Abstract

Closer examination of unknown Drosophila Melanogaster strain U3833 results in the observation of 3 mutant phenotypes. These observed mutations were white eyes, dark body, and mutant wing venation. Mutant wing venation resulted in certain longitudinal veins not fully traversing the wings. Variance in the expression of this mutation was also noted as the degree to which the individual vein was shortened varied. The dark body mutations was characterized with the body of the fly consisting of a black hue, while the eye color mutation was initially exemplified by white eyes, however, in the F2 generation both orange and brown became additionally evident. The location of these mutations was determined experimentally through the use of marker crosses and F1 backcrosses between F1 virgin females and unknown males. From these it was determined that the gene for body color was located on the third chromosome, while the gene for wing venation was on the second chromosome along with those controlling eye color. The mode of inheritance for eye color was determined from the F2 generation of cross A and B and I, which resulted in two additional phenotypes being observed. This corresponded to two genes controlling the expression of eye color. Furthermore, these genes were linked, as there is not a 9:3:3:1 ratio demonstrative of independently assorting genes in regards to eye color within these progeny. Between these two recessive genes homozygous b resulted in orange eyes and homozygous a resulted in brown eyes, while a fly homozygous for both genes expressed white eyes. Additionally, homozygous w resulted in mutant wing venation and homozygous d resulted in dark body both of which are recessive to the wild type allele. In regards to segregation, body color and wing venation consist of one gene and assort independently with a ratio of 9.7: 2.6: 2.1: 1. The recessive nature of each trait can be observed from the fact that all F1 progeny do not exhibit mutant phenotypes. Along with this, all mutations are autosomal as seen by the fact that in F1 progeny of cross A, no male fly exhibits mutant phenotypes.

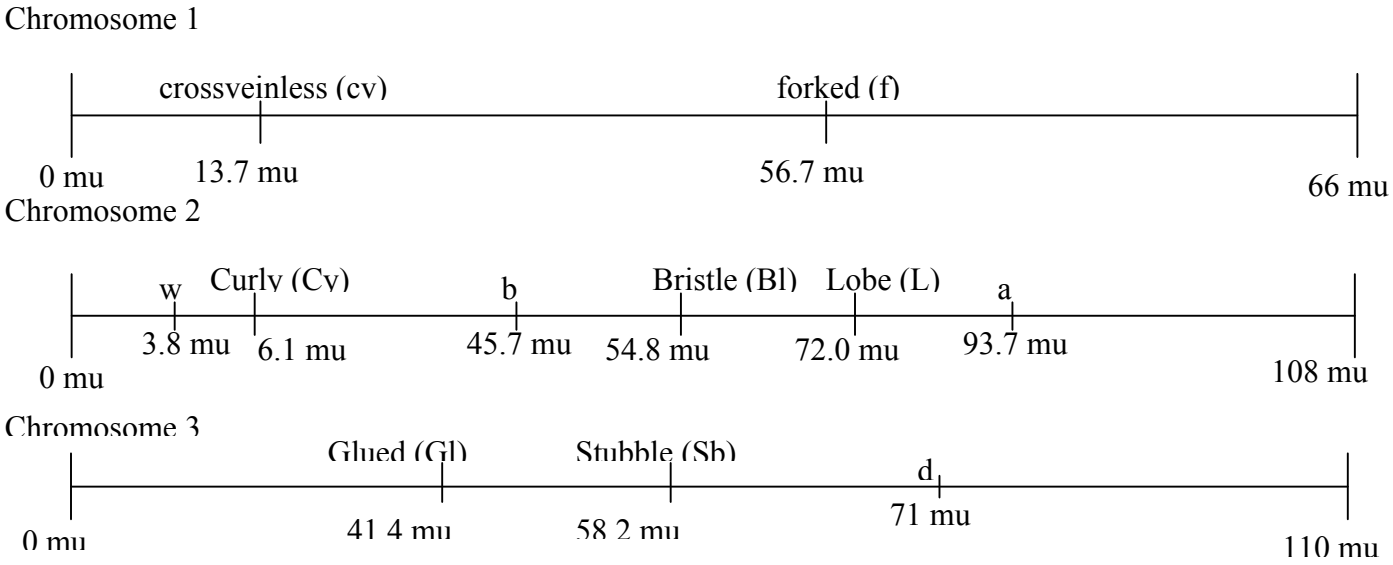

Figure 1: Proposed chromosome maps

Introduction

There are many reasons by which Drosophila Melanogaster is a model experimental model for genetic study. The first of which deals directly with the life cycle of this small insect. Only lasting 9 days, new generations are able to be generated quickly and coincide well with the timing of MCDB 306 class. Furthermore, as female flies can lay as many as 500 eggs in 10 days, the necessary population size for genetic analysis can be created and maintained. Another very important facet of Drosophila Melanogaster is the fact that upon emergence from pupa the female does not reach sexual maturity until approximately eight hours. This allows for the collection of virgin females for further crosses. There are also significant genetic advantages in using D. Melanogaster as well. Foremost is the fact that male flies do not undergo recombination and thus drastically reduce the complexity of scoring future generations. Additionally, the fact that this type of fly only has four chromosomes is also helpful in decreasing the number of chromosomal marker crosses.

The intent of this experiment was to identify the correct chromosome, locus, and mode of inheritance for three unique mutations through the use of designed crosses and statistical analysis. Given the dramatic increase in genetic study, experience in locating unknown mutations

within a genome can be very helpful in real world laboratories. This understanding was obtained via careful adherence to established laboratory protocol. The isolation of virgin females and the ability to produce viable crosses with the selected flies was a vital piece in the overall goal of establishing a chromosomal map of unknown mutations. This could be determined only after performing a series of specific genetic crosses between precise populations of D. Melanogaster. Cross A and B were designed to determine if the mutations were dominant or recessive, while cross A showed if the mutation was autosomal or sex linked. For the crosses designated as marker, after executing the initial parental and F1 crosses, F1 backcrosses were performed with flies originating from specific marker populations exhibiting mutations with known loci. As each marker cross utilized populations of flies with different known mutations as well as cross over suppressing inversions, using the principles of independent assortment, though the observation of the progeny from these crosses, allows for the determination of the correct chromosome. A mutation's location on a chromosome can be obtained by statistical analysis of three point crossovers of three mutant traits.

Materials/Methods

With exception to a few altered details the overlaying procedure for this lab is described in depth in the MCDB 306 Lab Manual. The major difference is that due poor viability, the marker 2 cross was not performed. As a result only four crosses were performed. Virgin females were used when necessary in order to have a controlled cross as the female cannot have already been fertilized by a male prior to the initiation of a new cross. The crosses performed are outlined in the chart below. Additionally in preparation of the marker one and three backcross an initial cross of unknown virgin females and the respective maker males was performed.

Cross	Participants	Significance	Reasoning
A	Unknown virgin females X Wild type males	Can determine if mutation is dominant or recessive and autosomal or sex-	If sex-liked all males will receive X chromosome with mutation from

		linked	mother. Thus one would observe mutation in males. If dominant would see mutation in both males and females.
B	Wild type virgin females X unknown males	Determine if mutation is Dominant or recessive	If dominant would see unknown mutation in both males and females. Cannot determine if sex linked because all females give wild type X chromosome
Marker I Backcross	F1 males and females of Cross 1	Determine if mutations are on sex chromosomes	If on sex chromosomes will observe mutations cv and f always separate from unknown mutation
Marker III Backcross	Virgin females from F1 of cross III X unknown males	Determine if mutations are on Chromosome 3	If on Chromosome 3 will not observe mutations on chromosome 3 with marker mutations.

Table 1: Cross summary

Results and Discussion

Cross A and B

Cross A F1	Wild Type		Mutant Type	
Trait	M	F	M	F
Body Color	63	37	0	0
Eye Color	63	37	0	0
Wing Venation	63	37	0	0

Cross B F1	Wild Type		Mutant Type	
Trait	M	F	M	F
Body Color	105	49	0	0
Eye Color	105	49	0	0
Wing Venation	105	49	0	0

	F_2 of A		F_2 of B	
Trait	wild type	mutant	wild type	mutant
Body Color	175	56	260	74
Wing Venation	184	48	269	65

Table 2: Cross A and B organized data

Body Color

From the F1 results noted above several conclusions can be drawn from the analysis of the progeny from both cross A and B. As in both crosses no mutant phenotypes were observed, one could determine that the mutation for dark body is in fact recessive. Furthermore, from cross A one is able to determine that this same mutation is autosomal. This is due to the fact that, were the mutation for body color located on the X chromosome, all male flies would receive only the

mutant allele from their mothers, as the female in the cross is homozygous for the mutation. This cannot be determined from the B cross as the females in this cross will always provide an allele with a wild type gamete.

From the F2 results noted above, it was determined that the mutation for body color undergoes normal Mendelian segregation and consists of only one gene. This was determined by the analyzation of the ratios of wild type to mutant flies. The observed ratio as noted in the skeletal appendix was 3.35:1. With this ratio being close to the typical Mendelian ratio of 3:1 a chi squared test was performed in order to make sure the hypothesis of normal segregation is not to be rejected. Obtaining a X^2 value of 1.18 with a degree of freedom of one and a probability greater than 0.05, it was clear that one would fail to reject the hypothesis of normal segregation.

Wing Venation

From the F1 data noted above it is clear that like body color, wing venation is also a recessive mutation found on an autosome. This can be supported by the fact that both the F1 progeny of cross A and B resulted in exclusively wild type flies being justified in a fashion similar to body color. Furthermore, similar to body color, one can conclude that the mutation is autosomal as all male progeny of Cross A were wild type for wing venation.

From the F2 results noted above, similarly to body color, it was hypothesized that this mutation undergoes normal segregation in a Mendelian fashion. Despite this, the observed ratio of wild type to mutant flies however was four to one compared to the expected ratio of three to one for normal single gene inheritance. Upon obtaining a X^2 value of 7.67 (degree of freedom 1) and a probability less than 0.05 the hypothesis cannot be accepted. Despite this, it is possible that due to the variance in expressivity of the wing venation mutation a counting error may have occurred, thus accounting for the rejection of the hypothesis.

Wing Venation and Body Color

Phenotype	Observed	Expected

	Actual #	Ratio	Actual #	Ratio
+ body color + wing venation	360	9.7	353.16	13.43
m body color + wing venation	98	2.6	105.45	4.01
+ body color m wing venation	78	2.1	88.1	3.35
m body color m wing venation	37	1	26.3	1

Table 3: Expected and observed ratios for wing and body mutation

In consideration of the relative location both wing venation and body color, as well as whether or not these mutations assort independently or are linked, it is necessary to analyze the phenotypic ratios. As shown above, the observed phenotypic ratio was 9.7 : 2.6 : 2.1 : 1, while the expected ratio of 13.43 : 4 : 3.35 : 1 was derived from the individual observed phenotypic ratio of each separate mutation. Having an initial hypothesis of independent assortment for each gene, a X^2 test was performed. The obtained value was 6.13 and with a corresponding probability of greater than 0.05 (three degrees of freedom) one would fail to reject the hypothesis. Therefore, one can assume the two individual mutations are not linked and undergo independent assortment in a Mendelian fashion.

Eye Color

Cross A, B, and I (F2 generation)		Eye Color							
		Red		White		Orange		Brown	
Body Color	Wing venation	M	F	M	F	M	F	M	F
Light	wild type	122	259	12	24	12	25	17	35
	mutant	12	20	16	11	14	15	9	11
Dark	wild type	43	40	6	4	6	2	9	13
	mutant	8	4	3	10	7	4	3	6

Table 4: Combined Data

From the F1 data obtained from the A and B crosses it was once again possible to establish that eye color was recessive and autosomal. This is due to the fact that in both cross A and B there were no mutant flies observed, illustrating once again the same pattern seen in the previous mutations of being recessive. Furthermore, from cross A it is possible to again

determine that as there are no males exhibiting the mutant eye color the mutant phenotype is not on the X chromosome for the previously stated reasons.

From the F2 results it becomes clear that due to the presence of more than two phenotypes, there must be multi gene control over eye color. In the F2 generation eye color was not limited to red and white, but rather orange and brown became evident among progeny. With a hypothesized genotypic and phenotypic relationship seen below.

Genotype	Phenotype
$a^+\_\ b^+\_$	Red eyes
$a^+\_\ b\ b$	Orange eyes
a a $b^+\_$	Brown eyes
a a b b	White eyes

Table 5: Proposed eye color genotypes and phenotypes

Phenotype	Observed	Ratio
Red	508	5.98
White	86	1
Orange	85	1
Brown	103	1.2

Table 6: Final eye color observation

From analyzation of the obtained data a hypothesis of two linked genes was proposed. One can justify the linkage, as were the two alleles for eye color assorting independently a ratio of 9:3:3:1 would be observed in actuality however, a ratio of 5.98: 1: 1.2: 1 for red: white: brown: orange. Were independent assortment occurring the ratio of orange and brown would be expected to be significantly larger than that of white. Given that they are linked the map distance between the two alleles was determined to be 48.1 mu.

Male Parent Backcross III and II

The male backcross allows for the determination of whether or not a mutation is located on a certain chromosome. One is first required to perform a cross between a virgin mutant female and a male from an individual marker stock. The male progeny from this cross are then mated with unknown virgin females. Upon examination of the progeny from this cross, one can

conclude that if the mutation in question can be observed with the marker mutations, then due to the principles of independent assortment the unknown mutation cannot be on the same chromosome as the marker mutations. Consequently if the unknown mutation is not observed with the marker mutations then one can assume that the mutation is on the same chromosome. Due to the low viability of marker stock II it was required that data for this cross be extrapolated from results throughout the lab.

Body Color	Eye Color	Wing Venation	Gl Sb	Gl^+ Sb^+
light	red	wild type	32	0
		mutant	0	0
	white	wild type	0	0
		mutant	27	0
dark	red	wild type	0	31
		mutant	0	0
	white	wild type	0	0
		mutant	0	24

Table 7: Cross III F1 male backcross

Body Color	Eye Color	Wing Venation	Bl L	Bl^+ L^+	Cy	Cy^+
light	red	wild type	25	0	25	0
		mutant	0	0	0	0
	white	wild type	0	0	0	0
		mutant	0	25	0	25
dark	red	wild type	25	0	25	0
		mutant	0	0	0	0
	white	wild type	0	0	0	0
		mutant	0	25	0	25

Table 8: Cross II F1 male backcross

From the given data, as the mutation for body color is not observed with the glued stubble marker for chromosome three, it can be concluded that this gene is on the third chromosome. Analyzation of the extrapolated data of marker cross two shows that both wing mutation and eye color are not observed with the maker mutations of Bristle Lobed or Curly. This can be determined since previous data concluded that these mutations are not sex-linked. Also from the data obtained from marker cross III it is clear that these mutations are not on chromosome three

as they are observed with the marker mutations. Finally, chromosome four is eliminated as a possibility as it was stated that no unknown mutations were located on chromosome four.

Cross A, B and I combined

Phenotype	8 Genotypes	Number observed
red eyes, + wings	+ + +	464
red eyes, mt wings	w + +	44
white eyes, + wings	+ b a	46
white eyes, mt wings	w b a	40
orange eyes, + wings	+ b +	45
orange eyes, mt wings	w b +	40
brown eyes, + wings	+ + a	74
brown eyes, mt wings	w + +	29

Table 9: Three point cross observed frequencies

Through the use of the previous marker crosses, it is clear that the two genes along with wing venation were on chromosome two. Due to the linked nature of the eye color genes and wing venation, one can use the frequency of single and double crossovers to determine the order and map distance of the genes in question. As seen calculated in the skeletal report appendix, the map distance between wing venation and mutant gene a was 89.9 mu. The distance between wing venation and b was 41.9 mu and the distance from gene a to gene b was 48 mu. Therefore the final gene order on chromosome two would be wing venation at 3.8 mu gene b at 45.7 mu and gene a at 93.7 mu.

Summary

The final assignments of each unknown mutation is as follows

Gene name	Symbol	Dom. of Rec.	Chromosome	Genetic Locus
dark body	d	Rec	3	71 mu
short vein wing mutation	w	Rec	2	3.8 mu
orange eyes	b	Rec	2	45.7 mu
brown eyes	a	Rec	2	93.7 mu

Table 10: Final mutation summary

From cross A it was determined that each mutation was in fact autosomal. This was due to the fact that as the female in the cross was homozygous for each displayed mutant phenotype,

were any of the mutant genes on the X chromosome, all male progeny would receive only the mutant gene and consequently express the mutant phenotype. As this was not the case, it is clear that each mutation was autosomal. From both cross A and B it was clear that as all F1 progeny were wild type, each mutation was recessive. From the F2 progeny of crosses A and B it was also made clear that body color was controlled by one gene and sorted independently. Wing venation, although controlled by one gene assorting independently from body color received a chi squared value that corresponded to a probability of less than 0.05 consequently rejecting the hypothesis of normal segregation. This error is most likely attributed to a counting error resulting from the varying expression of mutant wing venation. Marker Cross I, as it was already determined that no mutations were sex linked, instead was used to determine, along with cross A and B the mode of inheritance for eye color as well as the linkage between the two genes controlling eye color. From this information it was determined that eye color was controlled by two genes were only when both were homozygous were white eyes observed, when either gene a or b was homozygous the eyes were brown or orange respectively. Marker backcross III provides evidence regarding the chromosomal location of the dark body mutation, as it was not observed with the marker mutations in any progeny. Through extrapolation it was determined that both wing mutation and the two genes for eye color were located on chromosome two.

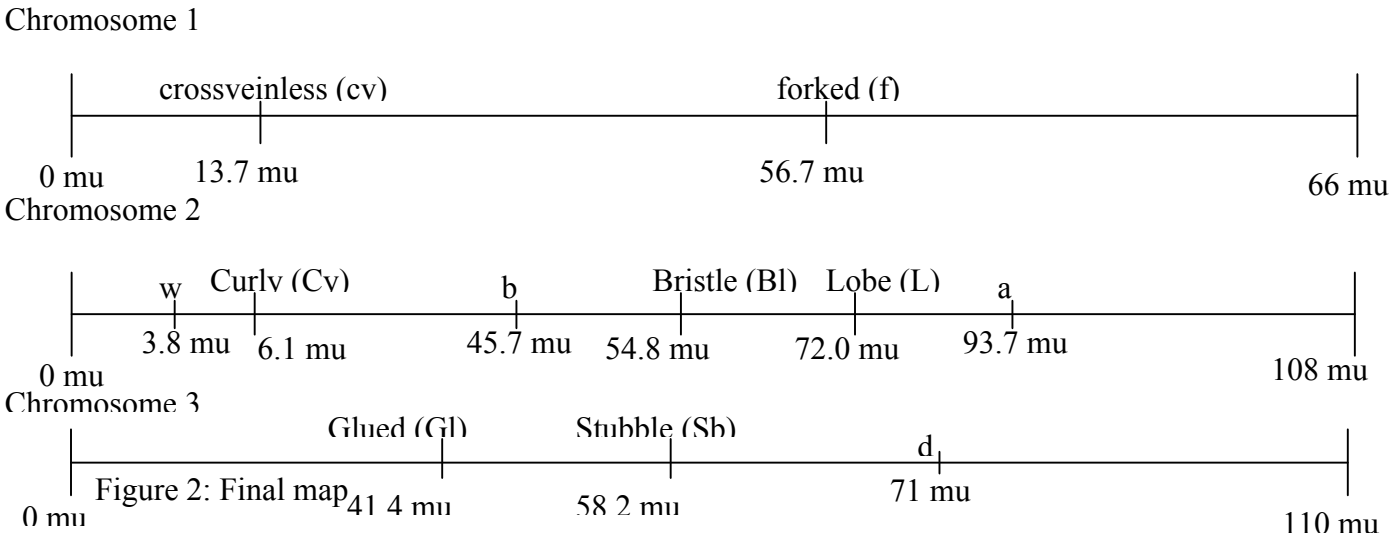

Figure 2: Final map