THE NATURE OF CLOSED *X*-CHROMOSOMES IN *DROSOPHILA MELANOGASTER*

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(With Four Text-figures)

The closed *X*-chromosomes in *Drosophila melanogaster* belong to two categories. One group, fairly well understood, consists of those cases of ring chromosomes which arise as the result of crossing-over between two attached *X*-chromosomes, one of which carries a segment inverted with respect to the corresponding segment of the other (Beadle & Sturtevant, 1935; Sturtevant & Beadle, 1936; Sidorov *et al.* 1935, 1936). The other and first studied group contains only two examples, both of which occurred as single individuals in the progeny of attached-*X* females, raised under normal culture conditions (Morgan, 1926, 1933; Beadle, 1934, Table I, p. 279; Boche, unpublished data). The origin of these two closed *X*-chromosomes as inversion cross-overs is unlikely; for the closed *X*'s which originate in such a manner occur in considerable numbers in the progeny of a given female. Moreover, in one of the two cases of the second group, the mother of the individual carrying the closed *X* was homozygous for Gowen's third chromosome suppressor of crossing-over, which makes cross-overs in euchromatic regions very rare. Her *X*-chromosomes were also marked by recessive genes throughout their length. The original closed *X*-chromosome carried all the mutant genes in one *X*-chromosome of its mother and none of those in the other of the two attached *X*'s. This makes it very unlikely that it originated as a cross-over, especially since in addition tests of similar attached-*X* females, heterozygous for the cross-over suppressor, showed no evidence of the presence of an inversion (Beadle, 1934).

Another mode of origin, considered by Morgan (1933), assumes a translocation between the two arms of the attached *X*'s, similar to that producing the ring chromosomes described by McClintock (1932; cf. also Rhoades & McClintock, 1935) in maize. The results of a translocation between the two arms of a V-shaped chromosome should be an acentric rod fragment, and a closed chromosome. The latter would be deficient for that part of the *X* between the chromosome end and the point of trans-

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location. It would carry in duplicate the portion between the spindle attachment and the translocation. These are, it is evident, exactly the same results as are given by crossing-over between attached X's carrying an inversion in one arm (Sturtevant & Beadle, 1936; Sidorov *et al.* 1935, 1936). The existence of such duplications or deficiencies in the two cases of the second group has, however, not previously been demonstrated. The study of their salivary gland chromosomes, which we report here, has accomplished this demonstration. From the consideration of their mode of origin, it follows that the two closed X-chromosomes of spontaneous origin arose by translocation between the two arms of the attached X-chromosomes present in their mothers.

X^{c1}

The closed X-chromosome of L. V. Morgan, designated as X^{c1}, appears in salivary gland nuclei as a loop[1] both of whose ends meet at the chromocentre in cells undamaged by smearing. This has somewhat complicated the study; but a comparison of females heterozygous for X^{c1} and males carrying it, with and without a Y-chromosome, has given mutually confirmatory results.

At what would be the left end of a normal rod X-chromosome, all the bands in section $1A$ are lacking (Figs. 1 and 2). This includes the section identified by Demerec & Hoover (1936) as one in whose absence viable and almost normally fertile individuals occur. These authors raise the question of the locus of a lethal ($l/1$) stated by Muller (1933) to be located to the left of scute. They consider its locus to be to the left of band $1B1$. On the basis of the X^{c1} data, the lethal (unless it results from a position effect of some kind) must be to the right of $1B1$; for males carrying the closed X survive, although they are less viable than the wild type (L. V. Morgan, 1933).

The existence of a duplication at the proximal end is strikingly manifested in the almost invariable attachment of the nucleolus to the yellow-bearing end of the X. This attachment persists even when the connexion with the chromocentre is disrupted in smearing (Fig. 2). The yellow end of the salivary chromosome, which is in contact with the nucleolus, is frequently broadened out fan-wise. Consequently, the dark bands of section $1B$ are broken up into fine dots, so that the bands lose their distinctness. The chromatic inclusions frequent in nucleoli are in these preparations unusually distinct, and display what might be inter-

[1] It may be remarked that we have seen no difference between the nature of the spirals in these closed X-chromosomes, and those of the rod X's.

preted as a banded appearance. The nucleolar region of the mitotic X-chromosome may be identified as lying in section 19–20, with which the nucleolus is associated in various chromosome rearrangements (Schultz, unpublished data). Thus the proximal break in this case lies within the nucleolar region; actually it is to the right of 20A, the duplication being, we believe, for 20C and D approximately.

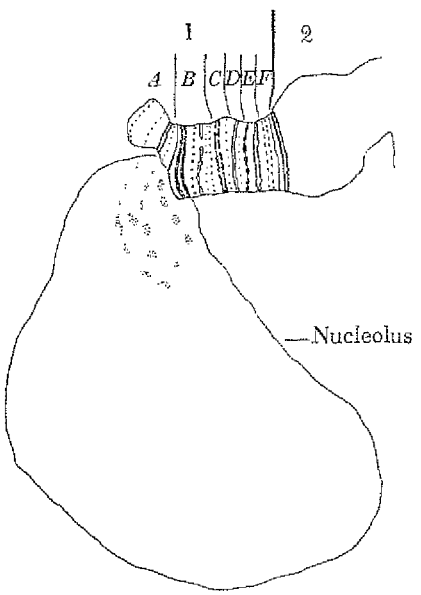

Fig. 1.

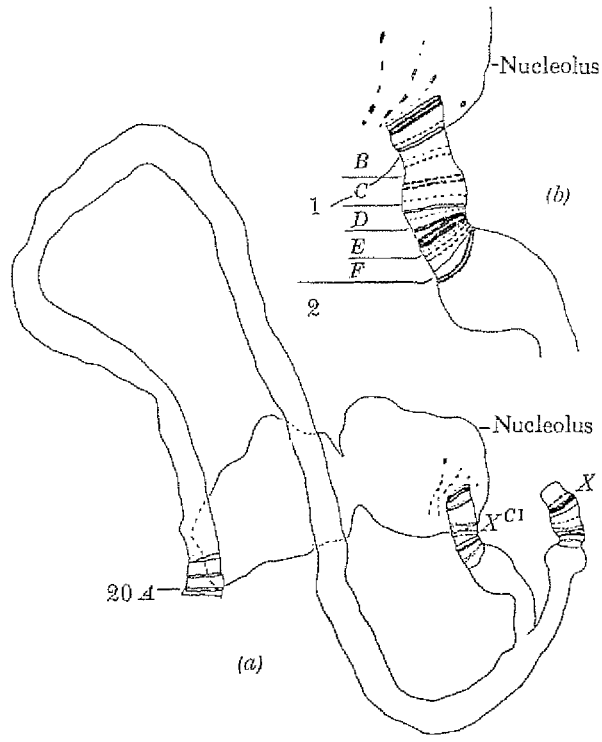

Fig. 2.

Fig. 1. Yellow end of the salivary gland chromosome of X^{c1}/X, showing that X^{c1} is deficient for region 1A and that the yellow end now has an intimate relation to the nucleolus. ×2000.

Fig. 2. (*a*) Salivary gland chromosomes of X^{c1}/X, showing the relation of the yellow end of X^{c1} to the nucleolus. ×1000. (*b*) The yellow end of X^{c1} from the same nucleus showing the 1A deficiency. ×2000.

X^{c2}

The second closed X (designated as X^{c2}) is considerably more viable than X^{c1}; correspondingly, the deficiency at the left end is less extensive. The precise extent of the deficiency is hard to establish, since the missing bands are faint and the broken end is in contact with heterochromatin.

Figs. 3 and 4 show cases in which 1*A*1–1*A*3 inclusive are absent from the X^{c2} chromosome. There appears to be some variation in the extent of the deficiency, which, if established, could be ascribed to cytological variegation.

The proximal break in X^{c2} is farther to the left than in X^{c1}, being at 20*A*1. We have made this observation, not only in heterozygotes for the ring and rod chromosomes, but also in the homozygous X^{c2} females. Fig. 4 shows a heterozygous X^{c2} female, which carries the proximal portion

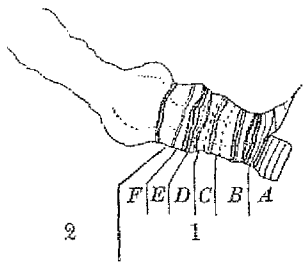

Fig. 3. Yellow end of salivary gland chromosome of X^{c2}/X, showing probable deficiency from X^{c2} of region 1*A*1–3. ×2000.

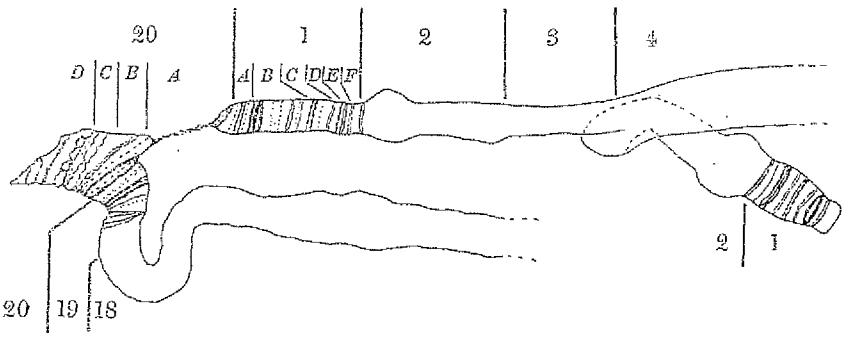

Fig. 4. Salivary gland chromosomes of X^{c2}/X, showing duplication and possible deficiency structure of bobbed and yellow ends of the X^{c2} chromosome. Region 20*A*–*D*, which is duplicate in X^{c2}, is triploid. The triplet in 20*A* that belongs to the duplicated region is here closely approximated to 1*A* at the yellow end. Section 1*A* of X^{c2} appears to be shorter than the corresponding region of the rod *X*, in spite of the greater stretching of the region in X^{c2}. ×2000.

of the *X* up to 20*A* in triplicate. The triploid character of section 20 may be recognized by its greater width when compared with the adjacent section 19. The triplet (20*A*1–3), belonging to the duplicated portion of X^{c2}, has been disrupted from the rest of region 20 in the process of smearing, but has retained a close connexion with section 1*A*. More frequently, disruption in smearing occurs between regions 20*A* and 1*A*. We have also been able to observe some nuclei in which the duplicated

region was not paired with the regular paired bases of the X-chromosomes; and a very few others in which all three heterochromatic regions were unpaired. There is in X^{c2} no association of the tip of the X with the nucleolus such as is characteristic in X^{c1}.

DISCUSSION

From the foregoing, it is clear that the nature of the closed X-chromosomes corresponds, as closely as could be expected, to the requirements imposed by the hypothesis of their origin as translocations between the tip of one of the attached X's and the base of the other. The existence of a break in the heterochromatic region of the X, to which its tip has now been joined, might lead one to expect variegation in the genes closest to the heterochromatin (Schultz, 1934, 1936; Noujdin, 1936). We do not consider it demonstrated in the case of X^{c2}, although the apparent variability of the bands in section 1*A* is suggestive. In the case of X^{c1}, however, there is an effect of the Y-chromosome on the viability of the male, wholly comparable to that found in other cases of variegation correlated with breakage in heterochromatic regions. The X^{c1} male without a Y-chromosome is completely inviable; death of such males occurs in the early pupal stages. Moreover, as L. V. Morgan has noted, the X^{c1} male, particularly when raised at low temperatures, shows the occasional absence of bristles characteristic for variegation at the scute locus. Thus in this respect also, the hypothesis of origin by translocation is in accord with the facts.

Neuhaus (1936) has obtained data which give an estimate of how frequent such an occurrence—translocation in specified regions of the two X's—may be. He found no "detachments" of attached X's in 29,460 flies from mothers which carried no Y-chromosome, thus excluding the "detachments" resulting from crossing-over or translocations with the Y.

It will be recalled that Muller & Dippel (1926) obtained what they then interpreted as detachments of attached X-chromosomes from females irradiated with X-rays. They pointed out that these "breakages" occurred at a different stage of oögenesis from that in which the effects of X-rays on ordinary crossing-over were produced. Whether these "breakages" were ring chromosomes, or translocations of other types (for example, with the Y-chromosome) is undetermined; repetition of the experiments is evidently desirable. It would seem, then, that the "detachments" of attached X-chromosomes can all be interpreted as resulting from the usual types of chromosome aberration, viz. (1) exceptional pairing and crossing-over with the Y, and (2) translocation.

SUMMARY

The two closed X-chromosomes of *Drosophila melanogaster*, that originated as single individuals from mothers carrying attached X-chromosomes, are shown to be duplicated at their bobbed, deficient at their yellow ends. This is in agreement with their origin as the result of translocations between the attached X's carried by their mothers.

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