A gene for viability in the X-chromosome of *Drosophila*

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(With 4 Text-figures)

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Introduction

The chromosomes of *Drosophila melanogaster* can be broken rather easily by X-rays. In making a study of the effects on development of such breaks in the X-chromosome, the writer has discovered the interesting fact that a small region, lying near the left end of this chromosome, is essential to the life of the female fly. It has been found that a female zygote inheriting one unbroken X-chromosome and a second X, from which this region is missing as a result of a break, will not develop into an adult fly. A similar zygote that has received a broken X-chromosome, in which this region is present, is viable, although the missing piece from another region of the chromosome may be considerably larger than the one eliminated in the first instance. The problem is to locate and to determine as accurately as possible the limits of this region. The solution of this problem may be approached by introducing into the chromosomal combination duplicated pieces of different lengths, and studying their effects on the viability of such zygotes.

We now have among our stocks a number of cases of broken X-chromosomes, and these furnish an opportunity to carry out a series of tests for the purposes just stated. Three of these cases deserve special mention at this point. These cases are known as Notch 172b, Notch 235a, and Mottled Notch 231b. All three of these cases arose as a result of X-raying the Theta-X-chromosome, which carries a duplicated fragment attached to its right end. The chromosome to which the fragment is attached has the wild-type genes, except at the left end where the mutant genes for yellow and scute are present. The attached fragment represents a deleted X-chromosome, and before attachment had the wild-type allelomorphs of yellow, scute, broad, and prune of the left end, and the gene for bobbed of the right end. When this fragment became attached, as a result of a second irradiation, part of it was lost, so that it now contains the normal allelomorphs for yellow, scute, and broad, but not that of prune.

The first two mentioned are very similar in character; in each case the left end of the X-chromosome is missing. The break, which was caused in each case by X-rays, occurred at some point lying between the loci of echinus and ruby. All of that part of the chromosome lying to the left of this point was eliminated at the time the break was produced. Since 172b and 235a are similar in character, we shall use the former case for the purpose of illustrating the principles involved.

Notch 172b has a broken Theta X-chromosome, from which a piece of the left end 5.5 to 7.5 map units in length is missing. A female fly that inherits this chromosome and a second X with the mutant genes for yellow and white, has gray body color, white eyes, and notched wings. She is not only viable, but she is also distinctly fertile. If the fragment at the right end is lost by a crossover during maturation, the female zygote receiving the broken crossover X-chromosome and a yellow white chromosome, would be yellow white notched. But numerous tests with this and several similar cases, has failed to give any such females.

The Mottled Notch stock, 231b, was produced in the same way as the other two, and the Theta X-chromosome had a piece approximately of the same length broken off of its left end. However, this fragment, instead of being lost by elimination, as in the other two cases, became attached to a fourth chromosome. The translocation thus produced is not stable, for it has been demonstrated that during somatogenesis the fragment occasionally becomes eliminated. The descendants of any cell from which the fragment has been lost, will, upon entering the embryonic rudiments of the eyes or wings, produce mosaicism in these structures. Both the mottling in the eyes and the notching in the wings, are variable, and vary independently of each other (PATTERSON and PAINTER, 1931). It was found, further, that a female inheriting the broken X-chromosome (from which the Theta fragment was removed by a crossover) a yellow white X-chromosome, and the translocated piece on the fourth, is viable and fertile. Such a female will produce four kinds of gametes, three of which when fertilized by X-bearing sperms, form viable and fertile females, while the fourth, which received the broken X-chromosome and a normal fourth, proved to be non-viable (PATTERSON, 1931b). A viable female can be produced from this gamete if it is fertilized by an X-bearing sperm that carries a duplicated piece from the left end of another X-chromosome, provided this piece is of sufficient length. This stock therefore gives an excellent opportunity for determining the location of the viability region of the X-chromosome.

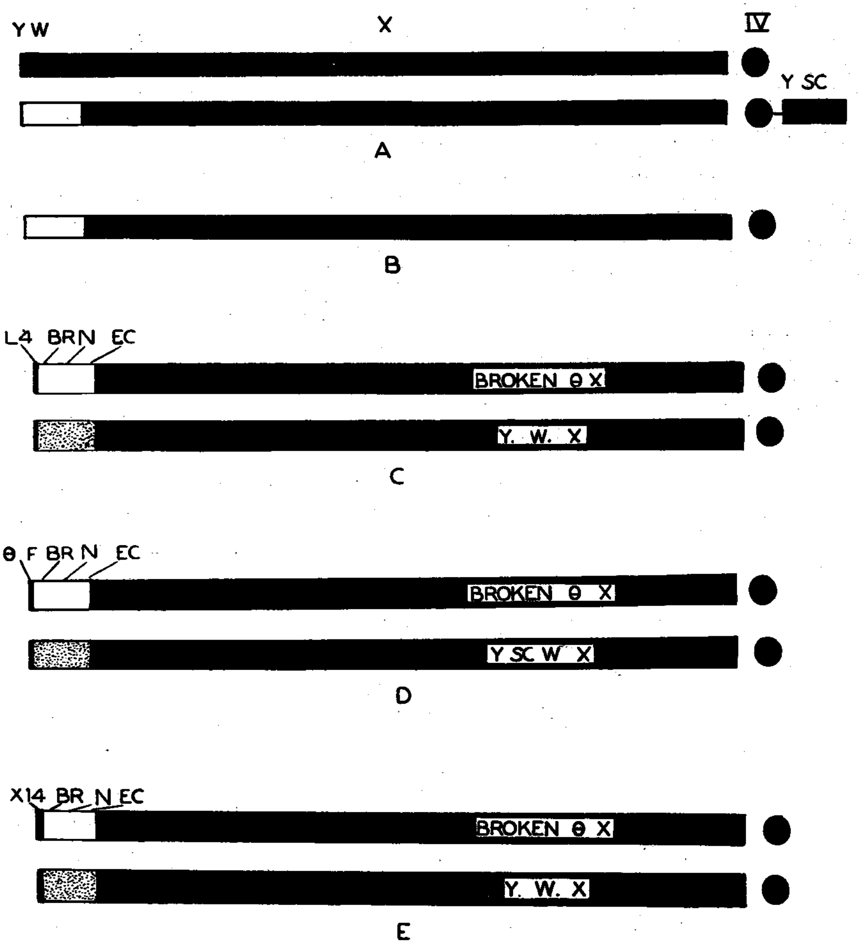

Fig. 1. Diagrams of the X- and IV-chromosomes. A, the compositions of these chromosomes in a mottled notched female of stock 231 b. B is the fourth gamete which inherits the broken X- and a normal IV-chromosome. C is the non-viable zygote which inherits the broken X-chromosome, a normal IV, and the gray L4 fragment. In this and the succeeding figures the parts of the two X-chromosomes which have the genes in duplicate are represented in solid black; the stippled part represents the genes that are in single dose; while the missing part of the X-chromosome is in white. From this it will be seen that the L4 fragment covers but a very small part of the extreme left end of the broken Theta X-chromosome. D is the non-viable zygote with the scute Theta fragment. E is the non-viable zygote with the Deleted X14 fragment

The composition of the X- and 4th-chromosomes of the yellow mottled notched mothers used in the tests is shown in Fig. 1A. The female has the broken X, one fourth with the attached fragment, a yellow white X, and a second fourth that is normal. She also has normal second and third chromosomes, but since we are not concerned with their distribution, they are not included in the figure. In maturation such a female will produce four types of gametes, with reference to the segregation of the X- and 4th-chromosomes. These four gametes will have, respectively (1) a yellow white X and a normal fourth, (2) a yellow white X and a fourth bearing the attached fragment, (3) a broken X and a fourth with the fragment, and (4) a broken X and a normal fourth. As suggested above, the first three types of gametes will, upon being fertilized by yellow white X-bearing sperms, produce the three expected classes of females, and these are all fertile. But the fourth type does not form viable zygotes. This gamete when fertilized by the same kind of sperm, should produce a yellow white notched female, but no such female is ever found in the cultures. The zygote is clearly non-viable.

The fact that this class does not appear gives the opportunity for making the tests for the postulated gene for viability. If this class can be made to appear, by the use of duplicated pieces, then the approximate location of the gene can be fairly accurately determined.

The experimental tests

In this section will be given the genetic results obtained from crosses between yellow mottled notched females (stock 231 b) and several different types of males, each carrying an extra piece of known length that duplicates a part of the left end of the X-chromosome, but leaves the locus of notched "uncovered". If we start with the shortest available deleted X-fragment and use successively longer and longer pieces, we should eventually reach one of a length sufficient to duplicate the locus for the viability region. This can be detected by the appearance in the F_1 generation of the class of female (gray white notched) which up to this point had proved to be non-viable.

The first type of male used belonged to MULLER's deleted stock "L 4". In this stock the extra duplicated fragment is an independent element, having its own fiber attachment, and contains the normal allelomorph of yellow and achaete from the left end. The fragment also has a part of the right end of this chromosome. The part from the right end does not contain the locus of carnation (point 65.5) or bobbed. The non-deleted X-chromosome of this type of male has the mutant genes for yellow and white. Yellow mottled notched females were crossed to L 4 gray white males, and the cultures yielded all of the expected classes of females, except the one to be derived from the fourth type of gamete (Fig. 1B). These females, if viable, would have had gray body color, white eyes, and notched wings, but no such female appeared in the cultures. Obviously, the zygotes formed by fertilization were non-viable. The composition of such a zygote is shown in Fig. 1C. The small section in the fragment from the left end of the X was not sufficient to save the fly; it did not contain the gene for viability.

The second type of male employed was from a stock known as "Scute Theta". This stock was produced by the writer by X-raying the Theta X-chromosome (MULLER's Deleted X 1). It will be recalled that the Theta fragment attached to the right end of an X-chromosome, contains the normal allelomorphs of yellow, scute, and broad. As a result of the irradiation a mutation to scute occurred at that locus, and at the same time the fragment was broken off from the X-chromosome and became attached to one of the second chromosomes. The newly translocated piece, was found to contains the locus of yellow and the new mutant gene for scute (Fig. 1D). The stock is carried with yellow scute white, and hence any male receiving the fragment on the second chromosome will be gray scute white. The fragment was tested for the loci of broad and prune, but both of these were found to be absent. The break in the fragment that occurred at the time the translocation took place lay at some point between the loci of scute and broad.

The test was made by mating yellow mottled notched females to the gray scute white males. The cultures gave a very large number of flies, and among these were found all of the expected classes of females, except the one that would have developed from the female gamete having the X-chromosome deficient at the left end. These females would have been gray scute white notched, but none was found. The duplicated piece was not long enough to bring through this class of female.

The next type of male used belonged to a stock known as Deleted X 14 (PAINTER and MULLER, '29, fig. 33). The lefthand part of the deleted X-chromosome contained the allelomorphs for yellow and scute, and the right hand part had that for bobbed. The left hand portion contains the normal allelomorphs of yellow and scute, but not that for broad, so that the left break of the deletion occurred at some point lying between scute and broad. Gray white males carrying deleted X 14 were mated to mottled notched females, and the F_1 generation examined for gray white notched females (Fig. 1 E). The culture gave 284 flies including 61 gray white females, but yielded no gray white notched females.

Since none of the three fragments, produced by breaks lying between the loci of scute and broad, was long enough to duplicate the viability region, we next used fragments produced by breaks between broad and prune. Three such cases were available, namely, Deleted X 2 (PAINTER and MULLER '29), Translocation X-III 2 (MULLER and PAINTER '29) and white Theta made by crossing white into the Theta X-chromosome.

In the test with Deleted X 2, yellow mottled notched females were crossed to males with a yellow white X-chromosome and having the independent gray fragment. The culture yielded 165 flies, among which were 17 gray white females, but none of these was notched. The left-hand part of the fragment was not long enough to produce viable zygotes with the fourth gamete (Fig. 2 A), although the break producing the fragment occurred between the loci of broad and prune. These results show that the viability region must be located to the right of the locus of broad[1].

Translocation X-III 2 is similar to the preceding one, but differs from it in that the fragment is attached to a third (III) chromosome, and does not contain any part of the right-hand end the broken X-chromosome. The test was made by crossing mottled notched females to males with a yellow white X and having the gray translocated fragment on a III-chromosome. The F_1 culture gave but 74 flies, but among these were found ten gray white females, of which two were *notched*. The most interesting point brought out in this test is seen in the appearance of the fourth class of females, the gray white notched females. This indicates that the zygote, which heretofore proved to be non-viable, has become viable through the effect of the duplicated fragment. The fragment of X-III 2 represents a larger section of the left end of the X-chromosome than is present in any of the other duplications used (Fig. 2 B), and therefore must include the viability region.

The next type of male used for testing was from a white Theta stock. The single X-chromosome of this type of male has the mutant

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[1] Since this paper was completed, Dr. H. J. MULLER has had occasion to retest Deletion 2, and now finds that the break lies between the loci of scute and broad, and not between broad and prune. Therefore, this case does not prove that the viability gene lies to the right of broad; it may lie to the left of that point.

genes for yellow scute and white at the left end and the regular Theta fragment attached to the right end. The same general plan was followed of crossing yellow mottled notched females to gray white Theta males. The F_1 cultures gave the four expected classes of females, which shows that each of the four types of gametes, produced by the mothers, formed viable zygote upon being fertilized by Theta white X-bearing sperms.

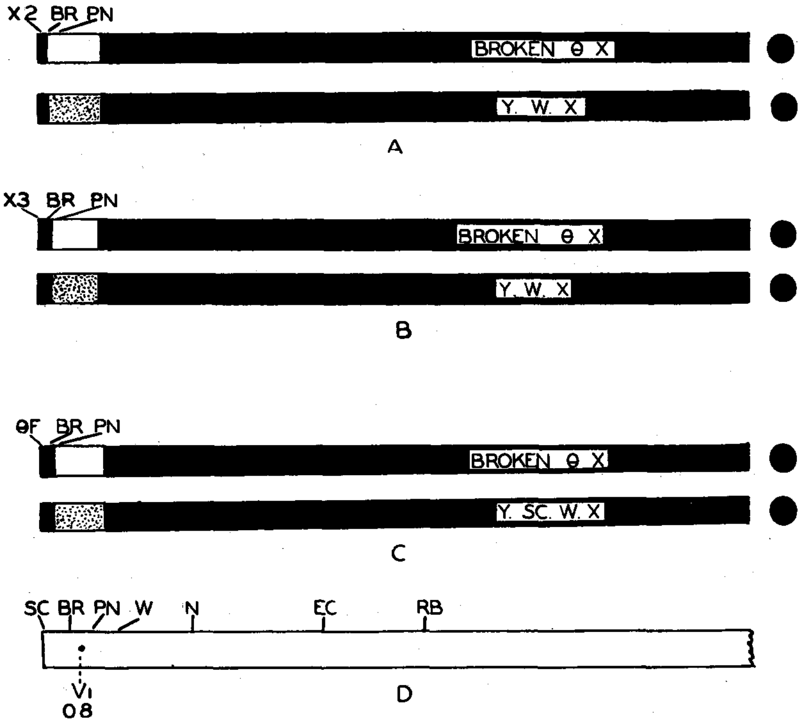


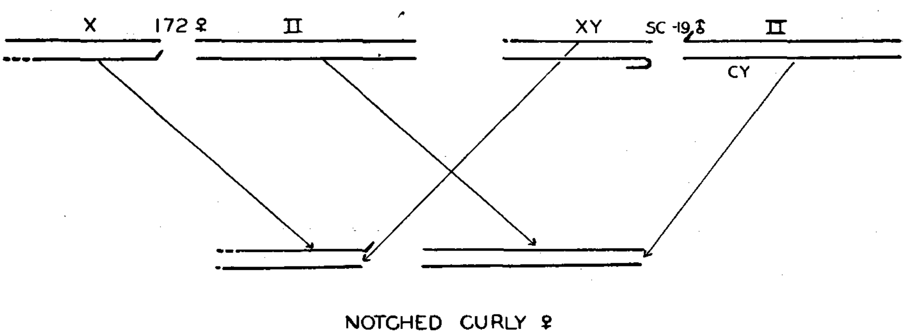
Fig. 2. Diagrams similar to those of figure 1. A is the non-viable zygote with the Deleted X2 fragment. B is the viable zygote with the Translocated X-III 2 fragment (X3). C is the viable zygote with the regular Theta fragment. D a diagram of the greatly enlarged left end of the X-chromosome showing the location of the gene for viability (Vi)

The number and character of the flies for the four classes of females (given in the same order as for the female gametes listed on page 128) were as follows: (1) 189, gray white class; (2) 97, gray mottled hyperploid class; (3) 158, gray mottled notched class; (4) 101, *gray white notched* class (Fig. 2C). As a matter of fact, not all of the females in class 2 were mottled, nor were all of those in class 3 mottled or notched. The phenotypic expression of these two characters in variable, and depends upon the occasional loss of the fragment from the fourth chromosome. The females of the first three classes are, in reality, all hyperploids, since each carries the extra Theta fragment; but the female of class 2 not only has two non-deleted X-chromosomes, but she also has two extra fragments, the Theta piece on one X and the mottling piece on one fourth chromosome. She is easily recognized by her abnormal wing venation, which is not seen in the females of the first and third classes.

The results from this test confirm the conclusion based upon those obtained in the preceding experiment, namely, that the viability region is located at some point lying between the loci of broad and prune. Since several of the fragments used in the experiments are composed of pieces derived both from the left and right ends of the deleted X-chromosome, the question naturally arises as to whether the right-hand part has any influence on the viability of the zygote. The evidence indicates that it does not have any such influence. Thus deletions L 4, X 14 and X 2 each has a part of the right end, and none of these produces viable zygotes. On the other hand, the translocation X-III 2 does not have the right-hand portion, and still produces viable zygotes. Furthermore, the fourth gamete when fertilized by a normal X-bearing sperm (non-fragment bearing) never produces a viable zygote, although the right ends of the two X-chromosome are present.

The results obtained from the experiments so far outlined demonstrate that a region of viability is located on the X-chromosome at some point lying between the loci of broad and prune. They further show that when this region is present in only one of the two X-chromosomes, the zygote is not viable, but when it is present in each of the two X-chromosomes, the zygote is viable. This raises the interesting point as to whether the zygote would survive if this region were present in both X-chromosomes, but with the parts of the chromosome lying adjacent to either side represented with non-duplicated genes. It also would be interesting to determine what would happen, if the viability region only were missing from one of the chromosomes. By combining broken X-chromosomes from different stocks, it is possible to settle both of these questions, and I am indebted to Dr. H. J. MULLER for suggesting the following experiments.

There were three stocks of females available for the first test, any one of which would be suitable. I have used Notched stock 172b, which has already been described. Notched 172 females were crossed to scute-19 curly males for the test. The latter stock was obtained (Dr. BESSIE LEAGUE) by X-raying Delta-49 males. A small piece from the left end of the ♂ 49-X was broken off and translocated to a II-chromosome. The chromosome was broken between scute and broad, and at the same time a mutation arose in the translocated fragment at the locus scute, producing a new allelomorph, or scute-19.



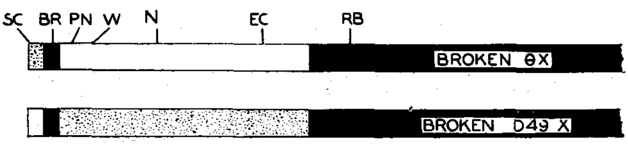


Fig. 3. A. Scheme of cross to produce a female zygote with the viability region duplicated. B shows the enlarged left ends of the two broken X-chromosomes

The scheme for this cross is shown in Fig. 3 A. The object was to find out whether a Notched Curly female would be produced, for such a female would inherit the two broken X-chromosomes and normal II-chromosomes (one having the dominant marker curly for identification). The F_1 culture gave 33 wild-type females, 19 notched females, 19 Delta 49 spectacled males (broken X is balanced to ♂ 49 lz^s in stock 172b), 29 Delta 49 spectacled curly males, and 15 *Notched Curly females*. This proves that a zygote receiving the broken X-chromosomes is viable, due to the fact that the viability region is in duplicate. The extent of the duplication near the left ends of the two chromosomes is shown in Fig. 3 B.

To test the second point raised above, required a more complicated series of crosses. The scheme for the crosses is shown in Fig. 4A. Bar (the ClB X) curly females were first crossed to scute-19 non-curly males, and the F_1 scute-19 bar curly females were then outcrossed to blonde

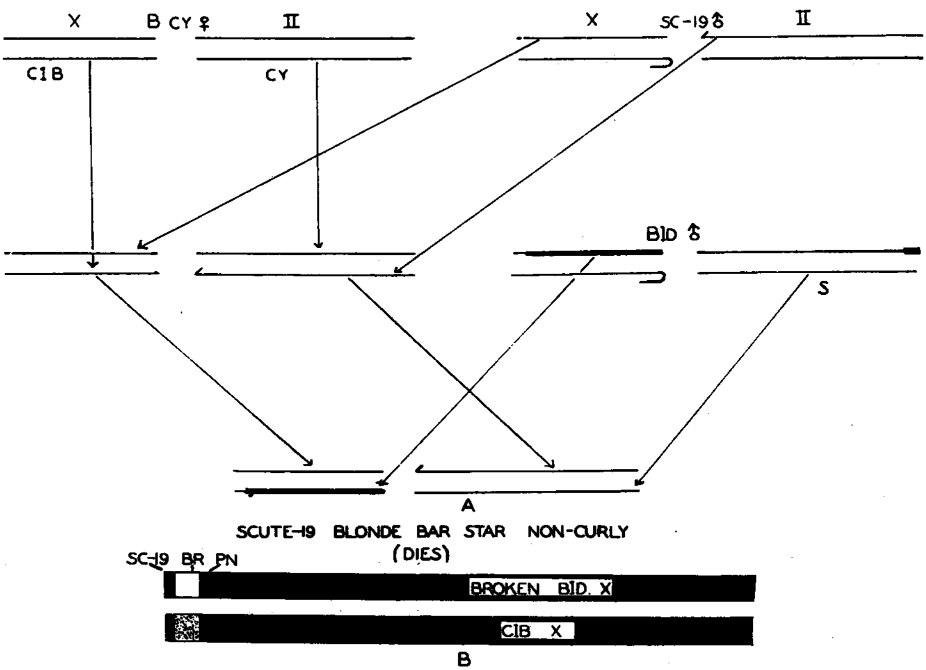


Fig. 4. A. Scheme of the crosses to produce a non-viable zygote inheriting the viability region not duplicated. The blonde stock (Bld) is the result of a mutual translocation between ends of the X- and II-chromosomes. The portions of the X-chromosome are in thick line, while those of the second chromosome are in thin line. The translocated left end of the X-chromosome on the second, has the loci for yellow scute and broad. B, shows the enlarged left ends of the two X-chromosomes

Star males. The F_2 cultures were examined for the presence of scute-19 blonde bar star non-curly females (the blonde stock has a mutual translocation). These cultures yielded 207 flies, but none having this combination was found. The amount of duplication and non-duplication at the left ends of the two X-chromosomes is shown in Fig. 4B, where it will be observed that the viability region is not duplicated.

Discussion and Conclusions

The facts developed above make it certain that a very small region, lying near the left end of the X-chromosome, must be present in duplicate if a zygote formed at the time of fertilization is to survive and develop into an adult female fly. If this region is not duplicated, the zygote is not viable, even though all other parts of the chromosomes are in duplicate. If this region is present in both X-chromosomes, a considerable portion of other regions of one of these chromosomes may be missing, and still the female survives. I have found several cases of this character.

Throughout the paper I have referred to the small section of the X-chromosome that is essential to the life of the female fly as a "viability region". I should like to suggest that this region may represent a single gene with a definite locus, and therefore may be designated as a gene for viability. The method of analysis followed in the experimental tests has enabled us to restrict this region to a very small section, lying between the loci of scute and prune, and this alone would justify the suggested interpretation. As a gene, its locus would lie at point 0.8 on the chromosome map (Fig. 2D), and its absence from one of the two X-chromosomes of the female zygote has the effect of a dominant lethal.

Whatever may be its exact nature, knowledge of its effect is essential for a correct interpretation of many results that have been obtained in x-rayed material. I refer especially to the studies dealing with the effects on development of breaking out pieces of the X-chromosome by irradiation. The writer has obtained over three hundred cases in which the female had a part of one of her X-chromosomes missing. A re-examination of the records on these cases shows that no valid exception to the rule, that the viability region must be in duplicate, has been found. The seven apparent exceptions recorded (all made before the discovery of the gene for viability) can now receive what is obviously a correct interpretation of their composition. We shall refer here to a single case, which was recently reported by the writer (1931a, p. 184). A yellow white crossveinless singed female was found in the F_1 generation of a cross between a treated yellow scute female and an untreated yellow white crossveinless singed male. Two explanations were suggested. The first was that the female had inherited the X-bearing sperm from the father, and a broken yellow scute X from the mother, the latter having the left end missing back to some point lying to the right of the locus of singed. The other suggestion was that the fly was the result of equational non-disjunction occurring in the paternal X-chromosome.

In the light of the present work, the better interpretation is that the yellow scute chromosome had had a piece broken out and eliminated by the X-rays, leaving the deleted X fragment with the mutant genes for yellow and scute, and the gene for viability from the left end, and all the wild type genes of the right-hand part. On the basis of this interpretation, the removal of the wild-type genes for white, crossveinless, and singed, would allow the corresponding mutant characters to appear. Scute would be covered by its normal allelomorph in the paternal X-chromosome, and hence the fly would show the four recessive characters of the father. The six remaining cases of apparent exceptions, mentioned above, can be explained in a similar manner.

During the course of this study, I have found occasion to use many different stocks, several of which were generously furnished by my colleagues in the laboratory. I have given credit for these in the main body of the paper; here I wish to express my appreciation for the use of indispensible data, much of which is still unpublished.

Austin, Texas.

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