genes and a mutant allele of the chromosomal inversion LVM on the other copy. As these three mutations are dominant, Marker III flies have small, smooth eyes and short blunt stubbles (LVM has no mutant phenotype). Both marker stocks are true-breeding because any progeny that do not have the same genotype as the parents lethally receive two copies of at least one mutation (Fig. 2).

In order to map body color and wing venation genes to chromosomes, we set up F_1 male backcrosses for Crosses II and III. In a F_1 male backcross, F_1 males showing a marker phenotype are crossed to unknown females. In the backcross progeny, if an unknown mutant trait shows up with a marker, then the mutant trait and marker are on different chromosomes; if they do not show up together, they are on the same chromosome. For Cross II – F_1 male

backcross progeny, dark mutant body color shows up with both Bristle Lobed and Curly markers, indicating that the body color gene is not on chromosome II with them (Table 7). Mutant wing venation never shows up with Bristle Lobed or Curly, indicating the wing venation gene is on the chromosome II with those markers. For Cross III F_1 male backcross progeny, dark mutant body color does not show up with Glued Stubble markers, indicating the body color gene is also on chromosome III (Table 8). Mutant wing venation does show up with the Glued Stubble markers, indicating that the wing venation gene is not on chromosome III. There is one exception of a dark bodied, Glued Stubble fly, but as it is only one fly, this is probably due to human scoring error or the presence of a random fly from the lab during scoring.

Overall the Cross II and III F_1 and male F_1 backcross data strongly support the conclusion that body color gene is on the chromosome III and wing venation gene is on chromosome II.

Cross I

We set up Cross I between unknown virgin females and crossveinless forked Marker I males and carried the cross through the F_2 generation. In the F_1 generation we expected to see all phenotypically wild type females and all crossveinless forked males (Fig. 3). However, we saw wild type males instead of crossveinless forked males and Glued Stubble males and females. Possibly some Marker III males were among the parents, which would account for the presence of Glued Stubble. Since every F_1 progeny should have received a mutant gene from the unknown parental female for crossveinless and for forked, all males should show both traits. We do not believe the absence of crossveinless forked males is from scoring error, as we checked many flies repeatedly. An alternate explanation is that the unknown females were not virgin when introduced and produced phenotypically wild type progeny, or that the parental females were not from unknown stock.

As we determined from Crosses A and B, the eye color genes are autosomal. By the combined F_2 data from Crosses A, B, and I (Table 9), if we hypothesize that the two eye color genes are independently assorting with each other, we get a X^2 value of 98, which corresponds to a p value less than 0.05. So, we must reject the hypothesis and conclude that the two eye color genes are linked. The F_1 progeny for all three crosses are all a^+b^+/ab (ab received from the unknown parent and a^+b^+ received from the wild type parent). Because crossing-over during meiosis only occurs in female gametes, all male gametes were either ab or a^+b^+, and recombinant female gametes were a^+b or ab^+, producing the following recombinant genotypes and phenotypes: a^+b/ab (brown), a^+b^+/ a^+b^+ (red), ab^+/ab (orange), and ab^+/a^+b^+ (red). Based on the frequency of these recombinant genotypes within the combined F_2 progeny, the two eye color genes are located 44.2 map units apart.

In Table 9, mutant eye color and mutant body color appear together less frequently than mutant eye color and mutant wing venation (54 versus 126). In our skeletal report we interpreted this to mean that the wing venation gene and eye color genes were more independent than the body color gene and the eye color genes, and we assigned eye color genes to the chromosome body color is on, chromosome III. We used the body color and eye color data in Table 13 to calculate map distances of 65.9 map units between d (dark body color gene) and a, 67.3 map units between d and b, and 44.2 map units between a and b. This produced the final results seen in Table 15 (p. 15 of Skeletal Report) and Fig. 4 (p. 15 of Skeletal Report). The map in Fig. 4 for chromosome III does not make sense (we have the distance between d and b calculated directly as 67.3 map

units, and indirectly by using the distances between d and a and a and b as 110.1 map units) because the eye color genes should really be on chromosome II (as clarified by Jessica Lehoczky). For this report we realized that we misinterpreted our data for the skeletal report. Since in the P generation, mutant eye color, body color, and wing venation all appear together, then linked genes will continue to appear together and unlinked genes will tend to appear separately. Since mutant eye color and mutant wing venation appear together more frequently than mutant eye color and mutant body color, eye color genes are linked to the wing venation gene on chromosome II. With this assignation in Table 14 we calculated map distances of 42.2 map units between sv (short longitudinal vein) and a, 86.4 map units between sv and b, and 44.2 map units between a and b. Table 16 and Fig. 5 show the final determined genetic map for our unknown *Drosophila*. Although the calculated map distances for sv, b, and a with respect to each other on chromosome II differ from the given values of sv (II:3.8), a (II:57.0), and b (II:104.5) (as clarified by Jessica Lehoczky), they are more logical than the distances found when body color and eye color genes are linked. The difference between our experimentally determined map distances and the actual numbers could be due to scoring error, crossing error, or an insufficiently sized pool.

Conclusion and Future Directions

Looking at Table 16, we can see that in U-4033 *Drosophila* the unknown genes orange (a), brown (b), dark body (d), and short veins (sv) are all autosomal recessive, with genes a, b, and sv on chromosome II and gene d on chromosome III. The linked genes a, b, and sv are mapped on chromosome II with respect to each other as shown in Fig 5. To help further clarify our data,

Materials and Methods

Oregon-R, unknown mutant, and marker stock *Drosophila melanogaster* were studied. Oregon-R have a wild type phenotype, unknown mutant have dark bodies, white eyes, and short longitudinal veins, and marker stocks have crossveinless wings with forked bristles, curly wings with small eyes and short bristles (although short bristle was undetectable), or small oblong eyes with short blunt stubbles. Flies were anethesized with CO_2 or Fly Nap and manipulated with paintbrush or pick under the microscope. They were sexed, collected, crossed, and scored as described in the Biology 306 Introductory Genetics Laboratory Manual.

Crosses A, B, I, II, and III were set up initially. Cross A had unknown virgin females crossed to Ore-R males and as a reciprocal cross, Cross B had unknown males crossed to Ore-R virgin females. The F_1 progeny were used to deduce whether the unknown mutations were autosomal or sex-linked and recessive or dominant. F_1 progeny in both crosses then produced F_2 progeny to examine the relationship between the mutant genes. Cross I had Marker I males crossed to unknown virgin females and a subsequent F_1 x F_1 cross, Cross II had Marker II males crossed to unknown virgin females and a subsequent male backcross, and Cross III had Marker III males crossed to unknown virgin females and a subsequent male backcross. All of these crosses were used to assign the mutant genes to chromosomes, and the Cross I F_2 progeny were used along with the Cross A and B F_2 progeny to map the two eye color genes.

References

Appendix A – Additional Tables

Table 10 – Body Color: F_2 results of A & B Combined

	+ type:mutant
Observed #	358:105
Observed Ratio	3.4:1
Expected Ratio	3:1
Expected #	347:116

Table 11 – Wing Venation: F_2 results of A & B Combined

	+ type:mutant
Observed #	361:102
Observed Ratio	3.5:1
Expected Ratio	3:1
Expected #	347:116

Table 12 – Body Color and Wing Venation Data from F_2 of Crosses A & B

	Observed		Expected	
	Actual #	Ratio	Actual #	Ratio
wt body color wt wing venation	277	13.2	278	11.9
m body color wt wing venation	84	4.0	82	3.5
wt body color m wing venation	81	3.9	80	3.4
m body color m wing venation	21	1.0	23	1.0

Table 13 – 3 Point Cross Between Body Color Gene and Eye Color Genes

d-a-b		
Phenotypes	Genotypes	#'s
Dark body, white eyes	dab	20
Dark body, orange eyes	dab^+	18
Dark body, brown eyes	da^+b	16
Dark body, red eyes	da^+b^+	85
Light body, white eyes	d^+ab	67
Light body, orange eyes	$d^+ ab^+$	57
Light body, brown eyes	d^+a^+b	60

Light body, red eyes	$d^+ a^+b^+$	360

d= body color gene
a&b=eye color gene

Table 14 – 3 Point Cross Between Wing Venation Gene and Eye Color Genes

sv-a-b		
Phenotypes	Genotypes	#'s
Short veins, white eyes	svab	52
Short veins, orange eyes	$svab^+$	42
Short veins, brown eyes	sva^+b	32
Short veins, red eyes	sva^+b^+	44
Long veins, white eyes	Sv^+ab	35
Long veins, orange eyes	$sv^+ ab^+$	33
Long veins, brown eyes	sv^+a^+b	44
Long veins, red eyes	$sv^+ a^+b^+$	401

sv=wing venation gene
a&b=eye color gene

Table 16 – Final Map of Unknown Mutations

Gene Name	Symbol	Dom. Or Rec.	Chromosome
orange	a	Rec.	II
brown	b	Rec.	II
dark body	d	Rec.	III
short veins	sv	Rec.	II

Appendix B – Figures

Figure 1 – Hypothesis of Interaction Between Eye Color Genes

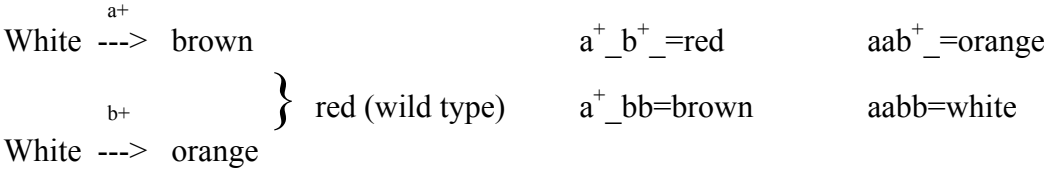


Figure 2 – Balanced Lethal System in Marker Stocks

Marker II

P: $\frac{Bl\ \ L\ \ Cy^+}{Bl^+\ L^+\ Cy}$ x $\frac{Bl\ \ L\ \ Cy^+}{Bl^+\ L^+\ Cy}$

F_1: $\frac{Bl\ \ L\ \ Cy^+}{Bl\ \ L\ \ Cy^+}$ (nonviable) x $\frac{Bl\ \ L\ \ Cy^+}{Bl^+\ L^+\ Cy}$

$\frac{Bl^+\ L^+\ Cy}{Bl^+\ L^+\ Cy}$ (nonviable) x $\frac{Bl^+\ L^+\ Cy}{Bl\ \ L\ \ Cy^+}$

Marker III

P: $\frac{Gl\ \ Sb\ \ LVM^+}{Gl^+\ Sb^+\ LVM}$ x $\frac{Gl\ \ Sb\ \ LVM^+}{Gl^+\ Sb^+\ LVM}$

F_1: $\frac{Gl\ \ Sb\ \ LVM^+}{Gl\ \ Sb\ \ LVM^+}$ (nonviable) x $\frac{Gl\ \ Sb\ \ LVM^+}{Gl^+\ Sb^+\ LVM}$

$\frac{Gl^+\ Sb^+\ LVM}{Gl^+\ Sb^+\ LVM}$ (nonviable) x $\frac{Gl^+\ Sb^+\ LVM}{Gl\ \ Sb\ \ LVM^+}$

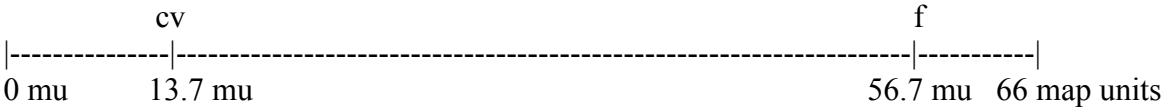
Figure 3 – Expected F_1 of Cross I

P: $\frac{cv\ \ f}{cv\ \ f}$ x $\frac{cv^+\ f^+}{cv^+\ f^+}$

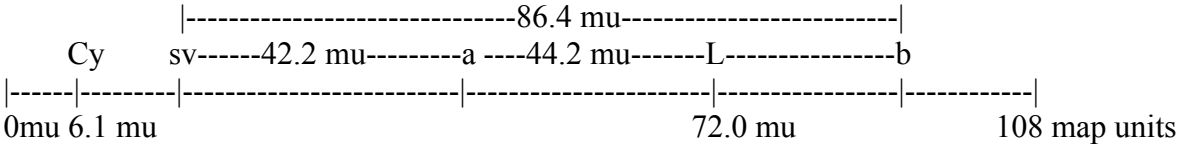
F_1: $\frac{cv^+\ f^+}{cv\ \ f}$ (females) $\frac{cv\ \ f}{\ }$ (males)

Figure 5 – Final Map of Unknown Mutations

Chromosome 1 (X Chromosome):



Chromosome 2 (Autosome):



Chromosome 3 (Autosome):