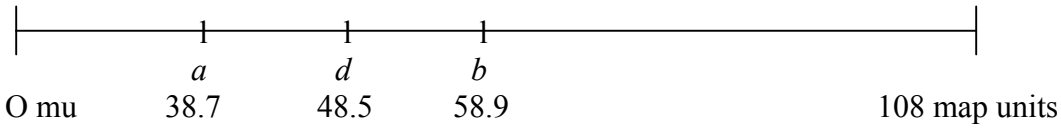
Mapping of Genes in a Mutant Strain of *Drosophila Melanogaster*

Abstract

Drosophila melanogaster (the common fruit fly) is convenient as a model organism for use in genetic experiments. By analyzing crosses in mutant and wild-type strains of Drosophila, the mapping of genes can be accomplished. In this experiment, U-4182 was the mutant strain used. This parental mutant strain possessed a dark body color, white eyes, and incomplete longitudinal veination. The purpose of this experiment was to determine the inheritance patterns of these genes as well as to locate the position of the genes on the chromosomes. To accomplish this task, numerous crosses were performed. Reciprocal crosses between mutant and wild-type strains established the patterns of inheritance of the mutant genes. The crosses also showed that the eye color was actually controlled by two genes: orange eye (a) and brown eye (b). Both genes were needed to create red eyes. Crosses with marker strains M1 and M3 were used to determine linkage relationships and the locations of the mutant genes. Body color (d), orange eye (a), and brown eye (b) were all established to be on chromosome 2. Specifically, body color was at locus 48.5 map units (mu), orange eye was at locus 38.7 mu, and brown eye was at locus 58.9 mu. The location of the wing venation mutation (w) was inferred to be on chromosome 3. All of the mutations are autosomal recessive.

Chromosome 2 (Autosome):



Chromosome 3 (Autosome):

Introduction

Drosophila melanogaster is useful as a model organism because it has a short life cycle of 10-14 days. Their short lifespan and small size make it easy to culture and relatively inexpensive to store large numbers of flies. Additionally, numerous mutant phenotypes are easily seen with or without the aid of microscopes so observing and separating the flies is not a painstaking task. Because *Drosophila* has a very long history in biological research, there is a tremendous amount known about its genome. The knowledge about wild-type and marker strains will help in identifying where the mutant genes are and how they are inherited.

Ultimately, the purpose of this experiment was to characterize unknown mutations in a mutant strain of *Drosophila melanogaster*. The goal was to determine the inheritance patterns of these mutations as well as to locate the specific position of the genes on the chromosomes. By comparing a pure line wild-type strain, known as the Ore-R strain, with the mutant strain, known as U-7812, distinct differences were found in the expression of body color, eye color, and wing venation. To find out how these three different phenotypes were created, various crosses were performed. In order to find out how the mutations were inherited (sex-linked verses autosomal; dominant verses recessive), reciprocal crosses between the Ore-R strain and mutant strain were performed. The progeny of the F1 generation underwent further reciprocal crossing to produce an F2 generation which was used to determine how the mutations are inherited. In addition to these reciprocal crosses, a series of marker crosses were performed using M1 and M3 marker strains (Note: Marker 2 was not available). The marker strains contain mutations with known positions on their respective chromosomes (M1 is the X-chromosome, M3 is

chromosome 3), so they could be used to decide whether the marker mutations were linked with the unknown mutations. Female unknowns were crossed with males from the marker strains. An F1 cross was completed for the M1 group, while a male back-cross was performed for the M3 group. These marker crosses provided information to establish linkage relationships of the unknown genes and to assign each gene to a specific locus.

Materials and Methods

Four different strains of *Drosophila melanogaster* were used. All of the flies in the stocks are true breeding. The Oregon-R (Ore-R) stock contains wild-type flies have red eyes, complete wing veination, and tan-colored bodies. U-7812, the unknown stock with white eyes, dark body, and incomplete longitudinal veination, was utilized in every cross. The Marker 1 stock had crossveinless (cv) and forked (f) mutations, which are located on the X-chromosome. These mutations were eventually found to be sex-linked recessive. The Marker 3 stock had glued eyes (Gl), stubble (Sb), and LVM mutations, which are located on chromosome 3. These mutations were eventually found to be autosomal dominant. LVM flies express the same phenotype as a wild-type fly would; the actual difference is that an LVM fly has an inversion on chromosome 3. LVM is homozygous lethal and therefore serves as a balancer lethal system in the M3 crosses.

Cross A

10 U-4182 virgin females were crossed with 20 Ore-R males.

Cross B

10 Ore-R virgin females were crossed with 20 U-4182 males.

Cross I

10 U-4182 virgin females were crossed with 20 M1 males.

Cross III

10 U-4182 virgin females were crossed with 20 M3 males

Cross A: F1 crosses

15 male and 10 female F1 progeny from the parental cross of A were transferred into a separate container and crossed.

Cross B: F1 crosses

15 male and 10 female F1 progeny from the parental cross of B were transferred into a separate container and crossed.

Cross I: F1 crosses

15 male and 10 female F1 progeny from the parental cross of I were transferred into a separate container and crossed.

Cross III: Male backcross

15 Glued-Stubble males from the F1 progeny of cross III were crossed with 10 U-4182 virgin females.

All crosses were performed twice and all protocol in performing the crosses as well as the handling of all drosophila strains can be found in the MCDB 306 lab manual.

Scoring of flies

All flies were put to sleep using carbon dioxide gas. After all F1 progeny were hatched, 100 flies were scored from crosses A, B, I, and III. After F2 and backcross progeny had hatched, 200 flies from each F2 generation were then scored with phenotype data recorded for all crosses.

Results and Discussion

Cross A F_1 Results

Trait	Wild type F	Wild type M	Mutant Type F	Mutant Type M	Pattern of Inheritance (comments)
Body color	55	45	0	0	Autosomal Recessive
Eye color	55	45	0	0	Autosomal Recessive
Wing venation	55	45	0	0	Autosomal Recessive

The F1 results as shown above support that there is an autosomal recessive pattern of inheritance for the mutant alleles for wing venation, body color, and eye color. If the mutations in the U-7812 strain had been dominant, all of the F1 progeny would have expressed the mutated phenotypes. This is because all of the F1 progeny are heterozygous for the mutant gene. Also, if the mutations had been sex-linked, then all of the male F1 progeny would be mutated. This is because males have one X-chromosome from their U-7812 female parent, meaning any mutations would be expressed. Neither of these patterns was supported by the data gathered, but autosomal recessive inheritance of the alleles was supported. Therefore, it can be concluded that all three mutant alleles show an autosomal recessive inheritance pattern.

Cross B F_1 Results

Trait	Wild type F	Wild type M	Mutant Type F	Mutant Type M	Pattern of Inheritance (comments)
Body color	52	48	0	0	Recessive
Eye color	52	48	0	0	Recessive
Wing venation	52	48	0	0	Recessive

The F1 results as shown above support that there is a recessive pattern of inheritance for the mutant alleles for wing venation, body color and eye color. Like in Cross A, if the mutations in the U-7812 strain had been dominant, all of the F1 progeny would have expressed the mutated phenotypes. Distinguishing between sex-linked and autosomal cannot be done, because it is impossible for either sex to express the mutant phenotype. All F1 male progeny will receive their X-chromosome from their wild-type mother, so males will always express the wild-type phenotype. Females will be heterozygous for the mutant gene. Therefore, from this data, it is only possible to conclude that the genes are recessive. However, with the data from Cross A and Cross B together, it is possible to conclude that the genes are definitely autosomal recessive.

Marker 1 Results

Phenotype	cv^+ f^+	cv f
Number of Flies	55 females 45 males	0

It is already known that crossveinless and forked mutations are sex-linked recessive. For the Marker 1 Cross, all of the progeny were wild-type phenotypically. The females were heterozygous for the mutant genes, and the males were all wild-type because they received the wild-type genes from the wild-type mothers. Therefore, the observed data makes sense.

Marker 3 Results

Phenotype	Gl Sb	LVM
Number of Flies	56	44

It is already known that Glued-eye, Stubble, and LVM are dominant mutations on chromosome 3. However, since Gl, Sb, and LVM are homozygous lethal, mutant flies in the stock must be heterozygous at those loci (Refer to skeletal report to see the actual arrangement of the marker mutant genes as their arrangement is crucial for successful backcrosses). This means that the father has the potential to give one of two different genotypes during mating. Since the unknown female is wild-type for the three genes, the resulting F1 progeny should have two different phenotypes in equal ratios. The data gathered reflects all of these facts, because the F1 progeny are about half Glued-Stubble and half LVM expressing. The observed data makes sense.

F_2 Results of Crosses A & B

Trait	F_2 of A		F_2 of B	
	+ type	mutant	+ type	mutant
body color	151	47	147	49
wing venation	148	50	147	49

The data above helped show three major things. First, it showed that the body color gene segregated properly and is controlled by one gene. The data corresponded to an observed ratio of 3.1:1 (wild-type:mutant), which was extremely close to the expected 3:1 ratio hypothesized to arise if there was one gene responsible, and the mutation was recessive. Using X^2 analysis, it was supported that body color is inherited through autosomes and that the mutation is recessive. The X^2 value of .081 correlated to a probability of 77.6% that the observed ratios would occur due to chance, given the expected ratio. Therefore, body color is autosomal recessive. Secondly, the data showed that the wing veination gene segregated properly as is controlled by one gene. The data corresponded to an observed ratio of 2.96:1 ratio (wild-type:mutant) which again was extremely close to the

expected 3:1 ratio hypothesized to arise if there was one gene responsible, and the mutation was recessive. Using X^2 analysis, it was supported that wing veination is inherited through autosomes and that the mutation is recessive. The X^2 value of .013 correlated to a probability of 90.9% that the observed ratios would occur due to chance, given the expected ratio. Therefore, wing veination is autosomal recessive.

Finally, the F2 data shown above helped determine whether the two genes, body color and wing venation, were linked or segregating independently (combined table found in skeletal report). The data presented showed an observed ratio of 10.9:3.8:3.95:1 which was close to the expected 9:3:3:1 ratio expected for two independently assorting genes with recessive mutations. Using X^2 analysis, it was supported that the two genes are assorting independently. The X^2 value of .898 correlated to a probability of 82.6% that the observed ratio would occur due to chance, given the expected ratio. Therefore, it was concluded that these two genes are localized to different chromosomes in the genome, so this accounts for the independent assortment observed.

Cross A and B F2 Eye Color Data Combined

	females	males
Red	143	133
White	40	39
Brown	9	8
Orange	12	13

The F2 data showed the expression of four phenotypes for eye color. The expression of the orange and brown phenotypes in the F2 progeny warranted the proposal that there were two genes controlling eye color. With four phenotypes, there must be at least two genes, but, because there are only four phenotypes, there should only be two genes

involved. The genes expressing the orange and brown phenotypes were assigned the names gene *a* and gene *b*, respectively. The following table shows how the genes interact to produce the different colors.

Red	White	Orange	Brown
$a^+\_b^+\_$	aabb	a^+_bb	$aab^+\_$

If the genes were independently assorting, there would be a typical 9:3:3:1 ratio expressed in the F2 generation. The data does not support this, as there are far more white-eyes flies than orange-eyed or brown-eyed. Since having white eyes requires that both genes are homozygous recessive, the genes are unlikely to be assorting independently. The data supports that the two eye color genes are linked, so crossing over may be occurring in the female flies. It makes sense that the brown and orange eyes are least expressed because they are recombinant phenotypes. The map distance between the two genes can be calculated using the combined data of crosses A and B. Orange-eyed and Brown-eyed flies are recombinant phenotypes, so crossing over had occurred in the mother flies. The two genes were shown to be 21.3 map units apart (refer to skeletal report).

Cross III -- F_1 Male backcross results

Body Color	Eye Color	Wing Venation	GI Sb	GI+ Sb+
light (wild type)	red	wild type	32	0
		mutant	0	25
	white	wild type	0	0
		mutant	0	0
dark (mutant)	red	wild type	0	0
		mutant	0	0
	white	wild type	23	0
		mutant	0	20

A male backcross is used to ensure that crossing over does not take place when it crossed to a pure-line unknown female. Glued-Stubble males were recovered from the F1 progeny, and they were crossed with U-7812 virgin females. Because of the way the genes are set up on the marker chromosome (refer to skeletal report), unknown mutations that are on chromosome 3 will never show up with Glued/Stubble (i.e. genes that are linked on chromosome 3 will not show up with Glued/Stubble). If the unknown mutation is on a different chromosome, then it is possible for the mutation to show up with Glued/Stubble. According to the data above, body color and eye color genes must be assorting independly from Glued/Stubble because the mutant forms of both genes show up with Glued/Stubble. On the other hand, mutant veination is never seen with Glued/Stubble, so wing veination must be on chromosome 3. The body color and eye color genes are therefore on chromosome 2 because they are neither sex-linked (which was found from Cross A) nor linked to chromosome 3.

Cross I, A, and B combined analysis

The data gathered (refer to skeletal report) shows that there is a linkage relationship between eye color and body color. If there had been no linkage, then the double-mutant, white eyes and dark body, would be expressed in the lowest quantity in the F2 progeny of crosses A, B, and I. This was not seen, as there were far more double-mutant flies than the recombinant orange or brown phenotypes. If the genes were *not* linked, then the expected ratio is 9:3:3:1. Using X^2 analysis, it was supported that the two eye color genes are linked on autosome 2. The X^2 value of 341.6 correlated to a probability of much less than 5% that the observed ratio would occur due to chance, given the expected ratio. This means the hypothesis of independent assortment must be rejected. This confirms that the

eye color genes are linked on chromosome 2. With the combined data, it was found that the map distance between the eye colors was 18.9 map units.

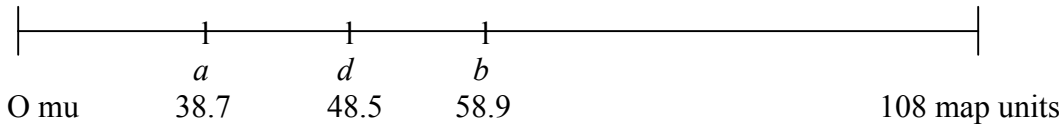
Performing a three-point cross established the map distances between the linked genes of eye color and body color. Refer to the skeletal report to see a table with data for the three-point cross. By dividing recombinant phenotypes by the total number of flies, the map distances between genes could be calculated. The calculations showed a 9.8 mu distance between body color and gene *a*, a 10.4 mu distance between the body color and the gene *b*, and 20.2 mu distance between gene *a* and gene *b*. Mapping of these genes to their assigned chromosomes was accomplished by acquiring the location of one of the genes. The other genes were then mapped in relation to that gene.

Summary

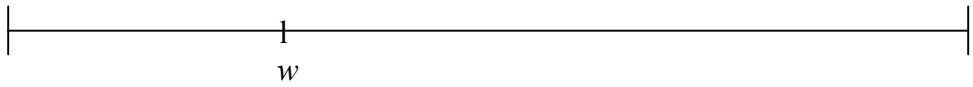
	Gene Name	Symbol	Dom. or Rec.	Chromosome	Genetic Locus
1.	Body Color	d	Recessive	2	48.5 mu
2.	Wing Venation	w	Recessive	3	N/A
3.	Orange Eye Color	a	Recessive	2	38.7 mu
4.	Brown Eye Color	b	Recessive	2	58.9 mu

The data presented above provides the organized conclusions of this experiment. It was shown that body color and eye color are linked on chromosome two, and that the wing venation trait was present on chromosome three.

Chromosome 2 (Autosome):



Chromosome 3 (Autosome):



An Appendix containing the skeletal report, with all chi-square calculations, can be found starting on the next page.