A TANDEM INVERSION IN DROSOPHILA MELANOGASTER

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INTRODUCTION

BY MEANS of X-rays it is possible to procure gene deficiencies and chromosomal aberrations in Drosophila which can be analyzed genetically and cytologically. The increasing refinement of technique in these fields is facilitating such detailed parallel studies. As a consequence there arises an ever-growing mass of problems concerning such questions as the origin of the changes and the relationships involved. In order to have a better understanding of these relationships we need to determine the types of changes which are occurring, and an analysis of different kinds of alterations may aid in a more complete understanding of the underlying mechanism involved. The present analysis was undertaken to obtain information about the relationship between chromosomal aberrations and deficiencies.

MATERIAL

With the intention of picking up lethal changes connected with known loci along the X chromosome of *Drosophila melanogaster*, *y Hw* males were treated with X-rays using a dosage of approximately 2500 r units. Following the treatment, these males were mated to females of the constitution *ct v dy g f*/In dl–49, *y Hw* m^2 g^4. The F_1 flies of this cross were examined and all females were saved which besides being heterozygous for *Hw* exhibited one of the mutants carried by the female parent. In the particular instance described in this paper an F_1 female was found with cut wings. The male off-spring of this female were only half as numerous as expected, and hence she was assumed to carry the cut mutant in the maternal chromosome, and either a lethal mutant for cut or a cut mutant plus a lethal in the paternal chromosome. Crossover tests were made by mating *y Hw* ct^1/*sc ec cv* ct^6 *v* s^2 *f car* bb^1 females with *ct v dy g f* males. No crossovers were observed among 141 males and 1007 females. This suppression of crossing over thus indicated that in addition to the change in the cut locus and a lethal which might or might not have been connected with cut, the treated X chromosome was involved in some chromosomal rearrangement. Therefore, the location of the lethal could not be determined by genetic means. Since it is known that lethals may be deficiencies (DEMEREC and HOOVER 1936; ALIKHANIAN 1937), salivary gland chromosomes of female larvae were studied cytologically in an attempt to determine the position of the lethal. The study revealed two inversions in the X chromosome—a

small one involving approximately 4.5 sections of the cytological map prepared by C. B. BRIDGES (BRIDGES 1935), and another larger inversion involving 11.5 sections. There is no uninverted portion of the chromosome between the two, so that we have here a case of what may be called inversions in tandem. This terminology may be conveniently used to designate two inversions, one following immediately upon the other in the same chromosome.

METHODS

In order to study this material, slides were prepared of the salivary gland chromosomes of female larvae according to the aceto-carmine method and were made permanent by the alcohol-euparol technique described by BAUER (1936). In analyzing the slides the optical equipment consisted of a 90X, 1.4N.A. apochromatic objective, an oil-immersed achromatic 1.4N.A. condenser and compensating oculars (12.5 and 15X). The source of illumination was a Bausch and Lomb research lamp (ANTHES 1936, BRIDGES 1936) equipped with a Wratton filter number 58A.

To guarantee the greatest accuracy in determining the breakage points of the inversions, observations were made on two types of figures, synapsed chromosomes and single haploid strands. The synapsed diploid chromosomes were studied to determine the homology of the bands present. However, in the complexity of even a very simple aberration, it is not easy to identify accurately the bands immediately bordering a break, so that confirmatory information must be obtained from stretched haploid strands. In these unsynapsed strands the band can be identified only by its relative position and intensity. Those working with similar problems are well aware of the risk involved here, for the amount of stretching of the chromosome and the particular quality of the illumination used may alter the general appearance considerably. Consequently in such an analysis as is attempted here, it is fair only to make suggestions as to what the conditions may be, suggestions based on as accurate statistical observations as were feasible.

RESULTS

The two inversions in heterozygous condition appear in the salivary gland chromosomes as two loops when both strands are completely synapsed. Complete synapsis occurs in 77 percent of the cells and complete asynapsis in 3 percent. By complete synapsis is meant the pairing of the two strands throughout their entire lengths with the possible exception of a very small region at the junction of the two loops, a region for which complete pairing would be quite difficult to determine. By complete asynapsis is meant the two strands separate throughout their entire lengths. This 77 percent may be compared with 90 percent complete synapsis for controls (X chromosomes not containing inversions), and the 3 per-

cent of complete asynapsis may be compared with 1 percent for controls. Similar observations have been made on six other inversions of differing lengths in the X chromosome (HOOVER 1937). Although the longest inversions (ClB and the tandem inversion) show the lowest percentages for complete synapsis, yet there is indication of very little, if any, correlation between inversion length and synaptic attraction.

When complete synapsis does take place, the inversions appear as indicated in figure 2. In each inversion one strand is inverted in relation to the other, so that, as the diagram shows, when pairing occurs, loops necessarily result. The two inversions use in their combined configurations almost the entire X chromosome leaving only small sections at each end in which both homologous strands are in their normal positions. There are actually two separate and distinct inversions, and, as the order of banding clearly reveals, not one inversion imposed upon or overlapping the other.

Three breaks in the X chromosome may account for the origin of these two inversions since one breakage point is common to both of them. In figure 1a are given diagrams of the three regions as they normally appear under optimal conditions of stretching. These normal maps have been furnished by Dr. BRIDGES as revisions of his 1935 maps (BRIDGES 1935). The first break, which is the left break of the first inversion, precedes 2F3; it follows 2E3. The second break in the chromosome which is common to both inversions follows 7B1, 2 and precedes 7B5. The third break follows 19A1, 2 and precedes 19B1, 2. Therefore, the first inversion extends from 2F3 to and including 7B1, 2 and the second inversion extends from 7B5 to and including 19A1, 2. The normal portions extend from the tip to and including 2E3 and from 19B1, 2 to the spindle fiber locus.

In figure 1b are represented these regions as they are recombined by the inversions. The 2E3 section thus comes in contact with the 7B2, 1 region, 2F3 comes in contact with 19A2, 1, and 7B5 with 19B1, 2. Since the critical lines at these breakage points are very fine, it has not been possible to obtain photographs of the figures which show them. Photographs and camera lucida drawings are given here only of figures which were reasonably good for reproduction. Figure 3 represents the first breakage region. The last synapsed band visible in that figure is 2E1, 2. Other figures have clearly shown that a fine line 2E3 completes this first normal section and that, as shown here, 7B1, 2 begins the first inversion. Comparing the two

bands of the second inversion followed by 19B1, 2, the first bands in the normal portion at the end of the chromosome. The absence of other bands between 7B5, 6, 7 and 19B1, 2 makes it probable that there are deficiencies for 7B3 and 4 and for 19A3, 4.

FIGURE 7. Third breakage region with 7B5, 6, 7 followed by 19B1, 2.

FIGURE 8. Scale referring to figs. 3 to 7.

Photographs and drawings by Rachel W. Parker.

strands, one normal and one inverted, the first inversion would seem to follow upon 2E3. Figure 4, however, shows that 2F1, 2 is not present at the other end of the first inversion where it would be expected to be if the breakage occurs following 2E3. Figure 4 again shows not only that 2E3 is followed by 7B1, 2 but moreover that 2F3 is followed by 19A1, 2 and, accordingly, there is a deficiency at this breakage for 2F1, 2. These two figures also show that 7B3 and 4 and 19A3, 4 are not present at these breakage points, making it necessary that all four of these bands be detected at the third breakage region if they are present at all. The second breakage region is illustrated in figure 5 showing an inverted haploid strand where it can be seen that 2F3 is immediately followed by 19A1, 2. It is clear from that figure that there is no band between 2F3 and 19A1, 2, as has already been indicated in figure 4. Finally, in figures 6 and 7 is illustrated the third breakage point. In figure 6, which again shows a haploid inverted strand, bands 7B5, 6, 7 are followed by 19B1, 2. Close examination of this and many other figures has failed to reveal any suggestion of a band or bands between. Similarly, in figure 7, 7B5, 6, 7 appear as the final bands of the second inversion and the normal stretch at the end of the chromosome begins with 19B1, 2. These observations therefore suggest deficiencies for 7B3 and 4 and for 19A3, 4. Negative evidence of this type is not entirely satisfactory. In the present instance, however, numerous well-stretched figures were examined without any sign of the bands in question, so that it seems probable that small deficiencies have occurred at each of the breaks. The following appears then to be the condition at hand:—*normal:* 1A1–2E3; *deficient:* 2F1, 2; *first inversion:* 2F3–7B1, 2; *deficient:* 7B3 and 4; *second inversion:* 7B5–19A1, 2; *deficient:* 19A3, 4; *normal:* 19B1, 2–20D.

DISCUSSION

One of the interesting problems connected with the study of chromosomal aberrations is to determine whether all chromosomal aberrations have small deficiencies connected with them. It is known that all deficiencies are not connected with other chromosomal alterations; it is also known that all chromosomal aberrations are not connected with lethals; but since it is being found that some deficiences are not lethals (DEMEREC and HOOVER 1936), it remains to be determined how many translocations and inversions are free from accompanying deficiencies.

The available data for the relationship of gene changes and chromosome rearrangements are recent. Although observations have been made on numerous deficiencies and numerous aberrations, statistical data on the interdependence of the two are just being accumulated. The existence of such an interrelationship is indicated from results obtained by DEMEREC

(1937) who found that of 61 lethals induced in known X chromosome loci by X-ray treatment, 26 carried a chromosomal aberration in the same chromosome and that in all but one case one breakage point of that aberration coincided with the region where the lethal change occurred. Of 30 visible changes induced by a similar treatment in the same set of loci only one chromosomal aberration was observed and in that case neither of the breakage points coincided with the region of the visible change. Since it is probable that lethal changes may be deficiencies, this suggests a close relationship between deficiencies and chromosome breaks. The present material gives additional evidence on this same problem. The two distinct inversions have apparently one breakage point in common at the cut locus which was simultaneously changed, and there are indications of a deficiency at each of the three breakage points.

Genetic evidence shows that the tandem inversion is lethal when homozygous. It has not been possible to localize the position of that lethal in relation to cut. There is every indication of a cytological deficiency in the cut region as well as deficiencies at two other places in the chromosome. It is interesting to note that tests applied to the hypodermal cells of females indicate that the cumulative effect of all the deficiencies is non-cell-lethal, an effect which has been limited to lethals of a very few of the known loci tested (DEMEREC 1934). In this respect the lethal here resembles other lethals at the cut locus.

The question naturally follows as to how these inversions and deficiencies have originated. Two alternative hypotheses are possible. One is that the X-ray treatment produced three breakages along the chromosome and that the broken pieces so rearranged themselves as to become fused together in a new arrangement. On the other hand, it is possible to visualize the chromosome as looped upon itself so that fusion of overlying strands occurred at the time of treatment, and following this fusion, breakages occurred resulting in a rearrangement of the gene order. The deficiencies which result would be conceived of as coincident with the breakages so that, as has already been suggested, the mechanism responsible for these abnormalities is also frequently responsible for lethal deficiencies. This material does not offer critical evidence for choosing between these two alternatives.

SUMMARY

A tandem inversion involving practically the whole X chromosome was obtained following X-ray treatment. Genetically this material involves a change in the cut locus, and a lethal, which while lethal in the homozygous condition to the organism as a whole, is non-cell-lethal to hypodermal cells of females. Cytologically three points of breakage have been determined

in the salivary gland chromosomes, and the heterozygous chromosomes have been observed to form two characteristically looped inversions. The two inversions have a common breakage point following 7B1, 2 and preceding 7B5. The first of the two other breaks occurred following 2E3 and preceding 2F3, and the second follows 19A1, 2 and precedes 19B1, 2. Cytological examination indicates that small deficiencies have occurred at each of the three breakage points.

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