TERMINAL DEFICIENCIES IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

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IN THE course of cytogenetic studies on the X chromosome in *Drosophila melanogaster* conducted in this laboratory by DR. M. DEMEREC, several terminal deficiencies have been found.

Three of these have already been described by DEMEREC and HOOVER (1936) and it is the purpose of this paper to describe others and to put forward evidence that they are really terminal deficiencies in which a broken chromosome has failed to rejoin either in the old or in a new combination, so that a new chromosome end is formed at the breakage point.

The standard nomenclature for genes and chromosomal changes in Drosophila (as in Drosophila Information Service No. 10) will be used throughout as follows: genes in the X chromosome with positions on genetic map—*ac*, achaete (0.0+); *bb*^{l}, bobbed lethal (66.0); *car*, carnation (62.5); *f*, forked (56.7); *Hw*, hairy wing (0.0+) *l J1*, lethal J1 (−0.0); *N*, Notch (3.0); *pr*, prune (0.8); *sc*, scute (0.0+); *spl*, split (2.9±); *w*, white (1.5); *y*, yellow (0.0).
genes in other chromosomes—
2nd chromosome: *bw*, brown; 3rd chromosome: *e*, ebony; 4th chromosome: *ey*, eyeless; *M-4*, Minute-4; *sv*^{n}, shaven-naked.
chromosomal changes—
Df, deficiency; Dp, duplication; T, translocation

METHODS

Slides of the salivary gland chromosomes of female larvae heterozygous for the different changes were prepared by the acetocarmine technique.

The equipment used in analysing the slides consisted of a 90 × 1.3 N.A. apochromatic objective, an oil-immersed 1·4 N.A. condenser, 12.5 × compensating oculars, and a Bausch and Lomb research lamp with the green Wratton filter number 61.

Cytological analyses were based throughout on BRIDGES' (1935 and 1938) maps of the salivary chromosomes.

ANALYSIS OF THE CHANGES

1 Df(1) 260.10.

This deficiency came from a mating of *y sc w* females with wild Swedish-b males which had been treated with X-rays. It was identified in an F_1 female which was phenotypically yellow, indicating that the *y* locus of the paternal

X chromosome was changed or deficient. The *ac* locus was found to be affected also.

Females carrying the mutant X chromosome (*y ac*) over normal *y sc w* were found to be heterozygous for a deficiency involving bands 1A1 and 2 of the salivary chromosomes. The appearance of the figures suggests that the whole tip to the left of 1A3 has been lost.

Males with the deficient X are viable and fertile, and must therefore carry the normal allele of the *lJ1* locus, which, according to MULLER, lies to the left of the *y* locus. Similarly, Df(1) 260.5 (DEMEREC and HOOVER 1936) has four bands missing, but is perfectly viable and fertile.

Data obtained in this laboratory show that the genes *y* and *ac* must both be located in the region 1A 5–8 and that their change was in this case due to "position effect." The work of MULLER (1935), and cytological results of MULLER, PROKOFYEVA and RAFFEL (1935) do not exclude the possibility that *lJ1* also is located in this region. This would allow deficiencies such as these to lack the extreme tip of the X chromosome and yet to carry the normal allele of the *lJ1* locus.

2. Df(1) 260.19.

This deficiency was picked up from cytological observations on a stock of STERN ($\widehat{yy}/g^2$ *B*) and must have occurred spontaneously. It resembles 260.10, cytologically, the break being in the same position between 1A2 and 3. It differs genetically as the loci of *y* and *ac* are unaffected. The males are viable and fertile.

3. Df(1) 260.25.

Genetical data. The stock carrying this deficiency was derived from an F_1 female carrying a normal *y sc w* chromosome and an X chromosome from an irradiated Swedish-b male. This female was phenotypically scute, and when mated with *y sc w* males she gave the following offspring:

	Females	Males
sc	11	—
y sc w	15	13
y sc	13	—
sc w	6	8

This progeny shows three peculiar features: (1) There is apparently an abnormally high crossover value for the *y-sc* and/or *sc-w* regions, giving relatively large numbers of *y sc* and *sc w* flies. (2) The *y sc* type is inviable in the hemizygous condition (no *y sc* males). (3) The mutant *sc* type is not recovered in the hemizygous condition (no *sc* males).

These results could be accounted for on the supposition that the tip of X including the y^+ gene had been translocated to some other chromosome region where it segregated with comparative freedom from *sc* and *w*, while

the break at the tip of X had caused a mutation in the *sc* locus accompanied by a recessive lethal change. The *sc* and *y sc w* classes would then represent the parental types (*sc* failing to appear among the males on account of the lethal). The *y sc* flies would be heterozygous for a deficiency including the *y* locus, this deficiency probably being lethal in males, which would in any case fail to survive on account of the lethal *sc* mutation. The *sc w* class would carry two normal *y sc w* chromosomes together with a duplication for the tip region including the y^+ gene.

On closer examination the *sc* and *sc w* flies showed a variegated body and bristle color, predominantly wild-type with patches of yellow, suggesting that the y^+ gene was inserted in some chromocentral region. Mosaicism of wild-type and mutant tissue is characteristic of such changes (SCHULTZ 1936).

A test mating with *y ac* showed that variegation of the achaete character also occurred. The break in the X was to the right of *y* and *ac*, therefore, but probably to the left of *sc*, because it showed no variegation.

In order to locate the translocated segment carrying y^+ and ac^+, a cross was made between females of a stock, (*yvf; bw; e; ey*) and *sc w* males assumed to carry a normal *y sc w* chromosome and a duplication for y^+ ac^+. The F_1 consisted of 309 *y v f* females and two wild-type females with 369 *sc w* males and one *y v f* male. The F_1 females receive both their X chromosomes (attached) from their mother, while the males receive their X chromosome from the father; the other chromosomes are distributed at random. Since all females (with two exceptions) were yellow and all males (with one exception) were wild-type with respect to the yellow locus, the duplication clearly segregates with the X chromosome. The exceptions could be accounted for by the rare formation of detached X's by crossing over in the female parent, whereby one son received a *y v f* chromosome from the mother, and two daughters received only one *y v f* chromosome from their mother, the second X chromosome with the y^+ duplication being derived from the father. These data showed that the tip region of the X was transposed to the chromocenter of the same chromosome.

A linkage test was made with genes at the proximal end of the X chromosome to determine the position of the duplication in respect to these genes. The back cross *y* Dp/*y f car* $bb^1 \times y$ *f car* bb^1 gave the results shown in table 1. In this experiment the flies were raised at 25°C in order to get crossover values comparable with the standard values.

From this mating all classes of females homozygous for bb^1 are lethal and thus lacking. Crossing over between *f*, *car*, and Dp is therefore shown adequately in the males only. The crossover value between carnation and the duplication calculated from the males is 3.41 ± 0.40 percent, so that it is not detectably different from the crossover value for *car*-bb^1 (3.5 percent).

Crossing over between the duplication and *bb*^{l} could be detected only in the females. Since double crossovers between *car* and *bb*^{l} would not be expected, the single yellow among these shows that the duplication is genetically to the right of, and very close to, *bb*^{l}, giving less than 0.1 percent of crossing over.

A cross was made between females carrying the deficient X chromosome and a normal X marked by the dominant gene *Hw* and males of the *lJ1 sc J1* stock in which the lethal change is covered by a deleted X chromosome. From this cross (Df/*Hw*×*lJ1 sc J1*−del 24), half of the F_1 females should have the deficient X chromosome and a *lJ1 sc J1* chromosome,

TABLE 1

Offspring of backcross y Dp/y f car bb^{l}×*y f car bb*^{l}.

FEMALES			MALES		
non-crossovers	Dp	1184	non-crossovers	Dp	1002
	y f car bb^{l}	—		*y f car*	815
region 1	*f* Dp	36	region 1	*f* Dp	69
(*f-car*)	*y car bb*^{l}	—	(*f-car*)	*y car*	43
region 2	*f car* Dp	35	region 2	*f car* Dp	33
(*car-bb*^{l})	*y bb*^{l}	—	(*car*-Dp)	*y*	32
region 3	*y*	1	regions 1, 2	*y f*	—
(*bb*^{l}-Dp)	*f car bb*^{l} Dp	—		*car* Dp	3
Total		1256	Total		1997

and only half of these should carry the covering deletion. If the deficiency includes the normal allele of *lJ1*, the *sc* flies which lack the deletion will be inviable, and the ratio of *Hw* to *sc* in the F_1 females will exceed 1:1. If the normal allele of *lJ1* is still present in the deficient chromosome, all *sc* females should be viable whether they carry the deletion or not, and the ratio of *Hw* to *sc* should be 1:1.

A very great excess of *Hw* females was actually found, the total numbers being 171 *Hw*:17 *sc*. It is evident that there is some factor disturbing the ratios other than the failure of the *sc* females which lack the deletion, and the test is therefore rather unsatisfactory. It is not apparent, however, that the normal allele of the *lJ1* locus is still present in the deficient chromosome.

On the genetical evidence, the deficiency may include all genes to the left of *sc*, and may therefore be a deficiency for the entire tip of the X chromosome.

Cytological data. Female larvae heterozygous for the *sc* change were obtained from the mating *sc/y sc w*×*y sc w*. Their salivary gland chromosomes showed an abnormality at the tip of X, where all bands to the left

of 1B 3.4 were missing in one of the strands. A further peculiarity was the presence of a nucleolus-like structure at the deficient tip, which looked as if material had oozed out from the end of the chromosome (Plate 1, fig. C). This phenomenon is common as a casual occurrence in figures where a partial or complete break has occurred (not induced by irradiation) in one of the "weak spots" of the chromosomes (for instance, in region 11A); but in this case it appeared consistently in every figure. This is regarded as evidence that the newly-constituted chromosome tip consists of the naked broken end, without any other material attached to it.

It proved impossible to locate the translocated segment 1A1 – 1B1.2 at the X chromocenter, so it is not known whether it is inserted in an intercalary position or attached terminally to the short arm.

It was considered desirable to test the capacity of the new end for rejoining with other broken ends, and for this purpose viable males carrying the tip deficiency were obtained by mating the *y sc* females (heterozygous for the deficiency) with Dp(1) 118, thus introducing a fragment, made up of the extreme distal and proximal regions of the X, which covered the lethal effect of the scute change and of the deficiency.

Males carrying the 260.25 deficiency and Dp 118 were treated with X-rays at a dosage of 4000r, and mated to wild Canton-S females. Slides were made from F_1 female larvae, which had both a normal X and the irradiated deficient X.

In 102 pairs of glands, 27 aberrations were obtained with a total of 63 breaks, but in no case had the broken tip of the deficient X become attached to one of the newly broken ends. It is apparent, therefore, that healing has taken place, so that the tip does not tend to rejoin as is usual within a short time after breakage has occurred.

4. T(1; 4) 258.53.

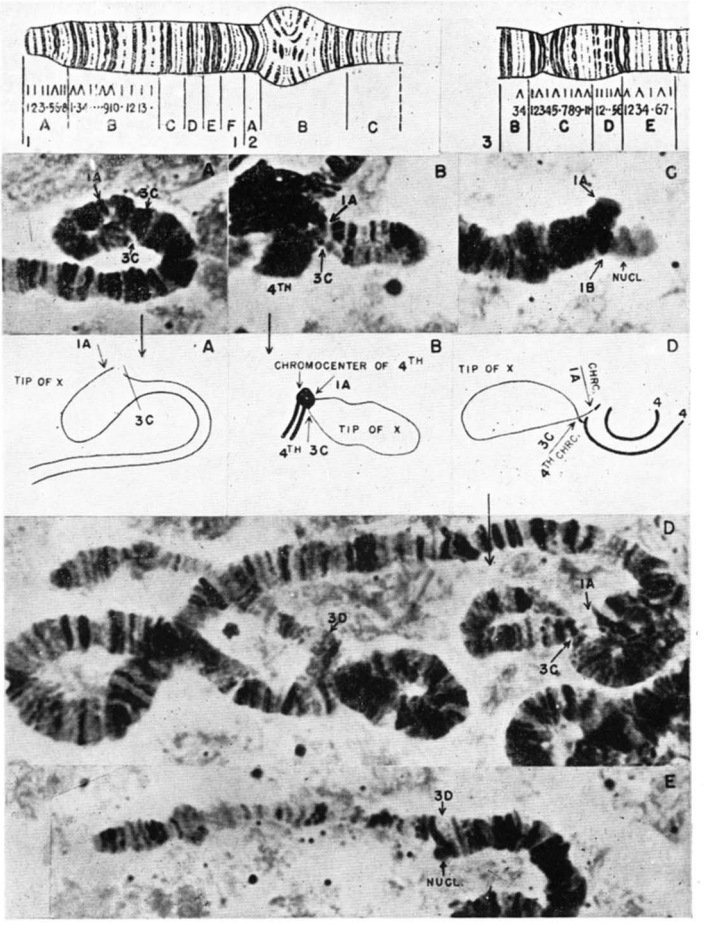
The existence of eye color variegation in an F_1 female from the mating of *y sc w* by X-rayed Swedish-b male indicated a translocation of the white locus (band 3C1 in the salivaries) to the chromocenter.

Observation of the salivary chromosomes showed that the tip of the X

LEGEND FOR PLATE 1

Top: Diagrams of regions of X chromosome from BRIDGES' salivary chromosome map.

Below: A. T(1; 4) 258.53, haploid tip of normal X bent back so that distal region (1A) pairs with distal end (3C) of deficient X (see diagram). B. T(1; 4) 258.53, tip of X attached to fourth chromocenter, distal end (1A) also pairing with chromocenter (see diagram). C. Df(1) 260.25 lower strand, deficient for 8 distal bands, shows nucleolus-like structure extending beyond normal tip of upper strand. D. T(1; 4) 264.113 at left, haploid tip of normal X and deficient strand ending at 3D; at right, tip of X attached to 4th chromocenter, distal end also bent back to pair with chromocenter (see diagram). E. T(1; 4) 264.113, haploid tip of normal X and, above, deficient strand ending in a small nucleolus-like structure.


A
B
C
D
E
F
1
2
3
1A
3C
4TH
1B
NUCL
TIP OF X
CHROMOCENTER OF 4TH
3D

up to and including 3C1 was attached to the chromocenter, apparently at the base of the fourth chromosome, but there seemed to be two normal fourth chromosomes, and there was no foreign material attached to X distal to 3C2.3 (Plate 1, figs. A, B).

The salivary configurations suggested two possibilities: (1) that the tip of X was attached to the short arm of the fourth, in which case it was possible that a piece of this arm, so small as to be practically invisible, had been translocated to the distal end of the X; or (2) that the tip of the X had been attached to the long arm of the fourth, near the proximal end, in which case the broken distal end of 4R must have been lost, the broken tip of the X remained unattached at 3C2.3, and non-disjunction of the fourth chromosome must have occurred subsequently to restore the second normal fourth chromosome.

A cross with the fourth chromosome recessive *sv*^{n} decided between these alternatives. The F_1 from the mating Df+w^{mot}−4/*y Hw w*; +^{sv}/+^{sv}×*w*; *sv* gave the following classes:

w^{mot}+	27
w^{mot} *sv*	13
w+	32

The presence of *sv* flies in the F_1 in the class which also carried the X-4 chromosome responsible for white mottling, can be explained only by the second alternative. The break in the fourth chromosome must have been proximal to the *sv* locus, so that the X-4 chromosome lacks the wild-type allele of this locus. The three fourth chromosome centromeres apparently segregate more or less at random, and some of the gametes carry no normal fourth, but only the X-4 chromosome, deficient for the *sv* locus. These gametes are responsible for the w^{mot} *sv* flies appearing in F_1 from the mating with the *sv* stock. These flies must be haploid for all of the fourth chromosome except the proximal region.

The subsequent discovery of Minute flies in the T(1; 4) 258.53 stock supported this explanation. These Minutes must be due to the same independent segregation of the X-4 chromosome from the two normal fourths, and they are comparable with BRIDGES' M-4, which is haploid for a part of the fourth chromosome owing to a deficiency (BRIDGES 1935).

In view of this evidence, the end of the abnormal X, broken to the left of 3C2.3, can be considered as having reconstituted itself as a normal end without the attachment of any previously existing end structure.

5. T(1; 4) 264.113.

This aberration was obtained in a Notch F_1 female from a mating of *y pn* females with irradiated Swedish-b males. It closely resembled the previous one, 258.53 (Plate 1, fig. D) but differed cytologically in two respects:

(1) the break in X occurred between 3C9·10 and 3D1; (2) the unattached broken end, terminating to the left of 3D1, frequently showed a nucleolar structure, similar to that found in Df(1) 260.25, except in that it was much smaller. This exudation sometimes obscured the distal bands of 3D (Plate 1, fig. E).

The cytological difference in the position of the break corresponded with a genetical difference. This stock showed a Notch mutation and mottling of *w* and *spl*, all of these loci being transferred to the fourth chromocenter, whereas in 258.53 only the *w* locus was affected.

DISCUSSION

The special behavior of chromosome ends, namely the fact that they do not as a rule become attached permanently to one another or to newly broken ends, but persist as ends, has led to a supposition that they must have a special structure which conditions their behavior. The name of telomere has been applied to this terminal structure, and it has been supposed that reproduction of the telomere maintains the identity of the chromosome end.

The literature provides occasional examples of behavior which is inconsistent with this view of the chromosome ends. Thus, although STADLER (1932) remarked on the absence of terminal inversions in maize, such an inversion has subsequently been found in *Drosophila ananassae* (KAUFMANN 1936). A further example of anomalous behavior of chromosome ends has been found by MCCLINTOCK (1939), who described chromosomes derived from the breaking of dicentric anaphase bridges during meiosis in maize. These chromosomes, though not deficient, have broken ends. In the endosperm the broken ends of half chromatids undergo a repeated cycle of fusion, dicentric bridge formation, and subsequent rupture. In the sporophytic tissue, however, no anaphase bridges are found. It is apparent that one of the chromosome set in this tissue was derived from breakage of a dicentric bridge, because of the absence of a characteristic knob; and it is therefore evident that the broken end has healed so as to form a normal, permanent chromosome end.

MULLER (1938) has questioned the existence of genuine terminal deficiencies in Drosophila, on the grounds that chromosomes with such deficiencies would lack a stabilizing telomere structure.

The simplest explanation of the chromosome ends described in this paper is, however, that they originated by loss of the whole distal portion of the X chromosome, and that the broken ends healed and became functionally normal. There is no evidence to contradict this explanation. On this view, stable ends can be formed de novo from broken ends without attachment of a previously existing telomere. There seems no reason to doubt this

interpretation of these and other apparent terminal deficiencies in Drosophila, as well as the results of McCLINTOCK in maize.

SUMMARY

Five apparent terminal deficiencies in the chromosomes of *D. melanogaster* are described. From genetical and cytological data it is concluded that these are genuine terminal deficiencies, in which the normal chromosome end has been lost and a new end has been formed at the breakage point. This conclusion is incompatible with the assumption that existing chromosome ends, or "telomeres," are essentially permanent and indispensable structures.

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