RECIPROCAL TRANSLOCATIONS BETWEEN THE SECOND AND THIRD CHROMOSOMES IN THE SOMATIC AND MEIOTIC DIVISIONS OF DROSOPHILA MELANOGASTER

By

Dale C. Braungart

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CHAPTER I

INTRODUCTION

Among the most perplexing cytological problems of the past decade has been the chromosome ring formation observed at the first meiotic metaphase in certain plants. Theoretical considerations have led to much speculation and discussion concerning the probable method of formation of such rings. The phenomenon was at first discussed in terms of parasynapsis and telosynapsis. Parasynapsis is the side by side pairing of the chromosomes, the homologous portions synapsing laterally. This has also been referred to as the "parallelism of the prophasic threads". Telosynapsis, on the other hand, is described as the end to end pairing, that is, the chromosomes are joined at their ends.

Belling (1927) suggested that the ring formation in Datura was attributable to segmental interchange or reciprocal translocation between non-homologous chromosomes. This has been confirmed in Oenothera (Cleland and Blakeslee, 1931) by both cytological and genetic evidence. The behaviour of reciprocal translocations in Drosophila (Sturtevant and Dobzhansky, 1930) is also in accord with Belling's hypothesis.

Belling's (1927) proposed concept of segmental

interchange was derived from a study of a hybrid between two lines of Datura Stramonium. This concept postulates that homologous chromosomes attract each other in synapsis through some affinity which exists between homologous regions, or more specifically, between homologous genes. If a portion of one chromosome exchanges with a portion of another non-homologous chromosome, then the pairing of homologous regions in synapsis will result in the formation of a circle of four. This is illustrated in Plate I, diagram 1 in which homologous end-segments are indicated by corresponding numbers. Further exchanges between chromosomes in the circle and paired chromosomes will increase the size of the ring.

The normal chromosome complement of Drosophila melanogaster (Plate I, diagram 2) consists of four pairs of chromosomes each having definite form, dimensions, and characteristics. The sex chromosomes are designated as X and Y, the female having two X's and the male having an X and a Y. The X chromosome is rod-shaped while the Y chromosome is slightly smaller with a bend at one end to give it the appearance of a hook. The remaining three pairs (autosomes) are designated by the numbers 2, 3, and 4. Chromosomes 2 and 3 are the ones dealt with in this paper. They are V-shaped in appearance with both arms approximately the same size.

Autosome 2, however, is slightly smaller than autosome 3. The fourth chromosome in Drosophila melanogaster is much smaller than the others of this species, and under the microscope gives the appearance of a small dot.

The translocations used in this investigation and designated as T 2-3 A and T 2-3 B were first described by Dobzhansky and Sturtevant (1931) in a somatic study of the chromosomes in Drosophila. Both A and B are somewhat different in nature and may be discussed separately.

In A, both chromosome 2 and 3 broke at their midpoints, that is, at the apices of the V's or the place of the spindle fiber attachment. The two left limbs then became attached to each other as did the two right limbs. If the normal chromosomes be designated as 2L 2R and 3L 3R (L-left limb, and R-right limb), then the new arrangement is 2L 3L and 2R 3R. Flies homozygous for the new arrangement do not survive as is the case for the majority of the translocations in Drosophila.

In translocation B, chromosome 3 broke at its midpoint, chromosome 2 somewhat to the left of its middle, and again the two left portions united as did the two right ones. Cytological examination reveals one unusually long chromosome and one short one, with

a disturbance of the somatic pairing. Both of these translocations may be better understood by referring to Plate I.

Except for a preliminary report of ring formation by Kaufmann (1935), maturation divisions in reciprocal translocation stocks of Drosophila melanogaster have not been systematically studied.

The cytological analysis undertaken in the present study was made on both the somatic and meiotic tissues of the vinegar fly.

CHAPTER II

MATERIALS AND METHODS

The Drosophila material represents the result of X-raying by means of which the reciprocal translocations were secured between the second and the third chromosomes.

Somatic studies were made from the ganglia taken from the larvae, while the meiotic tissues studied were obtained from the testes of both larvae and pupae.

The Drosophila cultures used were reared on an agar medium prepared as follows: Seventy-five cc's of water and thirteen and one-half cc's of molasses are mixed with ten grams of cornmeal and one and one-half grams of agar-agar. This material is heated and cooked till it reaches a firm consistency, and then it is poured into culture bottles and allowed to cool. A plug is taken out of the food in the bottle and a piece of filter paper introduced to absorb any moisture that may collect. The flies are introduced into the bottles after the agar has been richly coated with yeast. The bottles are closed with a plug of cotton wrapped in cheese cloth.

Drosophila eggs are white in color and about 0.6 mm. long. The ventral side of the egg is convex while

the dorsal side is somewhat flattened. There are usually two filaments attached to the dorsal side. The larva emerges from the anterior end of the egg, squeezing through an opening formed by a split in the chitinous shell. After 4 or 5 days of rapid growth, the larva attaches itself, usually to the filter paper, and enters the pupal stage. Pupation takes place within the larval skin. The skin, at first soft and white, hardens and turns brownish in the course of a few hours. After pupating 4 days the fly emerges with its wings bent and a few hours later becomes an adult.

This fly is particularly advantageous for genetical studies because of its relatively short life cycle, the whole life span requires only 9 or 10 days. Temperature, type of food, and the humidity, however, are all factors affecting the span of larval and pupal life.

The testes of larvae and pupae are separated from the rest of the abdominal organs in an isotonic salt solution. The process is one of teasing and separating the gonads from the foreign tissue. Fixation was accomplished by placing the testes in a solution of LaCours reagent. The material was embedded in paraffin in the usual manner and sectioned at 7 and 8 micra. A modification of the crystal-violet method (Carlson, 1936) was used in staining.

The ganglia likewise are dissected from the larvae in an isotonic salt solution, transferred to a small drop of aceto-carmine on a slide, and teased and flattened beneath a cover following the procedure of Kaufmann (1936). The cover-glass is then sealed with bees wax.

CHAPTER III

OBSERVATIONS

Somatic pairing in the ganglia proved to be more variable in expression than pairing in the testes. In a typical metaphase (Plate III, figure 2) the chromosomes may be seen to pair up in an atypical manner quite unlike that in normal somatic pairing. The chromosomes involved are only those that have undergone an exchange of segments. The sex chromosomes and chromosomes 4 play no part in the peculiar configuration. Their relations are normal as though the two V-shaped chromosomes had not undergone reciprocal translocation. In the figure mentioned and also in the other figures in the plate like segments can be seen to lie parallel to each other, indicating that somatic pairing, characteristic of the Diptera, is due to an attraction of like parts of the chromosomes rather than to the properties of the entire chromosomes, as thought at one time. The figures shown are of the same type as those seen in Oenothera.

In Plate III, figure 4, may be seen the ring formation in a slightly earlier metaphase so that the chromosomes are merely attached at their proximal ends while the chromosomes not involved in the translocation

are behaving in a normal manner. Chromosome 2 shows a very evident piece of 3 at its one end so that it is most easily recognized in this figure. The chromosomes, it is interesting to note, are definitely split.

Somatic pairing is variable, however, from cell to cell and these variations may be seen in the figures prepared. Figure 6 shows the chromosomes in a typical chain formation and here again we find that the chromosomes are split. A microphotograph of figure 2 is shown in Plate IV. The somatic pairing is quite different from that of the wild type fly and approximates more or less the cross-shaped arrangement shown in diagram 1 (Plate 1). This is what would be expected if like segments lie parallel to each other in somatic pairing.

Normal meiotic metaphase plates taken from Guyenot and Naville have been reproduced in Plate II. These show the typical alignment of the chromosomes in the testes and a normal movement toward the poles. They are in striking contrast to the figures shown in Plate 4 prepared from studies of the translocated chromosomes of first division metaphases. Here may be seen the atypical arrangement of the chromosomes during diakinesis in which is shown the peculiar "circle formation" previously described in Oenothera by Cleland.

First division metaphases were extremely difficult to find in the material available so that only a few of the best figures were prepared. Rings of four chromosomes are seen to appear in the first division metaphases of the testes. The figures prepared in Plate 4 throw some light on the problem and indicate the close similarity between somatic pairing and the meiotic pictures. No chain formations of the type described above for the somatic tissue were found in the present meiotic study. All the division figures seen were of the ring type (Plate IV, figures 10 and 11) with none of the end to end union as is most common in Oenothera.

In figures 12 and 13 may be seen a typical configuration which appears to be a double circle or figure eight, but apparently the four autosomes are twisted to give the appearance of two circles. In reality it is a single ring formation. The sex chromosomes and the fourth chromosomes are still closely approximated and behave in a normal manner. In figure 10 there appears to be a gray body near each pole. These are thought to be what Guyenot and Naville describe as a "sidereal mass of cytoplasm".

A somewhat later metaphase is shown in figure 11 and its similarity to the figures of the somatic tissue is striking.

CHAPTER IV

DISCUSSION

The cytological results and observations agree with those of Painter and Muller (1929) and Dobzhansky (1929 and 1930) indicating that somatic pairing is due to an attraction of like parts of the chromosomes. Somatic pairing is more variable in expression than meiotic pairing. In somatic mitosis, despite pairing, there is no segregation normally. Crossing over with somatic segregation was suggested by the chiasma-like configurations in Drosophila (Kaufmann, 1934) and affirmed more recently by the genetic studies of Stern (1936).

It has been assumed that corresponding parts of the homologous chromosomes have some property causing them to exert an attractive force on each other. Cytological examination of the phenomena reported in this paper further strengthens this hypothesis, for pairing of the corresponding segments of the chromosomes tended to persist in many cases even after one of the chromosomal parts had been translocated to another non-homologous chromosome. Such behaviour can only be explained by assuming attraction of homologous parts of chromosomes regardless of their relative positions in the chromosomal configurations.

The theory of this attractive force is well illustrated in metaphase plates of those testes where a mutual translocation involving an arm of the second and an arm of the third chromosome results in the radial arrangement of the large autosomes. Van Atta also demonstrates this in his work on induced eye colors in Drosophila. The fact that most of the individuals never survive is further evidence of the correctness of this hypothesis. Failure of alternate chromosomes to go to opposite poles will result in an incomplete complement of genes and the individual will fail to survive. This actually occurés in over 50 percent of the cases as would be expected if the segments had interchanged.

The results shown in this paper conform to those of Sturtevant and Dobzhansky (1930) who showed that homologous chromosomes do not lie parallel as they do in normal flies. This case parallels that in Oenothera and indicates the correctness of Belling's interpretation. The condition formerly designated as telosynapsis is a misnomer.

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Plate I

Diagram 1

a. Interchange between the non-homologous chromosomes.
b. Synapsis between interchanged and normal chromosomes.
c. Resultant circle formation.

Diagram 2

Normal chromosome complement in *D. melanogaster*.

Plate I

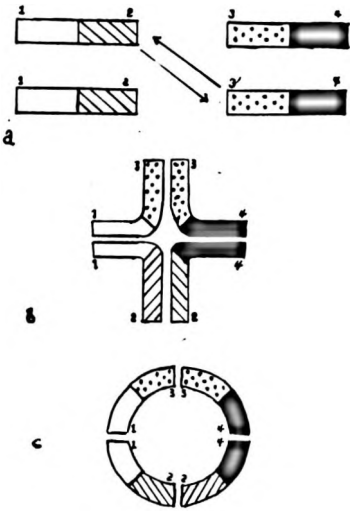
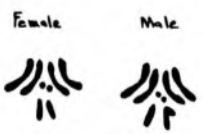


Diagram I



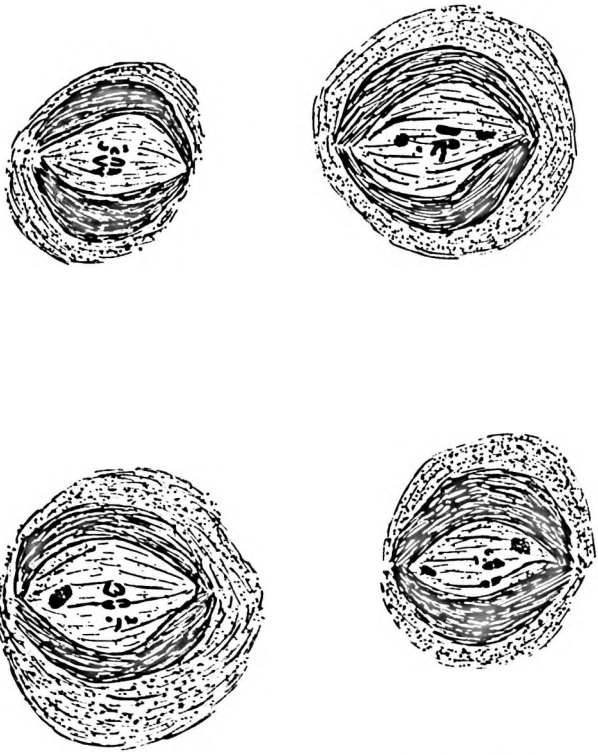
Lower pair in the female is
X chromosome — in the male is
X and y chromosomes — After Bridges

Diagram 2

Plate II

Normal metaphase plates in the first maturation divisions of D. melanogaster in spermatogenesis.

Plate II



Guyenot et Naville

Plate III

Explanation of figures

1. Typical A 2-3 translocation in female ganglion.

2. Metaphase. T 2-3 A in female ganglion.

3. Late prophase. Male ganglion, T 2-3 B.

4. Late prophase or early metaphase. T 2-3 B. All the chromosomes are split.

5. Somatic anaphase. T 2-3 B. The long translocated chromosomes are held together at their ends.

6. Apparent chain formation. T 2-3 B.

7. Metaphase of T 2-3 type A . 4th chromosome not seen.

8, 9. Both metaphase plates

Plate III

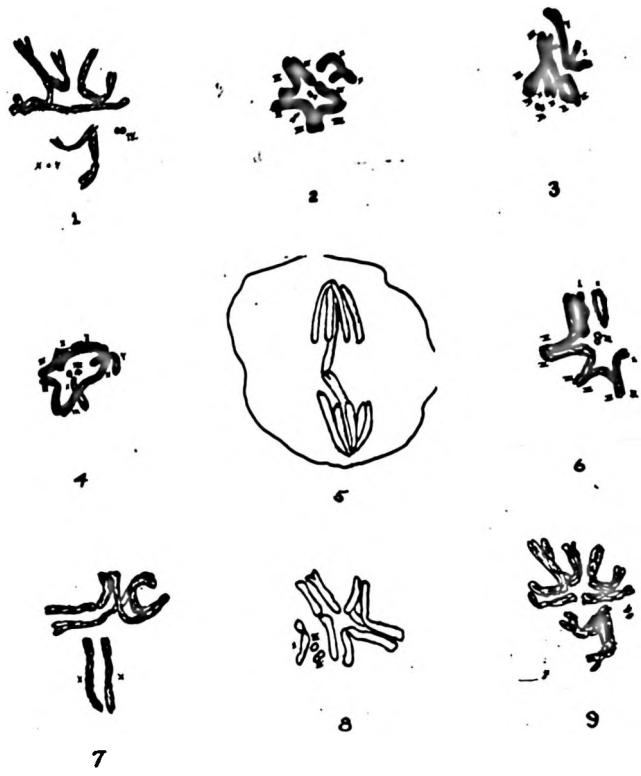


Plate IV

Explanation of Figures

10. Metaphases of the first division. Typical ring formation of chromosomes 2 and 3. Sex and 4th chromosomes seen above, " sidereal bodies " may be seen below.

11. Later metaphase.

12. Figure eight configuration.

13. Figure eight configuration.

Plate IV

10

11

12

13

Plate V