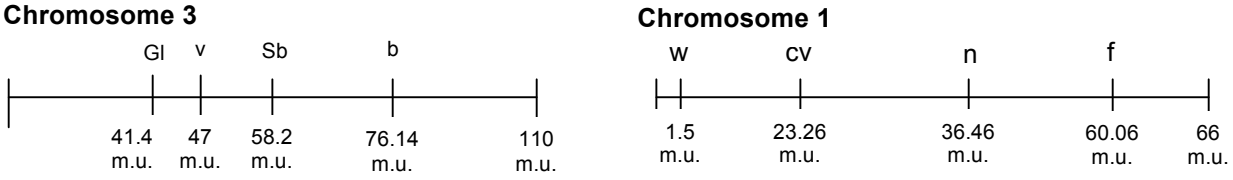
Genetic analysis of a mutant strain of *Drosophila melanogaster*

Abstract

Drosophila melanogaster was the organism used in this genetic investigation. The genetic mutations in unknown strain number 4254 were characterized and their genetic loci were mapped. Wild type flies have red eyes, gray bodies, and a normal wing venation pattern. The unknown strain had white eyes, a black body, and a mutant wing venation characterized by a gap in the second horizontal wing vein. Reciprocal crosses and marker crosses were conducted in order to determine the modes of inheritance for each gene and resolve map distances. The gene for body color, b, and wing venation, v, were both found to be recessive single gene traits that are linked on chromosome 3, refer to Figure 1 for the location of these genes relative to marker III mutations small Glued (Gl) eyes and short Stubbled (Sb) bristles. Eye color was found to be a two gene, w and n, trait characterized by a recessive epistatic relationship between the two genes. ww is is epistatic over n+_ or nn. A fly with a genotype of w+_n+_ has a wild type red eye phenotype, a w+_nn fly has orange eyes, and a wwn+_ fly and also wwnn fly has white eyes. The eye color genes were linked to the X chromosome and their loci were mapped along with marker I mutations for wings lacking crossveins (cv) and bent/forked bristles (f), according to Figure 1.

Figure 1: Genetic loci of mutations in unknown strain 4254



Introduction

This experiment used the model organism *Drosophila melanogaster,* more commonly known as the fruit fly, which is one of the most valuable organisms in genetic and developmental research today. *Drosophila* has been used for many years in research and its entire genome has been sequenced. The life cycle for the fruit fly is short, ten to fourteen days, with a developmental time of nine days and because of this, experimental results can be generated relatively quickly. *Drosophila* flies are inexpensive to

maintain in the laboratory setting and are easy to manipulate. All of these characteristics contribute to the wide use of this organism in genetic research today.

This experiment aimed to characterize an unknown mutant strain of flies through genetic analysis by answering several questions that this experiment was designed to answer. First, how many of the phenotypic differences in the unknown strain are inherited? And of the inherited traits, how many genes are involved in defining the trait? Moreover, how are the genes inherited, are they dominant, recessive, autosomal, sex-linked or do they have some other inheritance pattern? Finally, where are the genes for the identified traits located on the chromosomes? In order to answer these questions, scoring information from reciprocal crosses between the unknown flies and known wild type flies were coupled with scoring data from crosses with three marker strains that have mutations in genes of known chromosomal locations. Three marker strains were used corresponding to the three main pairs of chromosomes in *D. melanogaster* (*Drosophila* has a forth pair of chromosomes but they are very small and do not code for many genes). In order to completely characterize and map any unknown mutations F_2 progeny were generated from the reciprocal crosses and the sex chromosome marker cross and male backcross progeny were generated from the two other marker cross progeny.

Another supplemental objective of this experiment was for the experimenters to learn how to work with *Drosophila melanogaster*. Skills in the handling and maintenance of this frequently used organism are valuable tools to add to a future molecular biologists repertoire.

Materials and Methods

Eight bottles of unknown mutant flies were obtained and their phenotypes were recorded. Two bottles of wild type flies were also obtained and all unknown and known bottles were cleared of adults. The wild type strain used was Oregon-R (Ore-R) characterized by a gray body, red eyes, and normal wing venation. Virgin females (virgin ♀) and males (♂) were collected from the unknown, wild type, and marker strains. The marker number represents the chromosome number that the known mutations in that strain are on. Refer to the MCDB 306 Genetics Laboratory Manual for further details about these strains.

Reciprocal crosses A and B, and marker crosses I, II, and III were set up in duplicate according to Table 2 Phase I Crosses. Approximately ten females and twenty males were added to each of the crosses described. One hundred flies were scored from each of the F_1 crosses before the bottles were cleared and more virgin females and males were collected in order to set up the second round of crosses. In Phase II cross A, B, and marker cross I were used to create F_2 progeny by crossing the F_1 progeny. Phase II marker crosses II and III were male backcrosses between unknown females and mutant marker males. See Phase II Crosses in Table 2. The scoring results from phase I and II crosses were analyzed and the genetic inheritance pattern, linkage data, and map distances were determined.

Table 2: Phase I and phase II crosses

M1 – marker 1 ; M2 – marker 2 ;
U4254 – unknown strain #

Phase I Crosses		Phase II Crosses	
Cross A	U4254 virgin ♀ x WT ♂	**F_1xF_1 of Cross A**	F_1 ♀ x F_1 ♂
Cross B	U4254 ♂x WT virgin ♀	**F_1xF_1 of Cross B**	F_1 ♀ x F_1 ♂
Cross I	U4254 virgin ♀ x M1 ♂	**F_1xF_1 of Cross I**	F_1 ♀ x F_1 ♂
Cross II	U4254 virgin ♀ x M2 ♂	**Cross II** ♂ **Backcross**	Bottle 1: U4254 ♀ x F_1 Bl L ♂ Bottle 2: U4254 ♀ x F_1 Cy ♂
Cross III	U4254 virgin ♀ x M3 ♂	**Cross III** ♂ **Backcross**	Bottle 1: U4254 ♀ x F_1 Gl Sb ♂ Bottle 2:U4254 ♀ x F_1 WT ♂

Results and Discussion

A and B Cross

The F_1 progeny of cross A and B were tabulated, see Table 3. In both A and B crosses, all of the F_1 progeny (♂ and ♀) had wild type body color and wild type wing venation which suggests that these mutant phenotypes are recessive. Furthermore, males and females were distributed approximately equally for the body color and wing venation trait in both crosses which suggests that the genes for body color and wing venation traits are autosomal.

Table 3 shows that all cross A females have wild type eye color and all males have mutant eye color. This pattern of inheritance is indicative of a sex-linked gene since males receive only the mutant allele they have the mutant phenotype but females have one wild type allele and one mutant allele so they have a wild type phenotype given that mutant eye color is a recessive trait. Cross A F_1 females all inherit one copy of the wild type eye color gene which is enough to confer the wild type phenotype so eye color

is a recessive trait. Phase II of the A and B crosses involved a cross between the F_1 progeny, see Table 4 for body color and wing venation results.

Table 3: F_1 progeny of A and B Crosses

Trait	Wild type F	Wild type M	Mutant F	Mutant M	Pattern of Inheritance
CROSS A	unknown virgin ♀ x wild type ♂				
Body color	100	86	0	0	Autosomal recessive
Eye color	100	0	0	86	Sex-linked recessive
Wing venation	100	86	0	0	Autosomal recessive
CROSS B	unknown ♂x wild type virgin ♀				
Body color	86	96	0	0	
Eye color	86	96	0	0	
Wing venation	86	96	0	0	

Table 4: Phase II cross – F_2 progeny of A and B crosses

Trait	F_2 of A		F_2 of B	
	Wild type	Mutant	Wild type	Mutant
Body color	314	78	238	77
Wing venation	322	70	255	60

If body color and wing venation are two allele, recessive, single gene traits with complete dominance then they would be expected to segregate 3:1 wild type:mutant. The body color trait segregated 3.56:1 wild type gray body:mutant black body. A χ^2 test was conducted which did not reject the hypothesis that body color is a recessive single gene trait. The wing venation trait segregated at a ratio of 4.4:1 wild type:mutant wing venation and a χ^2 test rejected the hypothesis that wing venation is a recessive single gene trait (Skeletal Report 5). This data conclusion is not consistent with the F_1 progeny data that showed that wing venation was a recessive trait. However, since only a small number of F_2 flies were scored there is a chance of random error which may explain the unexpected result of the $\chi 2$ test. Scoring errors or expressivity/penetrance problems of the wing venation mutation could also have been factors in this unexpected result.

Since the genes for body color and wing venation have been characterized as autosomal, another χ^2 test was conducted to see whether the two genes were independently assorting. If two genes independently assort then the expected dihybrid ratio of F_2 progeny is 9:3:3:1, wild type:single mutant:other single mutant:double mutant. A modified dihybrid ratio had to be created based on the observed F_2 ratios in order to more accurately judge independent assortment based on the data generated from this experiment. See Table 5 for the modified expected ratios and expected number of flies for the

dihybrid cross compared to the actual observed numbers for each of the four phenotypes. If the genes were segregating independently then a ratio of 15.7 : 4.4 : 3.56 : 1 would be expected; however, there are approximately three times more double mutants than would be expected if the genes were segregating independently which logically suggests that the genes are linked. And indeed, a χ^2 test rejected the hypothesis that the genes for body color and wing venation are independently assorting so the alternative is that they are linked. The map distance between body color and wing venation was calculated to be 29.14 m.u. (Skeletal Report 6)

Table 5: Determining independent assortment or linkage between body color and wing venation (modified dihybrid ratio)

	Observed	**Expected**	
	Actual #	Actual #	ratio
Wild type body color Wild type wing venation	513	451.2	$\frac{3.56}{4.56} \times \frac{4.4}{5.4} = \frac{15.7}{24.6}$
Mutant body color Wild type wing venation	64	126.5	$\frac{1}{4.56} \times \frac{4.4}{5.4} = \frac{4.4}{24.6}$
Wild type body color Mutant wing venation	39	96.6	$\frac{3.56}{4.56} \times \frac{1}{5.4} = \frac{3.56}{24.6}$
Mutant body color Mutant wing venation	91	28.7	$\frac{1}{4.56} \times \frac{1}{5.4} = \frac{1}{24.6}$

The third unknown gene analyzed in this experiment was for eye color. The F_2 eye color segregation results for crosses A and B were recorded in Table 6.

Table 6: Phase II: F_2 progeny of A and B Crosses

Eye Color	**Cross A** ♀	♂	**Cross B** ♀	♂
Red	87	63	156	70
White	110	76	0	77
Orange	29	27	0	12

As Table 6 shows, a new orange eye color was observed in the F_2 generation and there are only wild type females in cross B F_2 progeny. Based on this data, the best hypothesis for the interaction that creates eye color is a two gene epistatic interaction where homozygous white eye mutations are epistatic over homozygous or heterozygous orange mutations. The proposed interaction is:

white $\xrightarrow{w^+}$ orange $\xrightarrow{n^+}$ red

Genotype	Phenotype
$w^+\_n^+\_$	red
w^+_nn	orange
wwn^+_	white
wwnn	white

The expected results based on this hypothesis are that the two

genes for eye color are linked on the X chromosome because the F_1 data showed that eye color is sex-linked and the only explanation for having only wild type F_2 females for cross B is if this is the case. See Page A of the attached Skeletal Report for the details of the crosses and how alleles segregate to give this pattern of inheritance. The map distance between the two eye color genes, w and n, was calculated from cross A F_2 data to be 28.57 mu.

Male Parent Backcross: Crosses II and III

Marker crosses II and III were conducted in order to map autosomal genes, which in the case of this unknown included the genes for body color and wing venation. Each of the mutations in the M2 and M3 strains was dominant and homozygous lethal. The marker stocks were part of a balanced lethal system which consisted of a true breeding heterozygote combination of known marker mutations. Crossovers are suppressed in this type of genetic setup and the original Bl L / Cy and Gl Sb / LVM genotypes for marker II and marker III, respectively, were conserved. The initial M2 and M3 marker crosses produced F_1 progeny that were expected from the conclusions based on A and B reciprocal cross progeny. All progeny were either Bristle Lobed or Curly in M2 or Glued Stubbled or wild type in M3 because those mutations were dominant in the marker stocks. All F_1 progeny had wild type body color and wing venation because these two genes were determined to be autosomal and recessive.

In phase II crosses, F_1 males were backcrossed to unknown mutant females and if the recessive mutation showed up with the male backcross progeny (Br L or Cy for marker II and Gl Sb for marker III) and four phenotypes were present in the F_1 male backcross progeny, then the gene for the mutation is *not* linked to that marker's chromosome. If a recessive mutation *is* linked to the markers chromosome, then the mutant phenotype would not show up with male backcross progeny and only two phenotypes would be present in F_1 male backcross progeny. The results of phase II male backcrosses are summarized in Table 7.

Table 7: Cross II and III F_1 male backcross progeny

Cross II (M2)

Body color	Eye color	Wing venation	Bl L	Bl+ L+	Cy	Cy+
Light	White	Wild type	25 / 0	31 / 9	0 / 3	56 / 6
Dark	White	Mutant	0 / 0	22 / 10	0 / 6	22 / 4

Cross III (M3)

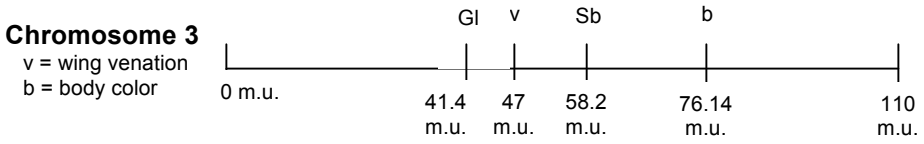
Body color	Eye color	Wing venation	Gl Sb	Gl+ Sb+
Light	White	Wild type	52 / 0	0 / 39
Dark	White	Wild type	1 / 1*	0 / 0
Dark	White	Mutant	0 / 0	51 / 28

Green colored numbers were scored from F_1 curly (M2) or wild type (M3) ♂s x unknown ♀s
Black colored numbers were scored from F_1 Bristle Lobed (M2) or Glued Stubbled (M3) ♂s x unknown ♀s

Table 7 data from cross II shows that mutant body color and mutant wing venation segregated with the male backcross curly progeny. This means that the body color and wing venation genes are not on the second chromosome. The mutations did not segregate with the Bristle Lobed marker mutations in this experiment, but it would be expected that a larger population of flies would show mutant body color and mutant wing venation showing up together in the F_1 male backcross progeny. Cross III shows that mutant body color and mutant wing venation do not segregate with F_1 male backcross progeny suggesting that these genes are located on the third chromosome. Two flies were scored to be Glued Stubbled, dark body, white eye, wild type wing venation, marked by an asterisk in Cross III. This unexpected result is likely due to a scoring error.

The mutant wing venation gene was given to be at locus 47 m.u. and was said to have a smaller map distance than the gene for body color. The map distance between the genes for wing venation and body color was calculated to be 29.14 m.u. This information is enough to assign a location on chromosome three to each of these three genes, see Figure 2.

Figure 2: Gene loci on chromosome 3



Cross I

Marker cross I was conducted in order to map sex-linked genes, which in the case of this unknown included the genes for eye color. The mutant marker genes present on the X chromosome are recessive and produce a crossveinless (cv) forked (f) phenotype. The initial M1, M2, and M3 marker crosses produced the expected mutant white eye color male and wild type red eyed female F_1 progeny. All M1 F_1 progeny were wild type for marker 1 mutations (cv f) since all males received a normal copy of these genes from the unknown female parent.

Based on the analysis of F_2 data of crosses A and B the two eye color genes were determined to be linked on the X chromosome. The possible genotypes and phenotypes for Cross I F_2 progeny are depicted in Tables 8 and 9 as well as the number of male flies scored in each of the phenotypic categories. Only males were included in this table and in determining map distances because it is only males that carry information about crossover events that are needed to map genes. The female progeny had approximately equal numbers (~64 flies) of red eye and white eye progeny and half that number (34 flies) of orange progeny. Table 8 and 9 are set up as three point crosses with only one of the marker I mutations included in relationship to each eye color gene. Table 10 is a two point cross for the marker mutations (cv and f) that shows the phenotype and number of male progeny in each category.

Table 8: Three point cross for cv-w-n of cross I F_2 male progeny

cv-w-n		
Phenotypes	**Genotypes**	**#'s**
White	cv+ w n	108
Red crossveinless	cv w+ n+	65
Wild type	cv+ w+ n+	0
White crossveinless	cv w n	8
White	cv+ w n+	108
Orange crossveinless	cv w+ n	14
Orange	cv+ w+ n	23
White crossveinless	cv w n+	8

Table 9: Three point cross for f-w-n of cross I F_2 male progeny

f-w-n		
Phenotypes	**Genotypes**	**#'s**
White	f+ w n	50
Red forked	f w+ n+	47
Wild type	f+ w+ n+	18
White forked	f w n	66
White	f+ w n+	50
Orange forked	f w+ n	7
Orange	f+ w+ n	30
White forked	f w n+	66

Table 10: Two point cross for cv-f of cross I F_2 male progeny

cv-f	
Phenotypes	**#'s**
Wild type	69
Forked	62
Crossveinless	29
Crossveinless forked	58

Table 11: Map distances between genes on the X chromosome

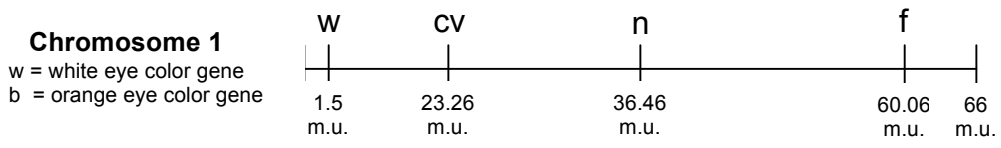
Genes	Map distance (m.u.)
cv-w	21.76
cv-n	13.2
f-w	58.56
f-n	23.6
w-n	34.96

These two and three point crosses were used to calculate the map distances between cv, f, w, and n on the X chromosome, see Table 11. For calculation details refer to page 11 of the Skeletal Report attached. After finding that the map distance between cv and f was slightly off from the actual known distance, the map distances between all other genes were corrected using the correction factor (43/41.7). The numerator in this factor is the actual distance between cv and f and 41.7 was the experimental map distance. Table 11 shows corrected map distances.

The map distances in Table 11 along with the relative number of flies in each category give enough information to determine gene order. The cv-w and cv-n distances add together to give the distance between w and n which tells that cv is between w and n on the chromosome. In the f-w-n three point cross the least number of flies were found in the orange forked phenotype which was assumed to be the double cross over event. In a double cross over the only gene that will be different from the parental genotype will be the gene in the middle of the two crossovers. The genotype of the F_1 female parent was f w+ n+ and the genotype of the double cross over was f w+ n. The gene that is different from the parent is n which means that n is in between f and w.

The locus of each gene was finally determined using the gene order and map distance data explained above coupled with the given locus of gene w at 1.5 m.u.. See Figure 3 for a diagram of the X chromosome and the locus of the eye color and marker I genes.

Figure 3: Gene loci on the X chromosome



Summary

- Cross A and B F_1 results showed that all three genes were recessive and the genes for body color and wing venation are on autosomes while the gene(s) for eye color are sex-linked.

- X^2 tests of cross A and B F_2 results did not reject the hypothesis that body color and wing venation* traits are specified by a single recessive autosomal gene. Another X^2 test showed that b and v are linked with a map distance of 29.14 m.u. *Unexpected results for wing venation were due to scoring errors or expressivity/penetrance problems of the wing venation mutation

- Male backcross progeny of the marker stock crosses showed that both body color and wing venation genes are on chromosome 3.

- Eye color was found to involve a two gene recessive epistatic interaction:
  white $\xrightarrow{w^+}$ orange $\xrightarrow{n^+}$ red
  The map distance between w and n was 28.57 m.u.

Genotype	Phenotype
$w^+\_n^+\_$	red
w^+_nn	orange
$wwn^+\_$	white
wwnn	white

- Two and three point crosses using the male backcross marker progeny provided the data needed to determine gene order and map distances of w and n on the X chromosome and their relationship to marker mutations, refer to Figures 2 and 3 for assignment of gene loci.

These results are summarized in Table 12.

Table 12: Experimental conclusions

Gene Name	Symbol	Dominant or Recessive	Chromosome Number	Genetic Locus
Black body color	b	Recessive	3	76.14 m.u.
Wing venation	v	Recessive	3	47 m.u.
Orange eye color	n	Recessive	1	36.46 m.u.
White eye color	w	Recessive	1	1.5 m.u.

Appendix

Butts, Darcy. Skeletal Report. MCDB 306: Genetics Laboratory. 2005.

References

Jeyabalan, S. Fall 2005. *MCDB 306 Genetics Laboratory Manual*. University of Michigan. Pp.3-28.