

*INTERCHANGE BETWEEN X- AND Y-CHROMOSOMES IN ATTACHED X FEMALES OF DROSOPHILA MELANOGASTER*

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*Introduction.*—Attached X-chromosomes ( $\widehat{XX}$ ) of *Drosophila melanogaster* separate occasionally (L. V. Morgan,<sup>1</sup> Anderson,<sup>2</sup> Muller and Dippel,<sup>3</sup> Dobzhansky,<sup>4</sup> Sturtevant<sup>5</sup>). The present cytological study was undertaken to compare the structure of detached X's with that of normal X-chromosomes.†

Seven separate detachments, occurring in cultures reared at about 25°C., were studied cytologically. Detachments *a*, *b*, *e*, *f* and *g* were detected as exceptional male progeny of  $\widehat{XXY}$  females. Such exceptional males, carrying the detached X's, were mated to attached X females. From the male larvae of these crosses, ganglion cells were selected for cytological study. The technique employed was a modified aceto-carmine method (Kaufmann<sup>6</sup>). Detachments *c* and *d* appeared in exceptional females, which were mated to wild-type flies. Ganglion cells of both male and female larvae from these crosses were investigated, since half of the members of each sex should carry the detached chromosomes.

*Structure of the Sex Chromosomes.*—The sex chromosomes of *D. melanogaster* during late prophases and metaphases of the somatic mitoses may be recognized by size and shape, and by the location of primary and secondary constrictions. A normal (unattached) X-chromosome (Fig. 9) shows an almost terminal primary constriction, or region of spindle fibre attachment. The most pronounced secondary constriction is slightly less than one-third of the distance along the chromosome from the proximal end. At this constriction in the X, one of the nucleoli develops (there are normally two per nucleus, although they may fuse to form one), so that during early prophases the X-chromosomes may be seen separated into two parts, usually by a distance equal to the diameter of the nucleolus (Kaufmann<sup>6</sup>). Another, less conspicuous secondary constriction exists about one-third of the distance from the distal end of the X. Knob-like portions often terminate the distal section.

The Y-chromosome at metaphase is a J-shaped body with two unequal arms separated by a primary constriction (Figs. 1, 4, 6, etc.). The longer arm shows a secondary constriction slightly distal to the middle. The shorter arm has a secondary constriction between a knob-like terminal region and a smaller, more or less spherical portion. A nucleolus develops

at this position in the Y and separates the short arm into two parts. This condition is clearly indicated during early prophases (Figs. 2, 5, 17, 18).

Attached X-chromosomes are held together at the primary constriction region (Fig. 21). During the prophases of the somatic mitoses the proximal attached ends are separated from the distal portions of the chromosomes by the nucleolus (Fig. 20).

All of the chromosomes are split longitudinally throughout the prophases and metaphases, although closely appressed or overlapping halves may obscure this detail.

*Structure of Detached X-Chromosomes.*—The seven detached X-chromosomes studied are not rod-shaped, but are either V-shaped or J-shaped. The V-type, represented by detachments *b, d, e, f*, is unequally armed, with one arm about three-fifths the length of the other (Figs. 10–18, chromosome  $\widehat{XY}^L$ ). The J-shaped X-chromosome, represented by detachments *a, c, g*, consists of a long arm, comparable in length to the long arm of the V-shaped detached X. The short arm of the J, however, is only about one-third of the length of the long arm (Figs. 1–9, chromosome  $\widehat{XY}^S$ ). V-shaped and J-shaped detachments seem to occur with about equal frequency, since four of the former and three of the latter type were encountered in the seven cases studied.

Comparison of detached X-chromosomes with the other chromosomes of the complement, and with unaltered X-chromosomes (Fig. 9), suggests that the longer arm of the V- or J-shaped structure is comparable in length to a normal X-chromosome. These detachments may be interpreted, therefore, as composed of a rod-shaped X-chromosome plus an extra arm. The nature of this extra arm was determined cytologically by a study of chromosome morphology in the giant cells of the ganglia and somatic pairing in the smaller ganglion cells. From this analysis it follows that the shorter arm of the V-shaped X corresponds to the long arm of the Y-chromosome, and that the shorter arm of the J-shaped chromosome corresponds to the short arm of the Y-chromosome.

The shorter arm of a V-shaped detached X often shows a nearly median secondary constriction. Such a constriction exists at a similar position in the long arm of the Y-chromosome. Further evidence that this type of detached X carries the long arm of a Y-chromosome is offered by somatic pairing in the smaller cells of the ganglia. In these cells side by side association of homologous parts is usually more complete than in the larger cells. In such metaphase plates as those shown in figures 11 and 12, the second, third and fourth chromosomes are paired. Pairing between X and Y presumably is between homologous regions, and exists between the long arm of the Y and the shorter arm of the V-shaped X.

Genetical evidence that the V-shaped X carries the long arm of a Y-

chromosome was secured by testing for fertility in males having this chromosome and the short arm of a Y-chromosome. Stern<sup>7,8</sup> has shown that the distal half of the long arm of the Y-chromosome has a fertility factor, or factor complex,  $K_1$ , and that the short arm of the Y possesses a supplementary factor, or factor complex,  $K_2$ . A male lacking either  $K_1$  or  $K_2$  is sterile.

The X-chromosome of detachment  $b$  of the present paper presumably carries the long arm of the Y-chromosome. Males with this chromosome and a normal Y were mated to  $\widehat{XXY}''$  females. (The  $Y''$  chromosome, discovered by Stern,<sup>7</sup> consists of the short arm and the proximal half of the long arm of the Y-chromosome.) Males from this cross have the detached X- and the  $Y''$ -chromosomes. Such males are fertile. Since the  $Y''$ -chromosome has only the  $K_2$  factor, the  $K_1$  factor was introduced with the detached X-chromosome. Therefore this chromosome carries the long arm of the Y, and is designated as the  $\widehat{XY}^L$  chromosome.

The J-shaped X-chromosome presumably represents a rod-shaped X plus the short arm of a Y-chromosome. This compound is designated therefore as the  $\widehat{XY}^S$ -chromosome. The short arm frequently shows a smaller proximal portion and a larger distal part. A similar structure exists in the short arm of the Y-chromosome.

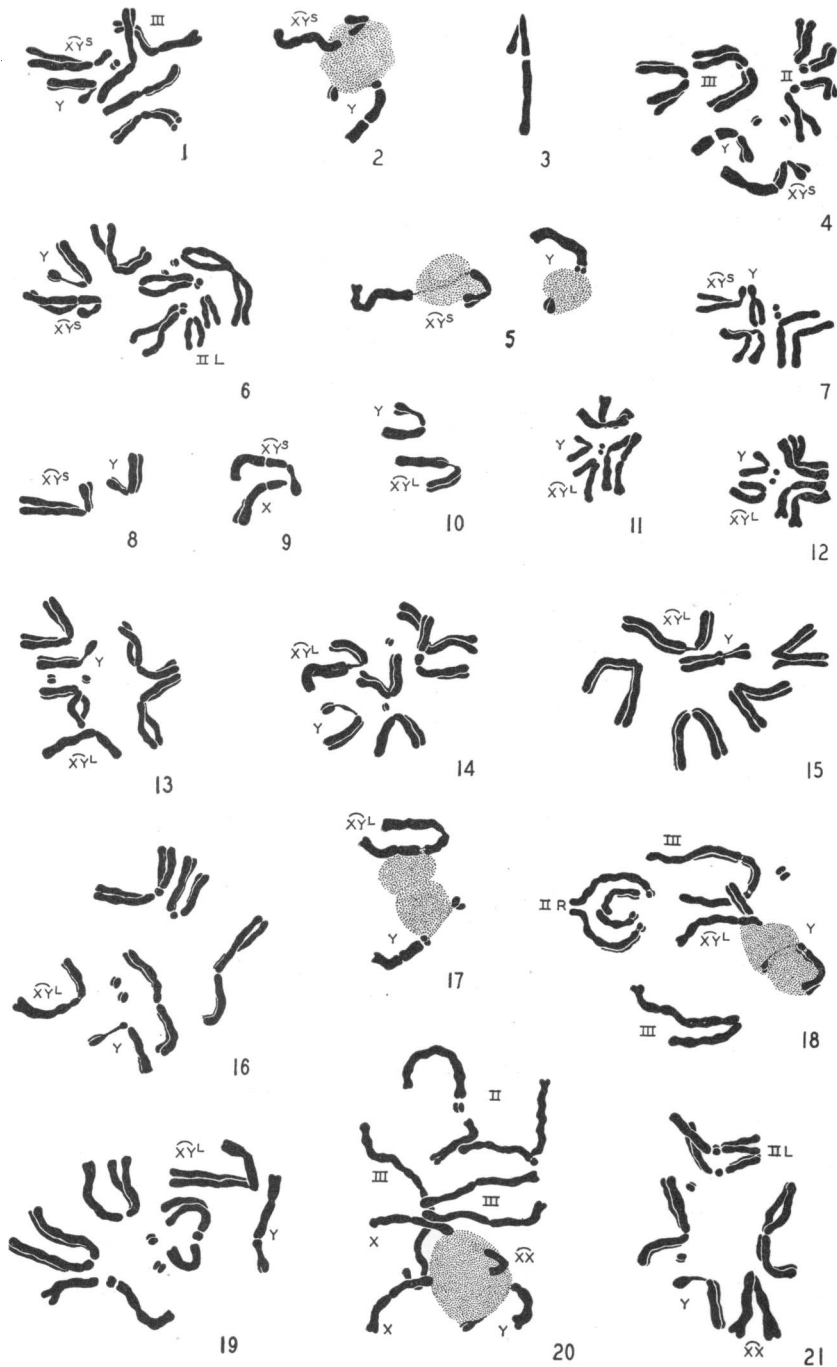
In ganglion cells of male larvae which have the  $\widehat{XY}^S$ -chromosome and a free Y, one of the nucleoli develops at the constriction in the short arm of the free Y. The other nucleolus apparently develops at the proximal secondary constriction in the long arm of the  $\widehat{XY}^S$ -chromosome (Figs. 2, 5). It could not be determined with certainty that the short arm of the  $\widehat{XY}^S$ -chromosome does not participate in this procedure, but in most prophase figures it is evident that there is no such wide separation as in the short arm of the Y. During late prophases and early metaphases, after the disappearance of the nucleoli as visibly staining bodies, the short arm of the Y may appear relatively long, because of the continued wide separation of the terminal satellite-like body from the proximal portion. This

#### DESCRIPTION OF FIGURES 1-21

Figures 1-5, detachment  $a$ ; 6-7, detachment  $g$ ; 8-9, detachment  $c$ ; 10-12, detachment  $f$ ; 13, detachment  $d$ ; 14-15, detachment  $e$ ; 16-19, detachment  $b$ . Figures 20, 21  $\widehat{XXY}''$ . Figures 9, 20, 21 from ganglia of females; all other figures from ganglia of male larvae.

Figures 2, 5, 17, 18, 20 prophases; figure 3, an  $\widehat{XY}^S$  anaphase chromosome; figure 15, inception of anaphase; all other figures from metaphase plates.

Y = Y-chromosome; X = X-chromosome;  $\widehat{XY}^S$  = detached X-chromosome bearing short arm of Y;  $\widehat{XY}^L$  = detached X with long arm of Y; II, III = second and third chromosomes; II L = left (al) limb of second chromosome. Nucleoli stippled. X ca. 2700.



FIGURES 1-21. (Description on opposite page.)

continued extension explains why some figures show the short arm of the Y considerably longer than the homologous short arm of the  $\widehat{XY}^S$ -chromosome (Fig. 6).

Pairing occurs between these homologous arms in the smaller ganglion cells (Fig. 7). Especially interesting in this figure is the Y-chromosome, which is "backed into" the plate, and does not occupy the normal position, with the primary constriction in a more central position.

A fertility test with the  $\widehat{XY}^S$ -chromosome was not possible, since no stock was available with a free long arm of the Y-chromosome.

*Exchange between X- and Y-Chromosomes.*—The foregoing data suggest that detached X-chromosomes of *D. melanogaster* possess either a long arm or a short arm of a Y-chromosome attached to an entire X-chromosome. Two possible methods of origin are suggested.

The first is related to the separation of parts of X and Y by the nucleoli (Fig. 20) and assumes a type of nucleolar development and intra-chromosomal separation in the auxocytes, or in gonial cells, similar to that found in somatic cells. If both  $\widehat{XX}$ - and Y-chromosomes develop a common nucleolus, then upon the disappearance of that body, the proximal and distal parts of one of the attached X's might join together, whereas the proximal part of the other X might not rejoin its distal part and therefore remain free, or it might join with the distal section of the short arm of the Y-chromosome. In either case a J-shaped chromosome would result. Likewise a V-shaped chromosome might arise from union of that part of the Y having the spindle attachment with the distal section of that X which did not rejoin its proximal portion. Such a V-shaped chromosome, however, should be smaller and more nearly equal armed than the type observed.

An alternative explanation, current in this laboratory, postulates an exchange between  $\widehat{XX}$  and Y during meiosis as the cause of detachment (Fig. 22). Either of two types of association of  $\widehat{XX}$  and Y might give, as a result of a single exchange, a V-shaped and a J-shaped chromatid. Those resulting from an exchange between the proximal portion of the X and the short arm of the Y (Fig. 22A) would resemble those resulting from an exchange between the proximal part of the X and the long arm of the Y (Fig. 22B). Although neither of the two types of association is excluded by the cytological findings, the position of *bobbed* in the proximal region of the X, and of its allelomorph in the long arm of the Y, suggests that these regions are homologous. From this evidence alone synapsis would be expected as shown in figure 22B.

The assumption that detached X-chromosomes arise as the result of an exchange is strengthened by a consideration of the effect of certain environmental factors on the rate of detachment. The frequency in x-rayed

flies is higher than in the controls (Muller and Dippel,<sup>3</sup> Bridges and Gairitschevsky<sup>9</sup>). The latter authors, and Dobzhansky<sup>4</sup> found an increase of the rate of detachment with higher temperature. Dobzhansky also found an increased rate at low temperature. X-rays and high and low temperatures are known to increase frequency of crossing-over. There is

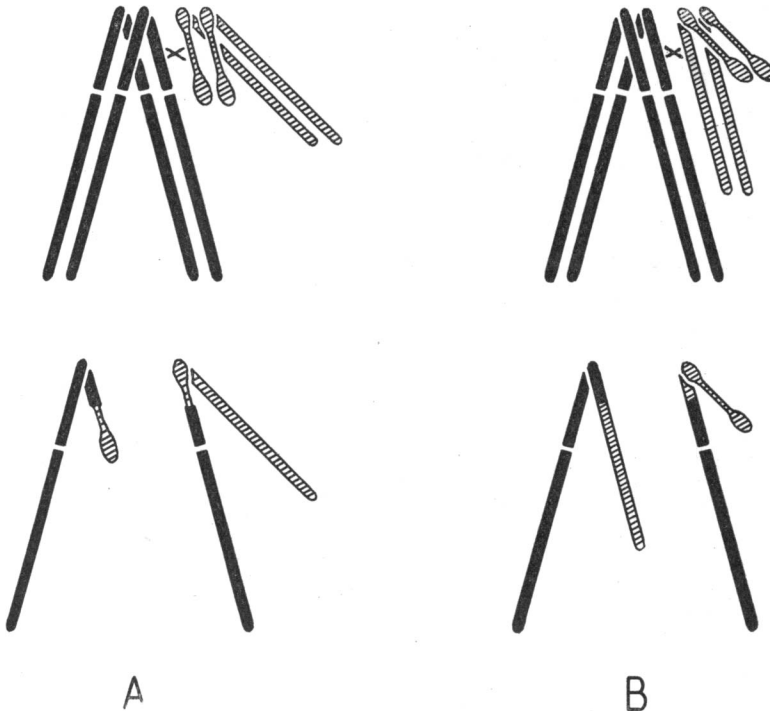


FIGURE 22

Diagram of two types of association of  $\widehat{XX}$  and Y which would give, as a result of a single exchange, chromatids similar to the types of detached X's shown in figures 1-19. A—above, association between X and short arm of Y; below, the two chromatids resulting from the exchange (unaltered chromatids not figured). B—above, association between X and long arm of Y; below, chromatids resulting from the exchange.  $\widehat{XX}$  in black; Y cross hatched.

of course the possibility that detachments resulting from x-rays are of a different type than those occurring in normal controls (Muller and Dippel).

The possibility of exchange between X and Y in the female was suggested by Stern<sup>10</sup> but was not verified. More recently Stern and Ogura<sup>11</sup> have demonstrated an exchange between  $\widehat{XY}'$ - and  $Y''$ -chromosomes of an  $\widehat{XY}'$ - $Y''$  male. This exchange occurred in the *bobbed* region of the two Y-chromosomes, and as a result a reconstructed Y-chromosome and a new combination ( $\widehat{XY}^3$ ) were secured.

The  $\widehat{XY}^L$  detachment of the present study is similar in structure to the  $\widehat{XY}'$ -chromosome discovered by Stern,<sup>8,12</sup> which arose during spermatogenesis of an XY individual, presumably through non-disjunction and attendant fusion of X and Y. This chromosome occurs with a frequency similar to that of the detached X's. Stern reported a frequency of the  $\widehat{XY}'$  of one in 1500-2000 times. Anderson<sup>2</sup> reported a frequency of detachment of attached X's of about one in 1300. Schultz (unpublished) found a frequency of detachment of about one in 1100, in flies mated to x-rayed males, and about one in 1500 in the controls.

From this comparison, the suggestion of the present paper that exchange may occur between the inert regions of X- and Y-chromosomes, and from the knowledge that crossing-over may occur in the male (Stern and Ogura), it seems probable that Stern's  $\widehat{XY}'$ -chromosome may have arisen by an exchange between X- and Y-chromosomes in the male. The simplest assumption involves a single exchange between the proximal region of the X and the short arm of the Y. From this exchange an  $\widehat{XY}'$  type of chromosome and a deficient chromosome, consisting of the spindle fibre region of the X, and a part of the short arm of the Y, could be obtained. Exchange between the long arm of the Y and the proximal region of the X could not be expected to furnish the  $\widehat{XY}'$ -chromosome, either on a basis of "homologous" pairing, or on the basis of inverted pairing with an inverted chiasma, suggested by Darlington<sup>13</sup> to explain this chromosomal aberration.

An inert region exists in the proximal portion of the X-chromosome (Painter,<sup>14</sup> Muller and Painter,<sup>15</sup> Dobzhansky,<sup>16</sup> Sivertzev-Dobzhansky and Dobzhansky<sup>17</sup>). The homology of the proximal portion of the long arm of the Y-chromosome with this region rests upon the location of the gene *bobbed* in the long arm. Probably the proximal regions of both long and short arms of the Y-chromosome represent "homologous" regions, either of which may pair with the inert region of the X-chromosome.

Since no exchange occurred between the *bobbed* region of the X and the "homologous" region of the long arm of the Y in Stern's test to detect crossing-over in that region in XXY females,<sup>10</sup> it seems probable that crossing-over between X and Y usually involves the short arm of the Y. This assumption is supported by a consideration of the probable origin of Stern's  $\widehat{XY}'$ -chromosome. It would also explain the origin of the types of detached X-chromosomes reported in the present paper.

Association of X- and Y-chromosomes in the first spermatocyte occurs over a short region only, in which the chromosomes assumedly are similar in construction (Metz<sup>18</sup>). Darlington<sup>19</sup> likewise found the sex-chromosomes of *D. pseudoöbscura* associated for a short region only. They were

held together by reciprocal chiasmata, either in the short arm or in the long arm of the Y. "In *D. melanogaster* the situation is probably comparable, except that the chiasmata can only be formed in the long arm." Whatever may be the situation in normal meiosis, the separation of attached X's and the compounding of X- and Y-chromosomes represent exceptional cases which may be explained by an exchange involving a single chiasma.

Detachments sometimes occur in groups of two or more from a single female. Sterling Emerson (unpublished) found one culture with 9 detachments. The grouping of detachments led Muller and Dippel<sup>3</sup> and Sturtevant<sup>5</sup> to suggest that detachment might occur in an oögonial division. This seems unlikely in light of the findings of the present paper, but cytological examination of a series of detachments of a single female should decide whether some other explanation than an exchange between  $\widehat{XX}$  and Y is necessary.

*Summary.*—Detached X-chromosomes from  $\widehat{XXY}$  females of *D. melanogaster* were observed to be of two types, either V-shaped or J-shaped. Both types seem to occur with about equal frequency. The former type is interpreted as a compound consisting of a rod-shaped X-chromosome and the long arm of the Y chromosome (the  $\widehat{XY}^L$ -chromosome). The J-shaped chromosome is regarded as a compound consisting of the X and the short arm of the Y (the  $\widehat{XY}^S$ -chromosome, a type of compounding of X and Y previously undescribed). The suggestion is offered that detachment occurs as a result of exchange between X- and Y-chromosomes. It could not be determined by cytological study of the detachments whether the exchange occurs between the long arm of the Y and the proximal region of the X, or between the short arm of the Y and the proximal region of the X. Other factors suggest that the short arm of the Y is usually involved.

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<sup>4</sup> Dobzhansky, Th., *Proc. Nat. Acad. Sci.*, **14**, 671-675 (1928).

<sup>5</sup> Sturtevant, A. H., *Carnegie Inst. Wash. Publ.*, **421**, 61-81 (1931).

<sup>6</sup> Kaufmann, B. P. (in press).

<sup>7</sup> Stern, C., *Naturwissenschaften*, **15**, 465-473 (1927).

<sup>8</sup> Stern, C., *Z. I. A. V.*, **51**, 253-353 (1929).

<sup>9</sup> Bridges, C. B., and Gabritschevsky, E., *Ibid.*, **46**, 231-247 (1928).

<sup>10</sup> Stern, C., *Ibid.*, **44**, 187-231 (1927).

<sup>11</sup> Stern, C., and Ogura, S., *Ibid.*, **58**, 81-121 (1931).



- <sup>12</sup> Stern, C., *Biol. Zbl.*, **46**, 505-508 (1926).  
<sup>13</sup> Darlington, C. D., *Jour. Genetics*, **24**, 65-96 (1931).  
<sup>14</sup> Painter, T. S., *Science*, **73**, 647-648 (1931).  
<sup>15</sup> Muller, H. J., and Painter, T. S., *Z. I. A. V.*, **62**, 316-365 (1932).  
<sup>16</sup> Dobzhansky, Th., *Biol. Zbl.*, **52**, 493-509 (1932).  
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*POLLEN TUBE ESTABLISHMENT AND THE DEFICIENCY OF  
WAXY SEEDS IN CERTAIN MAIZE CROSSES<sup>1</sup>*

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Investigations with the waxy gene of maize, described by Collins,<sup>2</sup> have been in agreement in that heterozygous plants (*Wx wx*) produced on an average a minus deviation from the expected 25 per cent of waxy seeds. Backcrosses of the type *wx wx* ♀ × *Wx wx* ♂ also produced a significant deficiency of the recessive type, while from the reciprocal combination the expected 50 per cent of waxy seeds were obtained.

Brink and McGillivray,<sup>3</sup> Demerec<sup>4</sup> and Brink<sup>5</sup> have shown that heterozygous plants produce *Wx* and *wx* gametes in approximate equality in both micro- and megasporogenesis. Brink<sup>6</sup> has shown further that the deficiency of waxy seeds is more marked on plants of the constitution *su su Wx wx* than on *Su Su Wx wx* or *Su su Wx wx* plants. The additional deficiency associated with the homozygous *su su* plants was interpreted as a cytoplasmic effect.

Of the many explanations advanced for these observed deficiencies of waxy seeds, available genetic evidence has permitted of the discredence of all hypotheses except differential growth rates or differential development of gametophytes carrying the *Wx* and the *wx* genes. Since no consistent differences have been observed in the percentages of waxy and non-waxy seeds on the upper and lower portions of the segregating ears, there is no indication of a constant difference in growth rate. Brink and Burnham<sup>7</sup> recognize two stages in the development of the pollen tube. In the first stage the tube is dependent upon its own food reserves, while in the second stage it is dependent upon the food material in the style. They suggest that the differential effect of the waxy gene is exerted during the first stage. Brink<sup>5</sup> also suggests that a chemical basis for differential development of waxy and non-waxy gametophytes may exist only during this stage.