SOME OBSERVATIONS ON CHROMOSOME PAIRING IN THE SALIVARY GLAND NUCLEI OF DROSOPHILA MELANOGASTER¹

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THE findings reported here are concerned with (1) the validity of observations on chromosome pairing made from slides prepared by the squash technique; (2) a relationship between pairing of a specific chromosome region and freedom of chromosome ends; and (3) an effect of the Y chromosome on chromosome pairing.

Does the squash technique affect the apparent degree of pairing of salivary gland chromosomes?

Observations on pairing of salivary gland chromosomes are open to question on the grounds that paired chromosomes may be separated in the process of squashing that is used routinely in the preparation of these chromosomes for microscopic observation. Some evidence is presented here that chromosome regions paired before squashing are not affected by this technique, but remain paired.

The material from which this evidence was obtained was a stock designated $N^{264-100}r7$, which was derived by irradiation from the stock $N^{264-100}$ (see BRIDGES and BREHME 1944). In $N^{264-100}$, a piece of the X chromosome, from 3C1 to 4B3-4 inclusive, is inserted in inverted order in the proximal heterochromatin of 3L in section 80 (see Figure 1a, and BRIDGES' salivary chromosome maps 1935, 1938). The stock used in these experiments had structurally normal X chromosomes, carrying the recessive w allele at the w locus (white eye, locus 1.5) and was hyperploid for the translocated 3C1-4B3-4 region for the X, which included the w^+ allele. Males and females heterozygous for the third chromosome carrying the 3C1-4B3-4 duplication are viable and fertile and have mottled eyes in which very little pigment is present.

In the original condition, males carrying the duplication were inviable (BRIDGES and BREHME 1944). It is assumed that selection has occurred within this stock for modifiers which increase their viability. The viability and fertility of flies (male or female) homozygous for the duplication is very low.

Males of the constitution $\gamma w/Y; Dp(1;3) N^{264-100}/+$ were irradiated with 5000r and mated to $\gamma w/\gamma w$ females (γ =yellow body color), and from the progeny, any flies whose eyes appeared darker than those of the $N^{264-100}$ stock

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FIGURE 1.—Altered third chromosomes in stock $N^{264-100}$ and its derivatives, $r1$, $r2$, $r3$, $r7$, and $r11$. Euchromatic regions are represented in outline, heterochromatic regions black, centromeres by circles and the duplication from the X chromosome, 3C1 to 4B3.4, by the rectangle marked x.

were selected and mated with γw flies to establish a stock. One such female proved, on cytological analysis of the stock, to have carried a pericentric inversion in the third chromosome in which the duplicated piece of X was present. The new breaks were immediately to the left of the duplication and close to the tip of 3R, between 99B3 and 99C1 (Figure 1e). The stock established from this female was $N^{264-100}r7$. The new chromosome could be studied only in heterozygous females, as males carrying the rearrangement are inviable.

The following observations were made on salivary gland chromosomes of $N^{264-100}r7$ females heterozygous for this third chromosome inversion and a normal third chromosome: (1) The tip of 3R distal to the duplication is nearly always (in 99 percent of 200 nuclei scored) paired with the homologous region in the normal third chromosome. (2) The piece of X translocated to the third chromosome is frequently (in 64.5 percent of 200 nuclei) paired with its homologues in the normal X chromosomes. (3) In nuclei in which both of these regions of the rearranged third chromosome (*viz.*—the tip of 3R and the adjacent piece of X) are paired, the paired regions may be several micra apart, connected by a thin strand of stretched unpaired chromosome.

Figure 2a shows the tip of 3R paired, the adjacent X duplication also paired and lying very close by, and the proximal heterochromatic regions of the third chromosome, including the centromere, which are unpaired. Figure 2b also shows the tip of 3R and the duplication paired, but in this nucleus they are about ten micra apart and the connecting chromosome region is stretched so that it has

FIGURE 2.—Microphotographs of salivary chromosomes in the stock *r7*. A: a relatively unstretched preparation; B: stretching of the region where the tip of 3R and the duplication from the X chromosome (Dp) adjoin one another, both being paired with their homologues. $\times 2700$.

only about one quarter of its normal width. In this nucleus the region including the centromere has broken away from the duplication, and is not visible in the photograph.

It is clear that although both the stretching and the breakage that have occurred in this second nucleus may be attributable to the squash technique, the pairing of the 3R tip and the X duplication has not been affected. It seems that regions which have been effectively paired remain paired in the course of cytological preparation, even though there is evidence of forces which would tend to separate them. This is not to say that chromosome regions which had been lying alongside one another, without being intimately paired, might not be separated by squashing. We should perhaps distinguish between intimate and effective pairing, or synapsis, and loose pairing or contiguity.

A relationship between free chromosome ends and pairing

It was noted in the previous section that pairing of the X duplication with the homologous region of the normal X occurred in 64.5 percent of the salivary gland nuclei in females of the $N^{264-100}r7$ stock. This frequency of pairing was markedly higher than that observed in the original stock carrying the duplication, $N^{264-100}$, where it was paired in only four percent of 50 nuclei scored. It was also higher than in four other stocks derived from $N^{264-100}$ by the same method as was $N^{264-100}r7$. In each of these stocks an inversion was present in the third chromosome, with one of breaks adjacent to the the duplication. The position of the duplication in the rearranged third chromosome of each of these stocks is shown in Figure 1 (b, c, d, f). The proportion of nuclei in which the duplication was found to be paired with the normal X chromosomes was as follows: in $r1$, two percent; in $r2$, zero percent; in $r3$, zero percent; in $r11$, four percent. Fifty nuclei were scored from each stock.

In seeking an explanation of the high frequency of duplication pairing in the $r7$ stock, it seemed possible that this was due to close proximity of the duplication to the free end of 3R. Association of non-homologous ends (in this case of the tips of X and 3R) as described by HINTON and ATWOOD (1941) might facilitate pairing of regions close to these ends. If this were so, the incorporation into the $r7$ stock of closed X chromosomes in substitution for the structurally normal X chromosomes would eliminate the possibility of association between X and 3R tips, and might be expected to reduce the frequency of pairing of the duplication.

Females from the $r7$ stock, of the constitution $\gamma w/\gamma w; In-Dp/+$ were mated with X^{C1}/sc^sY males. F_1 females which were $X^{C1}/\gamma w$ and might or might not carry the $r7$ duplication chromosome, were in turn mated individually with X^{C1}/sc^sY males. The larvae were raised at 18°C and slides were made from the salivary glands of $X^{C1}/\gamma w$ and X^{C1}/X^{C1} females. Cultures in which the $r7$ third chromosome was not detected cytologically were discarded, and counts of duplication pairing were made in slides from $X^{C1}/\gamma w; In-Dp/+$ and $X^{C1}/X^{C1}; In-Dp/+$ females. The two genotypes were distinguished prior to the counts by the frequency of pairing of the X chromosomes, which was much higher in the homozy-

gotes for the closed X. The results, together with those previously obtained from $\gamma w/\gamma w$; *In*Dp/+ females are given in Table 1, columns 1, 4, and 5. It will be seen that pairing is progressively reduced in the $X^{C1}/\gamma w$ and X^{C1}/X^{C1} individuals as compared with the $\gamma w/\gamma w$. In the $X^{C1}/\gamma w$ females the duplication is frequently paired with only one of the X chromosomes. This is the γw rod X whose tip is free from the chromocenter, while that of the ring X is in the chromocenter. In X^{C1}/X^{C1} females, the duplication is occasionally paired with only one X chromosome, which is not paired with its homologue in this region, but this is a much less frequent occurrence than in the $X^{C1}/\gamma w$ heterozygote.

TABLE 1

Pairing of an X duplication in the third chromosome of N²⁶⁴⁻¹⁰⁰r7 with the homologous region of a normal X chromosome (γw) and a ring X (X^{C1}), and an X chromosome with long inversion X^{C2} .

X chromosome constitution	$\gamma w/\gamma w$	$\gamma w/X^{C2}$	X^{C2}/X^{C2}	$\gamma w/X^{C1}$	X^{C1}/X^{C1}
Dp paired with both X's	36	5	3	14	5
Dp paired with one X	..	10	1	7	1
Dp unpaired	14	35	46	29	44
Total	50	50	50	50	50

A similar experiment was made with an X^{C2} stock, but it was found later that this ring X had opened up and now contained a long inversion. The results, shown in Table 1, columns 2 and 3, are much the same as those for the X^{C1} stock (which was checked cytologically for presence of a closed X in ganglion cells).

It can be concluded that the freedom of the X tip is an important factor in the frequency of duplication pairing manifested in the *r7* stock, for pairing is reduced both with X^{C1} , where the tip of X is not free, and with the opened and inverted X^{C2} , where the region homologous to the duplication is close to the centromere instead of close to the free tip. This conclusion supports, but does not prove, the hypothesis that the high frequency of pairing follows from non-homologous association between the X and 3R tips.

Effect of an extra Y chromosome on pairing of salivary gland chromosomes

In the course of experiments in which some larvae had an extra Y chromosome, it was noticed that the salivary gland chromosomes seemed to be unpaired more frequently in XXY females than in their XX sisters. The cross from which these larvae were obtained was between $w^{258-18}/\gamma Hw$ *In* dl-49 m^2g^4/sc^8 Y females and γw males. w^{258-18} denotes a reciprocal translocation between the X and fourth chromosomes with the break in X to the right of the *w* locus, between 3C4 and 3C5.6 (DEMEREK and SLIZYNSKA 1937). The *w* locus, reattached close to the centromere of chromosome 4, is responsible for mottling of the eyes and malpighian tubules in females heterozygous for w^{258-18} and *w*. The recessive γ allele of the yellow locus is also present in the translocated X. The sc^8 Y chromosome

carries the wild type (γ^+) allele at the γ locus and its presence can be detected in $w^{258-18}/\gamma w$ larvae by the color of the mouth parts—phenotypically yellow larvae are $w^{258-18}/\gamma w$ and phenotypically wild type larvae are $w^{258-18}/\gamma w/sc^8 Y$.

The experiment was set up for a comparison of pairing in the XX and XXY females, of the tip of X, in which the w locus is situated. It may be stated that this part of the X was unpaired in more than 90 percent of the nuclei in both types of females, and there was no significant difference between the two groups.

Pairing or nonpairing of heterochromatic regions was impossible to score, as they are usually associated in the chromocenter in any case. The further results relate, therefore, to the euchromatic regions of the major autosomes, excluding chromosome 4, and the proximal part of the X chromosome from 3C5.6 to the heterochromatic region (19–20). The counts are given in Table 2 (columns 1 & 2), which shows some increase in the frequency of unpaired regions in each

TABLE 2

Effect of Y chromosomes on the frequency of nuclei with unpaired regions in the euchromatic parts of 2L, 2R, 3L, 3R and the proximal part of X (XP) from 3C5.6 to 19

	XX larvae	XXY larvae	XXYY larvae
Total nuclei scored	200	200	50
All regions paired in	143 nuclei	102 nuclei	21 nuclei
Part of XP unpaired in	11 nuclei	16 nuclei	5 nuclei
Part of 2L unpaired in	5 nuclei	13 nuclei	3 nuclei
Part of 2R unpaired in	20 nuclei	28 nuclei	7 nuclei
Part of 3L unpaired in	10 nuclei	25 nuclei	8 nuclei
Part of 3R unpaired in	11 nuclei	16 nuclei	6 nuclei

XX larvae, $w^{258-18}/\gamma w$; XXY larvae, $w^{258-18}/\gamma w/sc^8 Y$; XXYY larvae, $w^{258-18}/\gamma w/sc^8 Y/bw^+$.

chromosome arm, (namely, the proximal part of X, 2L, 2R, 3L, and 3R) in the presence of the $sc^8 Y$ chromosome. The increase is in fact somewhat higher than is shown in the table, as the original intention was to score nuclei in which all regions were paired versus those in which one or more unpaired regions occurred. As soon as an unpaired region was identified in one chromosome arm, it was therefore entered as such in the record and other unpaired regions which might be present were ignored. The proportion of nuclei in which all regions were completely paired was 71.5 percent in XX larvae and 51.0 percent in XXY larvae. The limits of confidence for the difference of 20 percent between these values lie between 11 percent and 30 percent at a 95 percent probability level.

A further experiment was made in which a second Y chromosome was introduced into $w^{258-18}/\gamma w$ females along with the $sc^8 Y$. The second Y was the Y: bw^+ , identifiable cytologically by the inserted piece of 2R which forms a loop at the chromocenter (GERSH 1956). The results are shown in column 3 of Table 2. Failure of pairing in the euchromatic regions was somewhat more frequent than in the presence of the $sc^8 Y$ alone.

Figure 3 shows the unpaired regions in the 50 nuclei of $w^{258-18}/\gamma w/sc^8 Y$

Y:*bw*⁺ larvae that were scored (right) and in 50 nuclei scored at random in slides from *w*²⁵⁸⁻¹⁸/*y w* larvae (left). Two blacked-in areas on the same horizontal line indicate that there were two unpaired regions in the same chromosome within a single nucleus. There were also nuclei in which parts of two different chromosome arms were unpaired. These cannot be identified in the diagram. There were two of them in the 50 nuclei from XX larvae, and seven in the 50 nuclei from XXYY larvae. The latter account for the discrepancy in numbers between Table 2, column 3 and Figure 3(b) (see above).

The table and diagram indicate that all chromosome arms are affected, though one cannot, with the small numbers available, state confidently that they are equally affected. On the whole, the increase of unpaired regions in the presence of two extra Y chromosomes seems to be predominantly in the middle of a chromosome arm (see especially 2R at region 52 to 53 and 3L at region 68 in Figure 3), although the unpaired regions do often extend all the way down to the proximal heterochromatin of the chromocenter.

Data are being obtained on the pairing of heterochromatic regions which are not invariably associated in the chromocenter. Such information can be obtained from rearrangements with breaks in or close to, the proximal heterochromatin.

It is of interest to recall that SCHULTZ (1944) found that in *Drosophila* males the presence of one or two Y chromosomes progressively affected the shape of chromosome ends. The predominant "fan" shape in XO males was largely replaced in XY males by "straight" ends and bulbous nipple-like ends, while in XYY males the latter predominated. One might hazard a guess that this is also an effect of the Y on pairing, the "nipples" representing closely paired ends and the "fans" a lesser degree of pairing or even a failure of pairing at the extreme tip. If this were so, the effect of the Y on the ends would be opposite to that demonstrated here for the mid regions of the chromosome arms.

It is tempting to extrapolate to the pairing of chromosomes at meiosis and to speculate that such changes in pairing might be responsible at least in part for the altered crossover values found, for instance in inversion heterozygotes and in triploids. There is some evidence (REDFIELD 1953, for inversions; BEADLE 1934, for triploids) that the Y chromosome may enhance these changes in crossing over. Such a hypothesis would, however, require that the Y chromosome should *increase* pairing near the centromeres as well as at the chromosome ends; and that heterologous inversions and triploids should result in changes in pairing corresponding to the changes in crossover values which have been found. Evidence for these requirements is completely lacking as yet, apart from the data of SCHULTZ and HUNGERFORD (1953) which indicate that in the presence of heterologous inversions, pairing of the X chromosomes in the salivary glands is increased at both ends, but not in the middle of the chromosome. This result is consistent with the hypothesis outlined above, but it depends on the assumption that one can extrapolate from salivary chromosome pairing to meiotic chromosome pairing.

FIGURE 3.—Diagrams showing all unpaired euchromatic regions in the proximal part of X (Xp), 2L, 2R, 3L, and 3R in 50 nuclei of XX larvae (left) and XXYY larvae (right). The numbers in horizontal rows represent the numbered sections of the chromosomes as given in BRIDGES' maps (heterochromatic regions are omitted). The numbers to the right denote the number of nuclei in which unpaired regions of a given chromosome were found.

SUMMARY

It has been shown (1) that a synapsed region of the salivary gland chromosome is not likely to undergo separation in the course of the squash technique; (2) that pairing of a part of the X chromosome transposed to the third, and situated near the tip of 3R, with the homologous region in the normal X chromosomes, is dependent on the freedom of the distal ends of the X chromosomes; (3) that an extra Y chromosome increases the frequency of unpaired regions in euchromatic parts of chromosome X, 2 and 3.

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