The Nature of Gene Mutations in Drosophila

Jason E. Driscol

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ABSTRACT

In order to learn the actual techniques used in the field of genetics, I tried to find a project that would give me ample hands-on laboratory work. This was accomplished with the aid of Dr. Helen Salz, who operates a lab at Case Western Reserve University. Dr. Salz started by giving me a normal stock of fruit flies (Drosophila melanogaster) and three diverse stocks with unknown numbers of mutations. In each stock, I was to determine four specific points: 1) describe the mutations, 2) determine which chromosome each is on, 3) map the mutations relative to each other, and 4) determine whether there are any chromosomal aberrations. In the various stocks, I dealt with mutations of eye color, eye shape, and wing and bristle disfiguration. Using the technique of test-crossing learned in genetics courses, I uncovered the nature of each mutation.

INTRODUCTION

Like many students, I am able to learn more easily in a lab situation. The first time I was taught genetics in Freshman Biology, something was lost in the translation of the material. Since genetics is one of my possible fields of interest, I registered in an elective genetics course at Hathaway Brown School and hoped to begin my laboratory work in the same field. I felt that this time, my understanding of science would not be hindered by the fact that I could not learn for myself by producing my own results and applying them to previous knowledge.

The medium for this experiment was fruit flies (Drosophila melanogaster). Although my research was not designed to produce any breakthroughs in the field of genetics, work done in the past has helped to explain much about human genetics. Thus, there is not much difference between the two related fields, and the techniques are no different. Moreover, the flies are easy to work with. An ample number of flies to determine conclusive evidence for an experiment can be kept in few small vials and/or bottles. The tools needed are simple to use: a microscope, carbon dioxide (CO_2) gas, and a paintbrush. Most importantly though, Drosophila reproduce very quickly, in fact overnight. The first results from test crosses can be seen within one week of the day they were set. To begin the experiment, Dr. Salz chose three types of mutations and gave me vials of each.

METHODS/PROCESSES

The reproductive cycle of Drosophila lasts about ten days. (See Figure 1.) The cycle begins with an egg which the mother lays in the food at the bottom of the vial. The females lay constantly and can yield several hundred progeny in a matter of several days. The eggs continue to mature into instar larvae until they are ready to crawl up the sides of the vial. Next, the larvae begin to pupate when a hard pupid shell forms around them. After about four days in the pupid case, they hatch and become flies.

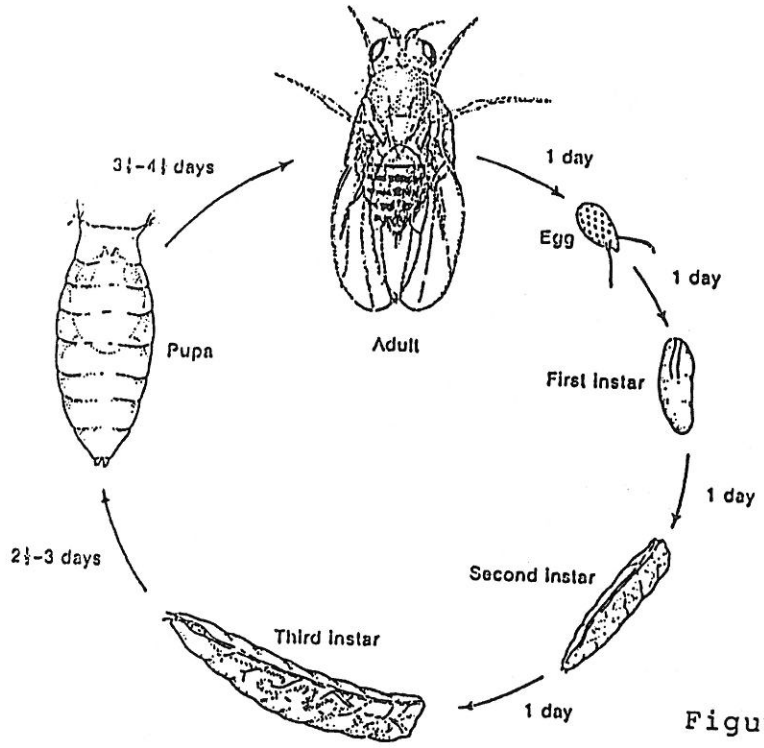


Figure 1.2.

The flies mature after twenty-four hours. This point is extremely important because in order to attain pure crosses, flies must be collected while they are still virgins.

My experiment, however, did not begin immediately with crossing the flies. First, I became acquainted with the equipment and began to observe what I should be able to remark about my stocks of flies. The basic tools I used were simple. They consisted of a paintbrush to move the flies, small glass vials with a thick layer of food on the bottom, a microscope from 100x to 400x opticals, and carbon dioxide (CO_2) gas with which to put flies to sleep. There are certain basic differences between the male and female Drosophila. Generally, the male has a smaller body and wings, black sex combs on its Tarses, and dark black coloration on the posterior portion of its abdomen. The usually larger female does not have the sex combs and has black and yellow rings at its back end. The three stocks of flies given to me contained mutations not in any of these basic areas.

In the first unknown, there was a noticeable variation in the eye color. A departure from the wildtype red eyes, these flies had a slightly brighter pink tint. Also, each fly had considerably broken or chipped wings and withered bristles on the dorsal part of its thorax. The second strain of Drosophila contained one clear defect; the flies were "blind" with pure white eyes, lacking any pigment. Lastly, the third group fostered two diverse types of males. There was the absence of the usual black coloration near the center of the eye in some flies, while others had the withered dorsal bristles and elongated, thin, white

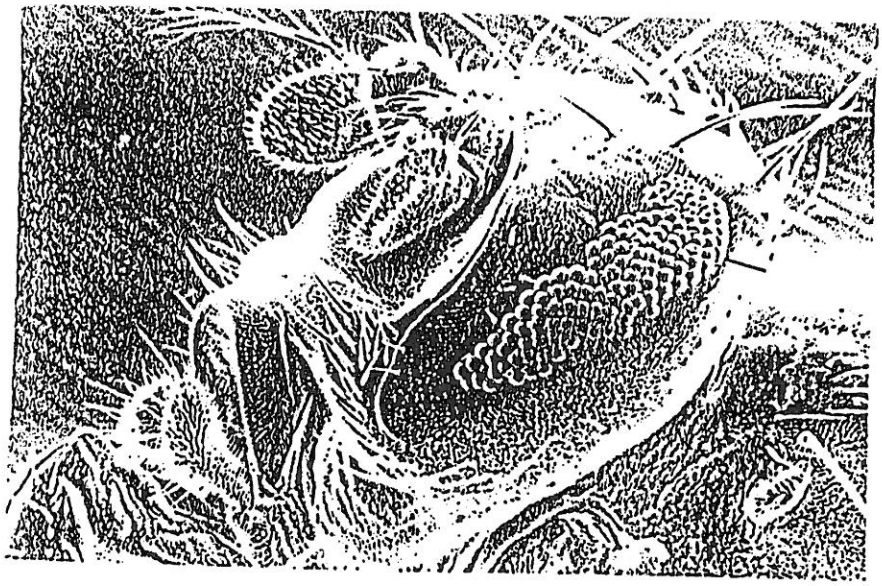


Figure 2.[3.]

"cat-like" eyes. (See Figure 2.)

As soon as all the variable traits were identified, I drew out the first possible results from a test cross and its reciprocal. (See Figure 3.) The method of using test crosses to determine genetic information was pioneered by the "Father of Genetics", Gregor Mendel. Crosses can be particularly useful because the geneticist can engineer the experiment in a certain direction to obtain a variety of expected results. For example, by drawing out all permutations and then setting a cross, a scientist can compare results with the theoretical basis to determine the pattern of inheritance.

The goal of the first cross was to determine the pattern of inheritance of each particular trait. To begin, I put each unknown and the wildtype stock in a refrigerator set at 18 degrees Celsius. This slows down the reproductive cycle so that it is possible to collect flies only once every

twenty-four hours. It is extremely important to collect virgin females to ensure that each one has purely bred with the flies laid out for the cross; any eggs fertilized by other Drosophila could throw off the results of the experiment. Once the virgins had been collected from each strain, for each individual cross I delegated a few virgin mutant flies and several wildtype males into a single vial. Then, I set the reciprocal cross for each unknown by using virgins from the wildtype stocks and mutant males.

By setting up these preliminary crosses, I was able to determine whether each trait is on a sex chromosome or an autosome as well as its dominance or recessiveness. However, it was not enough to stop here because in order to map each mutation against the others, I needed to determine which traits are controlled by the same gene. This is done through the technique of backcrossing. Traits within each stock tend to split up because a portion of a chromosome will <u>cross over</u> with its homologous pair. These are called recombinants and can be used to map the difference in location between the genes for these traits. Backcrossing the flies involved collecting virgin females from the F_o generation and mating them <u>back</u> with pure mutant males. (See Figure 4.) In the F_1 generation, there should be a certain amount of splitting up that allows the geneticist to calculate the locus of each in addition to discovering which traits operate from the same genes.

The final objective was to determine whether the chromosomes involved contained any visible defects that caused the various mutations. These could be in the form of an addition, a deletion, or an inversion. (See Figure 5.) To view the actual chromosomes of Drosophila, I performed "chromosome squashes". This is accomplished by dissecting the translucent white glands of a third instar fly larva in 45% acetic acid. Then, I squashed the chromosomes by pressing on the coverslip of a slide while the glands are inside. This breaks up the cells, making the chromosomes visible under a phase microscope lens. (See Figure 6.) I also attempted to photograph chromosomes by staining them with Lactic Acetic Orecin and taking pictures through an oil immersion lens. However, I was unable to discover any aberrations much due to my ineptitude with this technique. The glands are very difficult to find and remove, and I usually obliterated the chromosomes by pressing too hard or did not squash the glands enough. Dr. Salz informed me that I would only be able to notice visible defects in the chromosomes of the third stock, but I was unable to obtain a clear squash of whole chromosomes.

Figure 5.[4]

Figure 6.[5]

RESULTS

The results produced by this experiment took place in two stages for each stock of unknown mutations. The first cross determined the patterns of inheritance. The second developed which traits were controlled by the same genes and how far apart the genes for linked traits are. This technique is done by counting the number of recombinants (progeny of different phenotype than parents) and dividing them by the total number of progeny. The result (x 100) is the percent of recombination as well as the distance between the two genes in map units. It is easiest to explain the analysis of these portions by using one unknown at a time through the entire process.

UNKNOWN #1

The data obtained from the first cross and its reciprocal (Figure 6.) show that each trait, broken wings, broken bristles, and pink eyes, is x-linked recessive. All the males are affected and all the females are normal heterozygotes, just as Figure 3 explains. If we assume that there are three different genes:

Gene A--broken wings

Gene B--broken bristles

Gene C--pink eye color

then the heterozygous female is +++/abc (+ indicating a wildtype gene) and the male is abc/Y. To examine the recombinants, we map the data from female offspring:

+ + +/a b c	21\	
a b c/a b c	23 \	47
a b +/a b c	2 /	total
+ + c/a b c	1/	

Distance (A-B) = 0/90 = 0

Distance (B-C) = (8 + 3)/90 = 11/90 = 12.2 m.u.

UNKNOWN #2

Although the first cross and reciprocal for unknown #2 produce none of the original mutation, it surfaces again in the next generation. This and the fact that the trait does not exclude by sex led me to the conclusion that the gene (or genes as it turns out) is autosomal recessive. When different pigments surface in the next cross, this implies a case of polygenic inheritance, where there are in this case two genes that contribute to eye color. If each gene acts independently and is autosomal in nature, then the presence of a dominant allele "turns on" a pigment. Thus, both pigments yields red eyes, gene A gives pink, gene B produces brown, and zero gives white. A visual representation goes as follows:

AaBb = red

Aabb = pink

aaBb = brown

aabb = white

Since I determined that the mutations are on an autosome rather than the x-chromosome, it is possible to calculate the recombination data in males also. Thus, when one of the normal heterozygous females (+ +/a b) is crossed a mutant male (a b/a b), the representation looks like this:

+ +/a b x a b/a b

Females				Males			
+ +/a b	20	\		+ +/a b	17	\	
a b/a b	20	\	74	a b/a b	28	\	80
+ b/a b	19	/	total	+ b/a b	16	/	total
a +/a b	17	/		a +/a b	19	/	

Distance 1 (A-B) = (19 + 17)/74 = 48.6 m.u.

Distance 2 (A-B) = (16 + 19)/80 = 43.75 m.u.

UNKNOWN #3

Since the last cross has more than one type of male and, in the backcross, two types of females, it is more difficult. The basic patterns of inheritance still hold

though, and because of the splitting up between the sexes, all the traits are x-linked. Because the eyes without a dot, broken bristles, and white eyes are exclusively in the male, they are all recessive. The allele for cat's eyes, however, shows up in both sexes and is thus dominant. In two different backcrosses, I obtained these results to map the genes for cat's eyes (C), eyes without a dot (d), while eyes (w), and broken bristles (b).

cat's eyes	x	eyes w/o dot
Cwb+/+ + + +	x	+ + + d/Y
Cwb+/+ + + d	11	
+ + + +/+ + + d	17	
cat's eyes	x	white cat's, bristles
Cwb/+ + +	x	Cwb/Y
+ + +/Cwb	17	
C + +/Cwb	11	

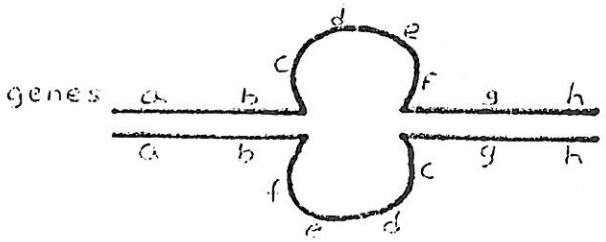
No recombinants were found in these cases.

DISCUSSION

The crosses performed gave results that did reflect the outcome of the experiment. Some inaccuracy must be due to the fact that no more than 90 flies were harvested for any particular cross. This is why my results for cross number one show no distance between Gene A and Gene B. With the small number of flies I collected, I did not pick up any recombinants. The distance between Genes B and C is map units, but Genes A and B are extremely close, and thus harder to split up by crossing over. The proper names of the traits (as known to geneticists) are cut wings, singed bristles, and vermillion eyes. The second cross gives an example of polygenic inheritance. As mentioned before, Drosophila melanogaster have been indispensable tools to geneticists, and this is one incident when human genetics has been enriched by experimentation. Just as the eyes of Drosophila contain pigments regulated by more thanone gene, the human integumentary system operates through polygenic regulation. At this point, there is speculation as to the number of genes determining skin color (4-6). Again, the difference in distances between male and female results can be explained by the relatively small number of flies.

Lastly, the third cross shows an example of sex lethal mutations. From beginning to end, no female flies surfaced with the trait "eyes without a dot". This is because the

trait linked to a gene that is necessary for life in females. When a mutation occurs, the vital allele is taken away, and no females with the trait are viable. Cat's eyes (properly "Bar eyes") is not sex-specific, but no recombination occurred. This must be because the nature of the mutation will not allow the crossing over that gives a recombinant fly. This is most likely an inversion of part of the chromosome. When a piece inverts, homologous chromosomes are unable to pair, and a loop forms. (See Figure below). This is the chromosome aberration I was suppose to look for. One final point worth mentioning is that normal flies should not have come out of the second cross. This is most likely due to a contaminant such as a non virgin fly.



ACKNOWLEDGMENTS

I have been provided with the opportunity to undertake an extensive research project which would never have been begun and completed without the aid of certain individuals. The fellowship established by Mr. and Mrs. James J. Strnad is respected by all who attend University School. It is for these reasons that I thank the Strnad family for the chance to accomplish my experiment and for the prestige associated with it. In addition, I owe my father, Dr. Thomas E. Driscol, many thanks for helping me to develop this project. However, I thank him more for his patience in having to deal with my schedule. Without the aid of these individuals, I would not have been able to even begin my fellowship.

Throughout the course of this project, I have been encouraged by my advisors. Dr. David Devore has guided me through the workings of a research project of this nature. Mostly, I owe Dr. Helen Salz, whose guidance and insight have been the primary fuel of my lab work. Also, I would like to thank Dr. Jane Petschek, Ms. Beth Albrecht, Mrs. Kristin Doll, and Ms. Barbara Wallenhorst for their aid, their patience, and for making each moment more tolerable.

FOOTNOTES

1. Suzuki, David T., and Anthony J.F. Griffiths, An Introduction to Genetic Analysis. (San Francisco: W.H. Freeman and Company, 1976.) front cover illustration.

2. Ibid, p. 29.

3. Ibid, p. 223.

4. Ibid, p. 169.

5. Ibid, p. 168.

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