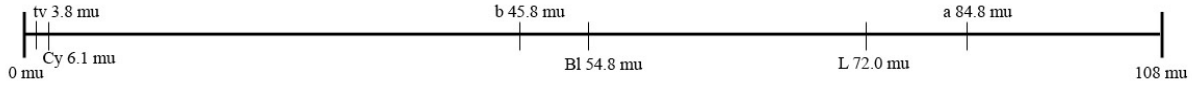
GENETIC ANALYSIS OF *DROSOPHILA MELANOGASTER* MUTANTS: TO DETERMINE INHERITANCE AND LINKAGE PATTERNS

ABSTRACT:

Ore-R wild-type (+type) *Drosophila melanogaster*, the common fruit fly, phenotypically displays red-eyes, full wing venation (five longitudinal veins and two cross veins), and a tan body color. The true breeding culture U-5343, however, varies from this wild type with white eyes, terminating pre-margin longitudinal veins LII and LIV, and a black body color. Experimentation was undertaken to investigate these mutations. Through crossing with the Ore-R type, and three marker cultures of *Drosophila* (Marker I, Marker II and Marker III cultures) the genetic differences between the +type and U-5343 were determined to be the following: black-body color is controlled by one autosomal recessive gene (expressed symbolically as bb), located on autosome three, with the exact locus undeterminable; mutant wing venation is also a single autosomal recessive gene (tv) on autosome two, located at 3.8 mu; the white eye color is controlled by two autosomal recessive genes (a and b), both located on autosome two and interacting epistatically to generate three mutant eye colors: white (aabb), orange (a+_bb) and brown (aab+_). Gene a is located at 84.8 mu and gene b is located at 45.8 mu. This data was gathered via five initial crosses, followed by three F_1 crosses and two male backcrosses. The specifics of these crosses are outlined below, but they will be distinguished as the follow: Cross A, Cross B, Cross I, Cross II, Cross III, F_1xF_1 of Cross A, F_1xF_1 of Cross B, F_1xF_1 of Cross I, Male Backcross II, and Male Backcross III. The genetic map of autosome two, including marker genes (Bl: bristle, L: Lobed, and Cy: Curly) was determined and is as follows:

INTRODUCTION:

Image 1: Autsome Two Map



Drosophila melanogaster, or the common fruit-fly, offers the geneticist a near perfect organism to explore the models of inheritance via simple, straight forward, cross breeding experiments. Short living – with a fast generation time – easy to handle and maintain in cultures and female only cross-over events, make *D. melanogaster* most desirable in the lab. As result much is known about the fruit flies genome; however, on occasion new mutations are discovered and experimentation is undertaken to determine the exact genes involved in these new mutants.

A true breeding culture of *Drosophila*, dubbed U-5343, is an example of such a scenario. The flies exhibit three genetically controlled novel phenotypic traits: black bodies, terminating pre-margin longitudinal veins LII and LIV, and white eyes. Ascertaining the nature of these mutations is an interesting problem and useful to the study of genetics in general. To answer ques-

tions, such as: what are modes of inheritance (dominant/recessive/sex-linked/autosomal); how many genes are involved; and, where are the genes located – cross breeding experimentation was undertaken. Specifically, five initial crosses and five secondary crosses were preformed in hopes of answering these questions.

Two of these initial crosses were reciprocal crosses. Cross A (unknown virgin female x +type male) and Cross B (+type virgin female x unknown male) were done to determine if the genes are recessive or dominate and sex-linked or autosomal. The secondary crosses, F_1xF_1 of Cross A and F_1xF_1 of Cross B, were done to determine: the number of genes controlling the trait; these genes interaction, if multiple present; the segregation ratios for each gene; independent assortment between genes, by a χ^2 test; and, the map distances between linked genes. Answering these questions was accomplished by scoring flies of both F_1 and F_2 generations of the two crosses.

Cross I (unknown virgin females x M-I males) and F1xF1 of Cross I were done to determine the relationship between the genes and the X-chromosome. The specifics of the marker type can be found in the MATERIALS AND METHODS section, however, these crosses were potentially helpful to determine if the genes are on the X-chromosome and, if yes, where they are located. Again, scoring F_1 and F_2 flies could be used to determine the loci of the genes on X-chromosome – it was important here to record gender in this scoring. However, if no genes are found on the X-chromosome (based on the results of Crosses A and B), then simply scoring F_2 flies would be helpful to later determine the map distance between linked genes not on the X-chromosome. This is possible because if the genes are not sex-linked, then ignoring the secondary marker characteristics of the F_2 would make them functionally the same as the F_2's of cross A and B – since then they become equivalent to the wild-type flies.

Finally, the Crosses II and III were done to determine where the genes were located in regards to the second and third autosome, with autosomal specific traits. Specifics of these marker types and their phenotypic expression can be found in the MATERIALS AND METHODS. In brief, however, these initial crosses, when scored would help show only if the mutant genes were dominant or recessive. A secondary backcross of the male progeny of these crosses with unknown virgin females was necessary to determine which chromosome the genes were on. Notably absent for all the crosses described here is an emphasis on autosome IV; since this autosome is so small it is not likely that these mutations' loci are there.

MATERIALS AND METHODS:

The crosses and scoring were done over a series of seven weeks and all flies for all crosses were grown at 24° C. All flies were anesthetized using CO_2 and inspected under a dissecting or stereoscopic microscope while on CO_2 pads. The average time sedated was minimized to prevent sterility. Flies were handled with small brushes and prodding needles; for specifics on fly handling please see Jeyabalan (2005).

In total five different cultures of flies were used: Oregon-R (Ore-R) type wilds, unknown culture U-5343, and marker stocks labeled M-I, M-II, M-III. Two Ore-R stocks and eight mutant stocks were initially provided. Two new cultures of U-5343 were made during the experiment to maintain the stock for later backcrosses. There was continuous access to all marker stocks. Wild-type flies were phenotypically described as: tan bodied, red eyed, full wing venation with straight wings, and normal straight bristles. U-5343 flies were observed to be black bodied with white eyes and lacked a complete LII and LIV longitudinal veins (failing to reach margin); they were wild-type for all marker genes. M-I flies were wild-type expect for the following traits: crossvienless (cv – X-chromosome @ 13.7) and forked, shortened bristles (f – X-chromosome @

56.7). M-II flies were wild-type expect for the following traits: short-thin bristles (Bl – autosome II @ 54.8), small 'lobed' eyes (L – autosome II @ 72.0), and with curled wings (Cy – autosome II @ 6.1). M-II was a balanced-lethal culture so all flies showed a Bl L/Cy genotype. M-III flies were wild-type expect for the following traits: small 'smooth' eyes (Gl – autosome III @ 41.4) and short blunt bristles (Sb – autosome III @ 58.2) with the inversion known as LVM present (autosome III lethal balancing inversion), a non-phenotypically expressed trait. Again, M-III was a balanced-lethal culture so all flies showed a Gl Sb/LVM genotype. All cultures were true breeding. For more details about mark stocks and traits see Jeyabalan (2005)

Concerning the crosses in general, when virgin females were needed all flies were removed from source bottle and, then, 6-8 hours later virgin females were harvested. For all crosses parent flies were removed from bottles 8-10 days after the cross and all flies were scored 14-20 days after the cross. All crosses were done in duplicate unless otherwise indicated. When scoring, body color, eye color, wing venation, sex, and relevant marker traits were all recorded. See appendix (skeletal report) for scoring data table structure.

Cross A: unknown virgin females were crossed with wild-type males, F_1 flies were scored. From the F_1 generation, flies were collected and placed into a new bottle for the F_1xF_1 of Cross A. F_2 flies were scored.

Cross B: unknown virgin females were crossed with wild-type males, F_1 flies were scored. From the F_1 generation, flies were collected and placed into a new bottle for the F_1xF_1 of Cross B. F_2 flies were scored.

Marker Cross I: unknown virgin females were crossed with M-I males, F_1 flies were scored. From the F_1 generation, flies were collected and placed into a new bottle for the F_1xF_1 of Cross I. F_2 flies were scored.

Marker Cross II: unknown virgin females were crossed with M-II type males, F_1 flies were scored. From the F_1 generation, Bl L/Cy+ male flies were collected and crossed with unknown virgin females – a male backcross. *Cross was not done in duplicate*. Additionally, Bl+ L+/Cy male flies were collected and crossed with unknown virgin females – *not done in duplicate*. The first cross yielded offspring which were scored. The second cross yielded no offspring, so no flies were scored.

Marker Cross III: unknown virgin females were crossed with M-III type males, F_1 flies were scored. From the F_1 generation, Gl Sb/LVM+ male flies were collected and crossed with unknown virgin females – a male backcross. The male backcross progeny were scored.

After all the data was collected, the unneeded flies were euthanized and discarded in a humane fashion.

RESULTS AND DISCUSSION:

In general, all but one of the crosses went as expected and there were no major problems with determining the nature of the genes involved in the U-5343 mutant culture. In the analysis below, to find any χ^2 calculations please see appendix (calculations); also, please see appendix (skeletal report) for raw data tables.

Crosses A and B:

Table 1: Cross A, F_1

Trait	+type ♂	+type ♀	mutant type ♂	mutant type ♀
Body Color	56	46	0	0
Eye Color	56	46	0	0
Wing Venation	56	46	0	0

Table 2: Cross B, F_1

Trait	+type ♂	+type ♀	mutant type ♂	mutant type ♀
Body Color	43	47	0	0
Eye Color	43	47	0	0
Wing Venation	43	47	0	0

These two tables are very revealing: first, since these crosses were reciprocal and there is no significant differences between male and female expressed phenotype, it is most likely that *all* mutations are autosomal in nature. Secondly, since no mutant types are present in any flies, it should be that the mutations are *all* recessive. These conclusions can only be drawn, however, because it was given that the unknowns were true-breeding homozygous mutants. Further analysis of the F_2 generation helps confirm these initial observations.

Qualitatively, in the F_2 generation, mutant wing venation and normal wing venation were both observed. Additionally, mutant body color and wild-type body color were observed. However, four eye colors were observed: red, white, orange and brown – suggesting that more then one mutant gene is affecting eye color.

Table 3: Body Color Segregation

	wild-type:mutant
Observed #	574:179
Observed Ratio	3.2:1
Expected Ratio	3:1
Expected #	564.75:188.25

Table 4: Wing Venation Segregation

	wild-type:mutant
Observed #	581:172
Observed Ratio	3.38:1
Expected Ratio	3:1
Expected #	564.75:188.25

Table 5: Body Color and Wing Venation Assortment

	observed #	obs. ratio	expected #	expected ratio
wt body color and wt wings	439	9.38	421.31	9
mt body coilor and wt wings	142	3.03	140.45	3
wt body color and mt wings	131	2.8	140.45	3
mt body color and mt wings	37	0.79	46.81	1

The organized data of the F_2 crosses are presented in **Tables 3-6**. These tables combine both Cross A with B, and ignores gender; there should be no differences in the F_2 sexes where the genes are autosomal recessive. These tables were compiled from the raw data tables present in the appendix. **Table 3** establishes the segregation pattern of the body color gene. The χ^2 here is .606 ($0.455 < \chi^2 < 2.706$ for 1 df), thus $0.1 < p < .5$. This confirms proper segregation of the blackbody mutant color – supporting the null hypothesis. The gene, then, for the black body color is bb. **Table 4** verifies the segregation pattern of the wing venation mutation gene. The χ^2 here is 1.87 (again, $0.455 < \chi^2 < 2.706$ for 1 df), thus $0.1 < p < .5$. This suggest proper segregation of the wing venation mutation – again, supporting the null hypothesis. The gene, then, for the mutant wing venation is tv. **Table 5** helps determine the relationship between these two genes, tv and bb. Assuming a null of independent assortment, a χ^2 test reveals a value of 3.45 ($2.366 < \chi^2 < 6.251$ for 3 df), yielding $0.1 < p < 0.5$. Thus, one cannot reject the null hypothesis of independent assortment, signifying that no linkage exists between these two genes and they are on different autosomes.

Finally, **Table 6** explores the assortment of the two eye color genes. Qualitative observation suggests that two genes – a and b – interact epistatically to control eye color, thereby, producing four observed eye color possibilities: white (aabb), orange (a+_bb), brown (aab+_), and wild-type red (a+_b+_). To test for assortment, the χ^2 for the eye color genes based on cross A and B combined was calculated at 87.59, giving a $p < .005$ for 3 df of freedom. This a significant deviation from the expected results, implying that the two genes are indeed linked. Furthermore, here the orange and brown types represent a recombination event, allowing for the calculation[‡] of a mapping distance between the two genes at 37.4 mu.

Table 6: Eye Color Data

	Observed #	Obs. Ratio	Expected Ratio	Ept. #
Red	527	7.75	9	432
White	86	1.26	3	144
Orange	73	1.07	3	144
Brown	68	1	1	48

Male Parent Backcross:

Observations of the male parent backcross help to reveal the autosome associated with the mutant genes. As aforementioned, the two marker crosses represent a balanced lethal system, where heterozygosity is maintained, even in the face of crossing over. With such marker systems, one can easily determine the autosome upon which an unknown mutation lies. Backcrossing the males (hybrid M-II, or M-III, and unknown flies) with the unknown female culture produces male backcross progeny. If the recessive mutation(s) is linked to the markers, then it will not show-up with the marker phenotype in the male back cross progeny – since crossing over does not occur in the male *Drosophila*. Alternatively, if the recessive mutation(s) does show up with the marker type, then it must be independently assorting and, therefore, on a different autosome than the marker.

[‡] 2 * (73 + 67)/754 = 282/754 = .374 or 37.4 mu

For the male backcross progeny observed here, compelling results allow for autosomal assignment of all mutant genes. M-II backcross progeny were observed phenotypically as bristle, lobed, and black bodied – showing that the body color is not on the second autosome, but on the third. Also, neither the wing mutation nor the eye color mutation were present with the markers in the M-II backcross progeny, implying their presence on the second autosome. However, the curly-type backcross did not produce any viable offspring; most likely because balanced lethal systems often create very fragile males, easily sterilized by overexposure to CO_2. Nevertheless, the M-III backcross showed glued, stubbled, white-eyed, wing mutant flies, confirming the presence of these three mutant genes (a, b, and tv) on the second autosome and supporting the results of the successful M-II backcross. Additionally, the mutant body color did not show up with the marker phenotypes, also corroborating the assignment of this gene to the third autosome.

In summary then, the mutant genes a, b, and tv are on the second autosome and the mutant gene bb is on the third autosome. Thus, using a three point cross and the combined data of the F_2 progeny from crosses A, B, and M-I, one can complete[※] the map of autosome two, by determining the mutant positions. However, from these experiments, the map of autosome three cannot be completed; though, a *female* parent back cross of M-III would allow for the missing mapping data to be scored.

※ Given that tv is at 3.8 mu

Cross I:

As all other crosses have indicated, no unknown mutations are present on the X-chromosome. This, however, does not mean that the M-I cross was done in vain – by ignoring the marker phenotypes, the M-I cross effectively becomes the same as the A/B crosses. Thus, by combining all of these crosses (**table 7**), a robust data set is created where a three point cross can determine the map distances between a, b, and tv. From the table then:

Table 7: Three Point Cross

Phenotype	tv	a	b	#'s
wt wings, red eyes	+	+	+	589
wt wings, orange eyes	+	+	b	46
wt wings, white eyes	+	a	b	46
wt wings, brown eyes	+	a	+	65
mt wings, red eyes	w	+	+	88
mt wings, orange eyes	w	+	b	50
mt wings, white eyes	w	a	b	64
mt wings, brown eyes	w	a	+	27

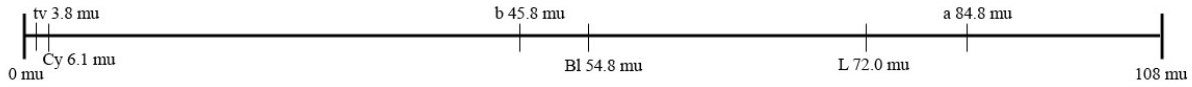
a—b = (46+65+50+27)*2/975 = .386 or 38.6 mu
(note: this is very close to the previous estimate of 37.4 from Cross A/B alone)

a—tv = (46+65+46+88+50+27+46+27)*2/975 = .810 or 81.1 mu

b—tv = (46+88+46+27)*2/975 = .420 or 42.0 mu

From these map points, we see that the proper gene order is tv-b-a and we can now map the autosome. The mutation tv is was given at 3.8 mu. Therefore, b is 3.8+42.0 = 45.8 mu and a is 3.8+81 = 84.8. The map, with all marker genes included, is represented by **image 1**:

Image 1: Autsome Two Map



SUMMARY:

Through well thought-out cross breeding experiments, the previously unknown genetic components of a true breeding *Drosophila m.* culture (U-5342) was determined. In all, four new genes were discovered and described, in brief, that control body color, wing venation, and eye color. The gene bb ('black body') is autosomal recessive and located on the third autosome; further tests are needed to establish the exact location. The genes a and b epistatically controlling eye-color, they are linkage with a distance of about 38.6 mu. They express themselves in four ways phenotypically, red (a+_bb), orange (a+_bb), brown (aab+_) and white (aabb). And are

mapped at the locations in the figure above. Finally, the gene tv (pre-terminating LII and LIV longitudinal veins) is autosomal recessive and on the second chromosome, also mapped above with a and b.

REFERENCES:

Jeyabalan, S. (2005) *MCDB 306: Genetics Laboratory Manual* GradeANotes:Ann Arbor

APPENDIX:

Calculations:

Body Color Segregation:

$\chi^2 = (574-564.75)^2 / 564.75 + (179-188.25)^2 / 188.25 = .606$

Wing Venation Segregation:

$\chi^2 = (581-564.75)^2 / 564.75 + (172-188.25)^2 / 188.25 = 1.39$

Independent Assortment of tv and bb:

$\chi^2 = (439-421.31)^2 / 421.31 + (142-140.45)^2 / 140.45 + (131-140.45)^2 / 140.45 + (37-46.81)^2 / 46.81 = 3.45$

Independent Assortment of a and b:

$\chi^2 = (527-432)^2 / 432 + (86-144)^2 / 144 + (73-144)^2 / 144 + (68-48)^2 / 48 = 87.59$