Mapping of Unknown Mutations In *Drosophila Melanogaster*

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Abstract

Introduction

Drosophila Melanogaster, commonly known as fruit fly, was the organism studied in this experiment. *Drosophila* were ideal because they were small and had a life cycle of about 10 to 14 days. This allowed for many generations to be produced from crosses. *Drosophila* are diploid organisms that only have four pairs of chromosomes which includes three pairs of autosomes and one pair of sex chromosomes. *Drosophila* were also ideal to study because they possess traits, which can be observed with a microscope, that were characteristic of the sex and genotype of the flies. In this experiment the pattern of heritance and the genetic loci of the mutations in body color, eye color, and wing venation in an unknown strain **u4184** of flies. The unknown strain was crossed with a wild-type strain in a set of reciprocal crosses to determine if each trait was recessive or dominant and autosomal or sex-linked. The unknown strain was then crossed with three strains that each had different known marker mutations. The results from the F_1 generation of these crosses and the results from their subsequent male backcrosses or F_1 x F_1 cross determined on which chromosomes the genes for body color, eye color, and wing venation were. The genetic distance between these genes was determined by the results in the F_2 generation of the reciprocal crosses combined some of the results from the marker crosses by calculating the percentages of recombinants and parental types.

Methods and Material

During the first lab period, the traits of eye color, body color, and wing venation were observed in the unknown strain and wild-type strain using the microscope and CO_2 according to the procedure described in the lab manual. The unknown flies had whit eyes, dark brown body color, and wing venation with short longitudinal veins. The wild-type flies of Ore-R had red eyes, tan body color, and wing venation with longitudinal veins that went to the end of the wing. During this time the differences between the female and male flies was determined. The male flies have rounder abdomens with the last two dorsal segments darkly pigmented, genital claspers ventrally on the abdomen, and sex combs on the first pair of legs. The female flies have pointier abdomens with dorsal triangular dark pigmentations and lack genital claspers and sex combs. After observing, two new cultures of unknown flies were made with about 15 females and 20 males each. The cultures bottle was placed on its side until the flies wake up so that they do not get caught in the food and die. These new cultures supplied a fresh source of unknown flies

for later weeks during the experiment. Shortly after this, the adult flies in the eight unknown bottles and three wild-type bottles were cleared. Eight hours later 80 unknown virgin females and 20 wild type virgin females were collected as described in the lab manual on page 20. Clearing the adults and then waiting no more than eight hours to collect the females ensured that the females were virgins because they were not mature yet for mating with the males.

The next step in the experiment was setting up the set of reciprocal crosses and the marker cross using the collected virgin females. The first cross was Reciprocal Cross A which consisted of 15 unknown U4184 virgin females and 10 wild type Ore-R males. To perform the crosses the same procedure was used as described in the lab manual for the culture, but in this case the flies were from two different strains instead of one strain. The vials for each cross were labeled for identification. The second cross was Reciprocal Cross B which consisted of 15 unknown U4184 males and 9 wild type, Ore-R females. The third cross performed was Marker Cross I, which consisted of 25 males from marker stock I and 10 unknown U4184 females. The marker I male flies had the genotype cv f on their only X chromosome, which produced a phenotype of cross veinless wings (with allele cv) and forked bristles (with allele f). These mutations are sex-linked, recessive. The fourth cross was Marker Cross II which consisted of 30 marker stock II males and 10 unknown U4184 virgin females. The marker stock II males had the phenotype of short thin bristles (with allele Bl), lobed eyes (with allele L), and curly wings (with allele Cy) and a genotype of Bl Bl+ L L+ Cy Cy+. The fifth cross was Marker Cross III and consisted of 30 marker stock III males and 10 unknown 4184 virgin females. The marker stock III males had a phenotype of smooth ("glued") eyes (with allele Gl) and short, blunt bristles (with allele Sb) and a genotype of Gl Gl+ Sb Sb+ LVM LVM+. The LVM gene did not produce an effect on phenotype.

One week later and once the vials all had a sufficient amount of pupae, the parents from all the crosses could be removed, killed with CO_2, and disposed in the fly morgue. Because the fly life cycle is about 9 days, this step removes the parental generation and prevents breeding between the parental and F1 generation. During this time, the phenotypes of the marker crosses (which were noted above) were observed under the microscope so that offspring could be scored correctly. Also 40 unknown virgin females were collected according to the same procedure done before.

The next step in the experiment was to score the F1 generation of all five crosses and make a record. The scoring took looked at the phenotypes for sex, body color, eye color, wing venation, and the marker mutations. At least 100 flies from Reciprocal Cross A, at least 100 from B, and at least 50 each from Marker Cross I, II, and III were scored.

After scoring, the second round of crosses was set up. For Reciprocal Cross A 30- 40 male and female flies from the F1 generation were taken and put into a new bottle to make a F1 x F1 cross. This was done again to make a duplicate. The same was done for Reciprocal Cross B and Marker Cross I and their duplicates. For Marker Cross II, first a cross was made between 30 bristle, lobed males of its F1 and 10 unknown 4184 virgin females, and then a second cross was made between 30 curly males of the F1 and 10 unknown virgin females. For Marker Cross III, a cross of 30 glued, stubble makes from the F1 and 10 unknown virgin females was made and duplicated. These crosses for Marker Cross II and III are known as male back crosses. And one week later the adults were removed just as in the first round of crosses. Finally, the last step in the experiment was to score the F2 generation and the male back cross progeny according to their eye color, sex, body color, wing venation, and marker mutations.

Results & Discussion

Reciprocal Crosses A and B

The results from the Reciprocal Crosses A and B in the F1 and F2 generations shed light o the genes controlling eye color, body color, and wing venation. The reciprocal crosses allowed for the determination of the traits as sex-linked or autosomal, and dominant or recessive. For example, if a trait were autosomal, then the progeny of both Cross A and B should be the same because it would not matter which parent had which genotype since each is passed on equally to both sexes of children. This is not true for a sex-linked trait, in which the son inherits the x chromosome and its genes only from the mother. These crosses F1 offspring also indicated whether the traits were dominant or recessive, because the offspring of these pure-breeding lines would show the dominant allele in their phenotype. The results for the F1 and F2 generations for the Reciprocal Crosses A and B are discussed individually below for body color, wing venation, and eye color.

Body Color

Table 1: Body Color Results from Crosses A and B	Wild Type (tan)	Mutant (dark brown)
Cross A F1 results	59 females & 41 males	0 males & 0 females
Cross B F1 results	90 females & 89 males	0 males & females
Cross A F2 results	217 (males & females)	54 (males & females)
Cross B F2 results	159 (males & females)	45 (males & females)

From Cross A F1, the dark body color gene can be determined as autosomal, recessive. It is recessive because all the F1 is wild-type. This indicates that the wild-type allele masks the mutant dark color allele. The trait is autosomal because both the males and females are wild-type. If the trait were sex-linked then the males would be mutant because they would only have one X chromosome which they inherited from their mutant mother from a pure-breeding line. The F1 from Cross B only indicated that dark body color trait was recessive because the males would be wild-type even if the trait were sex-linked since they would inherit a normal allele on the X chromosome from the mother. The results form the F2 generations of Crosses A and B determined if the dark body trait alleles were segregating properly. If this data were tabulated again to look at the ratios, the following would be observed:

Table 2: Ratios for Body Color based on F2 From Crosses A and B	wild type : mutant
observed #	376 : 99
observed ratio	3.80 : 1
expected #	3: 1
expected ration	356.25 : 118.75

Upon Chi- Square analysis, the chi- square value was 4.38 with a p-value less than 0.05. This indicated to reject the hypothesis of light body color being completely dominant to dark with no more segregation the two alleles for body color. The rejection may have been because the dark body mutation does not have 100 % penetrance, or perhaps during scoring the newly hatched dark bodies were scored as light instead of dark.

Wing Venation

Table 3: Wing Venation Results from Crosses A & B	wild type	mutant (4 short longitudinal veins)
Cross A F1 results	59 females & 41 males	0 males & females
Cross B f1 results	90 females & 89 males	0 males & females
Cross A F2 results	196 (males & females)	75 (males & females)
Cross B F2 results	159 (males & females)	45 (males & females)

Based on the F1 results of cross A, the mutant wing venation can be determined as a recessive, autosomal trait based on the same reasoning used for the body color trait. The F1 results from Cross B only determined the trait as recessive, as it did for the dark body color gene. The F2 results determined if the alleles for the wing venation were segregating properly. Once the F2 results were combined and looked at in ratios, the hypothesis of normal

segregation for a complete dominance of wild-type wing venation over the mutant could be tested. The following is the combined ratios:

Table 4: Ratios for Wing Venation based on F2 results of Cross A and B	wild-type : mutant
observed #	355 : 120
observed ratio	2.96 : 1
expected ratio	3 :1
expected #	356.25 : 118.75

The chi-squared value for this test was .0176 with a p-value between .5 and .9. Thus, the hypothesis of complete penetrance and dominance of wild type wing venation over the mutant venation with no more segregation between the alleles cannot be rejected.

Analysis of body color and wing venation genes together

To test if the alleles for wing venation and body color were independently assorting or if the genes were linked, the two traits have to be looked at together in the F2 results from the Crosses A and B combined. These numbers must then be made into ratios that can be compared to the expected ration for independent assortment. The ratio for independent assortment of the alleles for wing venation and body color must be derived from the obtained ratios, obtained earlier, of the segregation of the individual traits. The observed and expected ratios are tabulated below. Wt indicates wild-type, and m indicates mutant.

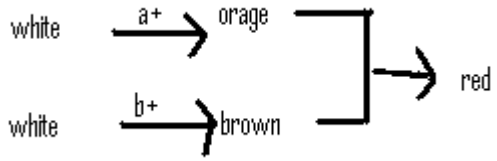
Table 5: Body Color and Wing Ven. Data from F2 of A& B	observed actual #	observed ratio	expected actual #	expected ratio
wt body color wt wing venation	278	278 : 198	282.0	11.4 : 7.8
m body color wt wing venation	77	77 : 398	74.2	3 : 16.2
wt body color m wing venation	98	98 : 377	94.0	3.8 : 15.4
m body color m wing venation	22	22 : 453	24.7	1 : 18.2

The chi-squared value for testing the hypothesis of independent assortment was .628 with a p-value between .5 and .9. Thus, the hypothesis of independent assortment cannot be rejected. The genes for body color and wing venation were not linked.

Eye Color

Table 6: Eye Color Results from F1 of Cross A & B	Wild Type	Mutant
From Cross A	59 females & 41 males	0 males & females
Form Cross B	90 females & 41 males	0 males & females

From the same reasoning used for the determination of body color and wing venation, the results from the F1 of cross A determined eye color as a autosomal, recessive trait, while Cross B F1 determined eye color as just a recessive trait. Looking at the F2 results for the eye color trait, however, made this trait a bite more complicated than the others. When observing the F2 of Crosses A and B, four eye colors were identified. There was red, white, and also brown and orange. Because there were four different eye colors, there could be two genes involved in the expression of eye color. Because there are two genes involved, the F2 results will determine the interaction between these two genes; the F2 results will conclude if the genes were linked or not. A hypothesis for the possible interaction is described by the following diagram:



The presence of the a+ allele with the b+ allele complements to make the wild-type red eye color. However, brown was possible with the genotype of aab+_, orange was possible with the phenotype of a+_bb, and white was aabb. The data eye color from F2 of Crosses A and B is tabulated below.

Table 7: Eye Color Data from F2 of Crosses A &B	From Cross A & B F2
Red	318
White	63
Orange	44
Brown	50

From just looking at this data it is reasonable to say that the two genes for eye color are linked because the number of number of white eye progeny (aabb) the double mutant offspring for the cross between two a+ab+b parents, is higher than the number of single mutant offspring (orange of brown eyes.) If the two genes were independently assorting, the double mutant of white eyes would be the least of the F2 progeny. A chi-square test with this data and the data from the F2 of Marker Cross I for the hypothesis of independent assortment gave a chi-squared value of 115.51 with a p-value <<< 0.05. Thus, the hypothesis was rejected. And the data above was used to find the map distance between the two eye color genes, a and b. The calculated map distance was 39.6 map units.

Male Parent Backcross: Cross II and III

The marker II and III stocks were balanced marker stocks to keep the heterozygous stocks pure line. The pure-line was established with a balanced lethal system, in which being homozygous for the mutant alleles was lethal. For example, flies in marker stock II had a one chromosome 2 carrying Bl L Cy+ and another chromosome 2

with Bl+ L+ Cy. These alleles are for the dominant, autosomal traits of short thin bristles, lobed eyes, and curly wings, respectively. When a male and female from marker stock II mate, the offspring with both chromosomes carrying Bl L Cy+ are killed and so are those both carrying the Bl+ L+ Cy chromosomes. Recombination among the genes in the chromosomes were controlled by inversion, an introduced cross-over suppressor for females, and males do not undergo recombination. This balanced marker system was also used in marker stock III with their chromosomes: one carrying Gl Sb LVM+ and the other carrying Gl+ Sb+ LVM.

Marker Cross II and III used male backcrosses instead of F1xF1 crosses. In a male back cross, the male progeny from the F1 are selected and mated to certain homozygote genotype female (in this case an unknown virgin.) In Drosophilia, male flies do not under go recombination. By selecting the males form the F1 generation and using the homozygous unknown females, recombination should not occur. These crosses also used male back crosses so that all the offspring would be viable. This was true for the kind of backcrosses employed in this experiment because an unknown female was always used. The unknown female carried the wild-type alleles for the marker mutations and would pass them on to the offspring. So the male back cross offspring would never be homozygous for the lethal marker mutations. The back crosses conducted would indicate which genes were on the marker chromosomes 1, 2, and 3. If a certain mutation does not show up with a marker mutation in the male backcross progeny, then that mutation is linked with the marker mutation, and thus on the same chromosome. This is a result because no recombination should occur in a male backcross in *Dosophilia.* However, if the mutation does show up with the marker mutation in the back cross progeny then this would be a sign that the two mutations are not on the same chromosome. In this experiment Cross II did not produce any male back cross progeny, but the results for Cross III male progeny are below.

Table 8: Male Backcross Progeny results for Marker Cross III

phenotype	# observed
wt body, wt eye, wt wing, Gl Sb	24
wt body, wt eye, wt wing, Gl+ Sb+	9
wt body, wt eye, m wing, Gl+ Sb+	43
m body, m eye, wt wing, Gl Sb	16
m body, m eye, wt wing, Gl+ Sb+	2
m body, m eye, m wing, Gl+ Sb+	27

The mutant body color of dark brown and mutant eye color of white showed up with the glued eye and stubble bristle marker mutations, and this means that neither the body color gene or eye color genes were on the third chromosome. However, the mutant wing venation of four short longitudinal veins did not show up with the glued eye and stubble bristle mutations, so the wing venation gene was on the third chromosome.

Cross A, B, and I Combined

The results from the F1 of Cross A determined that the eye color genes were autosomal. From Marker crosses II and III, the placement of gene for wing venation was found to be on chromosome 3, while body color was not. Since body color was not sex-linked, the gene for body color must be on chromosome 2 (since chromosome 4 does not carry many genes.) The same was true for the two eye color genes. Another clue that the eye color genes and body color gene are on the same allele was the fact that only 13 white eyed light body and 69 white eye dark body F2 progeny were observed for the crosses A, B, and I combined. This was very different from the expected results associated with independent assortment of about ¾ of the white eye progeny to be light body and only ¼ to be dark bodied. In a tree-point cross between d-a-b (d is allele for dark body, a and b are alleles for eye color), the distance between genes was found by doubling the number of recombinants because the F1 x F1 crosses hides half of the recombinants. The distance between d-b was found to be 10m.u, a-b was 39.5 m.u, and d-a was 49.5 after taking the twice the number of double crossovers into account. Thus, d and a were on the ends and b was in the middle.

Summary

With the information from the five performed crosses and the information of the location of one mutation of the chromosomes given by the instructor, the genes for mutations for dark body color, mutant wing venation, and eye color may be mapped on the chromosomes of the *Drosopholia.*