FINE-STRUCTURE ANALYSIS AND GENETIC ORGANIZATION AT THE BASE OF THE *X* CHROMOSOME IN *DROSOPHILA MELANOGASTER*[1]

ELIEZER LIFSCHYTZ[2]

Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Manuscript received May 20, 1976
Revised copy received November 18, 1977

ABSTRACT

Genetic organization at the base of the *X* chromosome was studied through the analysis of X-ray-induced deficiencies. Deficiencies were recovered so as to have a preselected right end "anchored" in the centric heterochromatin to the right of the *su(f)* locus. "Free" ends of deficiencies occurred at any of 22 intervals in Section 20 and in the proximal portion of Section 19 of Bridges' (1938) polytene chromosome map. The distribution of 130 such free ends of deficiencies induced in normal, *In(1)sc*^{8}, and *In(1)w*^{m4} chromosomes suggests that on the single section level, genes are flanked by "hot" or "cold" sites for X-ray-induced breaks, and that occurrence of the hot spots is dependent on their interaction with the fixed-end sites in the centric heterochromatin. In the light of these results, it is argued that long heterochromatic sequences separate the relatively few genes in Section 20, and thus endow it with several characteristics typical of heterochromatic regions. Section 20 is considered to be a transition region between the mostly heterochromatic and mostly euchromatic regions of the *X* chromosome; the differences between them are suggested as being merely quantitative.

FINE-structure analysis of the chromosomal level was used to define a long array of linearly arranged complementation units at the base of the *X* chromosome for mutagenic analysis of X-ray and EMS-induced lethal mutations (LIFSCHYTZ and FALK 1968, 1969) and in the characterization, by means of recombination, of two short subsegments on both sides of the boundary between Bridges' (1938) polytene chromosome Sections 19 and 20, (LIFSCHYTZ 1971, 1975). In this article, analysis of the distribution of X-ray-induced deficiency breaks at the base of the *X* chromosome will be presented in order to reveal the arrangement of heterochromatic and euchromatic sequencies in a short chromosome segment.

Section 20, the base of the Bridges' *X*-chromosome map, has been the subject of several studies concerned with the genetic organization of chromosomes. KAUFMANN (1946) and COOPER (1959) assigned to this section a heterochromatic nature, primarily because of its conspicuous high breakability. Cooper called

[1] This work was supported by a grant from the Israel Commission for Basic Research.
[2] Present address: MRC Laboratory for Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

upon cytological evidence regarding the location of aberration breakpoints in mitotic and polytenic chromosomes to establish this point. Recently, SCHALET and LEFEVRE (1973) reopened the question of the heterochromatic nature of Section 20. On the basis of their cytogenetic study of lethal complementation units and deletions that map to Section 20 (20A to 20D-E), they argue that this Section should no longer be considered heterochromatic, and the polytene chromosome heterochromatic-euchromatic junction, if any, is located at or proximal to 20D-E. However, I have suggested (LIFSCHYTZ 1971) a working hypothesis according to which no heterochromatic-euchromatic junction at all exists, and that the base of the *X* is composed of heterochromatic segments of different lengths alternating with euchromatic ones. This hypothesis was based on the detection of a "hot spot" for X-ray-induced breaks in the middle of a region that encompasses the 30 most proximal genes in the *X* chromosome. This "hot spot" was surmised to be associated with a comparatively long, intercalary heterochromatic segment that, through ectopic pairing, forms a "site" for X-ray-induced chromosome breaks and reunions (LIFSCHYTZ and FALK 1968).

If this hypothesis is valid, one should be able to show that (1) more than one "hot spot" is intercalated in Sections 20 and 19, and (2) the preferential breakpoint is indeed dependent on a heterochromatin-heterochromatin interaction. Implied in this hypothesis is that in addition to "hot-spots," "dead" spots occur that are more or less refractory to X-ray breakage. Experiments will be described that fulfil these prerequisites.

Deficiencies at the base of the *X* chromosome were selected so as to have their right ends fixed in the proximal heterochromatin to the right of *su(f)* (suppressor of forked). The distal breakpoints were allowed to occur in any of 22 contiguous intervals as defined by lethal gene mutations to the left of *su(f)*. The distribution of the distal "free" ends was studied in a normal-sequence *X* chromosome and in two different inverted chromosomes (see Figure 1).

In(1)sc^{8} and In(1)w^{m4}, the inverted chromosomes, differ from the normal with respect to the position of the proximal heterochromatin in which the preselected (fixed) end break may occur. *In(1)w^{m4}* also differs in respect to the amount of heterochromatin available for the induction of the fixed-end breaks (Figure 2).

MATERIALS AND METHODS

The following Drosophila strains were used:

(a) Marked chromosomes: *y*; *y^{2} f su(f)*; *y^{2}sc;w^{a} f su(f)*.

(b) Inverted chromosomes and balancers: *In(1)sc^{8}*, *sc^{8}*; *In(1)w^{m4}*, *y w^{m4}*; *FM6*; *In(1)dl-49*.

(c) *Y*-chromosome derivatives: *Ymal+*; *y+mal^{126}*; *B^{S}Y*; *w+Y*.

(d) *In(1)sc^{4L}sc^{8R}*, *dl49*, *y w^{a} B*. This *bb^{-}* inverted chromosome was used to classify *su(f)–bb^{-}* deficiencies.

For detailed descriptions of the mutants and chromosomes, see LINDSLEY and GRELL (1968).

Recovery of Df(1) su(f) *mutations*: Although *su(f)* males and females are phenotypically normal, it was found (LINDSLEY and GRELL 1968) that the *Df(1)su(f)/su(f)* genotype is characterized by such visible phenes as narrow and rough eyes, spread wings and confluens-like wing veins, fine bristles and late emergence, as well as low viability. Thus, *su(f)* deficiencies could be easily and directly recovered among the F_1 progeny of X-irradiated males. Accordingly,

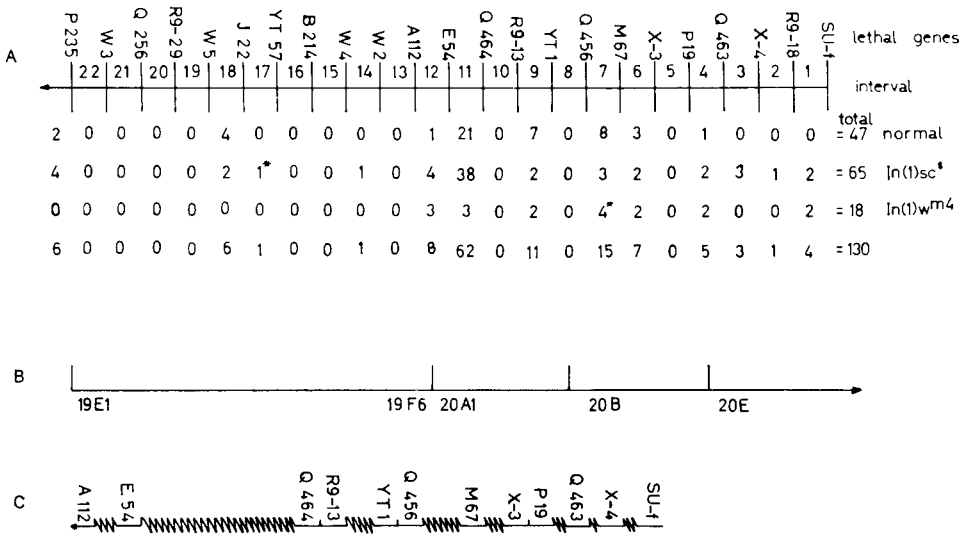

FIGURE 1.—The distribution of 130 "free ends" of *su(f)* deficiencies. Sections 19E-F and 20.

(a) Intergenic intervals defined by lethal gene mutations. *Su(f)* marks the most proximal interval. Order of genes that is not evident from overlapping deficiencies (*e.g.*, X-3, P19) conforms to that of SCHALET and LEFEVRE (1973).

(b) Parallel arrangement of polytene chromosome sections after SCHALET and LEFEVRE.

(c) Schematic visualization of "heterochromatic" and "euchromatic" sequences in Section 20. Significance of comparisons.

(1) The differences between the number of breaks in Section 20 *vs.* 19 in the three chromosomes, excluding interval 11 breaks.

For the normal chromosome $p < 0.01$ ($\chi_1^2 = 7.54$) for *In(1)sc⁸* $p < 0.05$ ($\chi_1^2 = 4.07$) and the w^{m4} $p \ll (\chi_1^2 = 15.003$.

(2) Since there is no question as to the hot spot in interval 11, breaks in this interval were excluded from the calculation. Even so, on the assumption of an expected Poisson distribution, $p = 0.0074$ for getting a zero-break site in Section 20 and $p < 0.0001$ for three zeroes in this Section. $p < 0.005$ for having a site with 15 breaks or more. If the three zeroes are excluded (and the expected number of breaks per site is 6.75), $p < 0.05$ for getting 15 or more breaks in any of the remaining 8 intervals.

(3) Homogeneity test for the distribution of breaks in normal and *In(1)sc⁸* chromosomes in Section 20 yields ($\chi_3^2 = 14.497$) $p < 0.005$.

(4) Comparison of interval 11 breaks in the normal and *In(1)w^m4* chromosome: 21 out of 41 breaks in the normal and 3 out of 18 breaks in the w^{m4} chromosome. $p < 0.025$ ($\chi_1^2 = 6.129$).

y²sc/Y and *In(1)sc⁸/Y* males were X-rayed and crossed *en masse* with *y² f su(f)* females. X-irradiated *In(1)w^m4* males were crossed with *w^a f su(f)* females. Two- to four-day-old males irradiated with 3400r were used throughout. Parents were discarded after four days. Recovered *Df(1)su(f)*-bearing chromosomes, almost all of which are lethal, were balanced to either *FM6* (for the normal sequence) or *In(1)dl49* (for the inverted chromosomes). The *Df(1)su(f)* deletions so recovered along with *y* or *sc* mutations in the *In(1)sc⁸* or *w* and *w-N* in *In(1)w^m4* chromosome were also employed in the study of heterochromatin dominant male-sterility factors to be reported elsewhere.

Mapping procedure: The left breakpoint of the *su(f)* deficiency may reside at any point distal to *su(f)* and can be accurately determined by allelism tests with lethals located to the left of *su(f)* and "covered" by the duplication *Ymal+*. Some 22 intervals in which breaks could

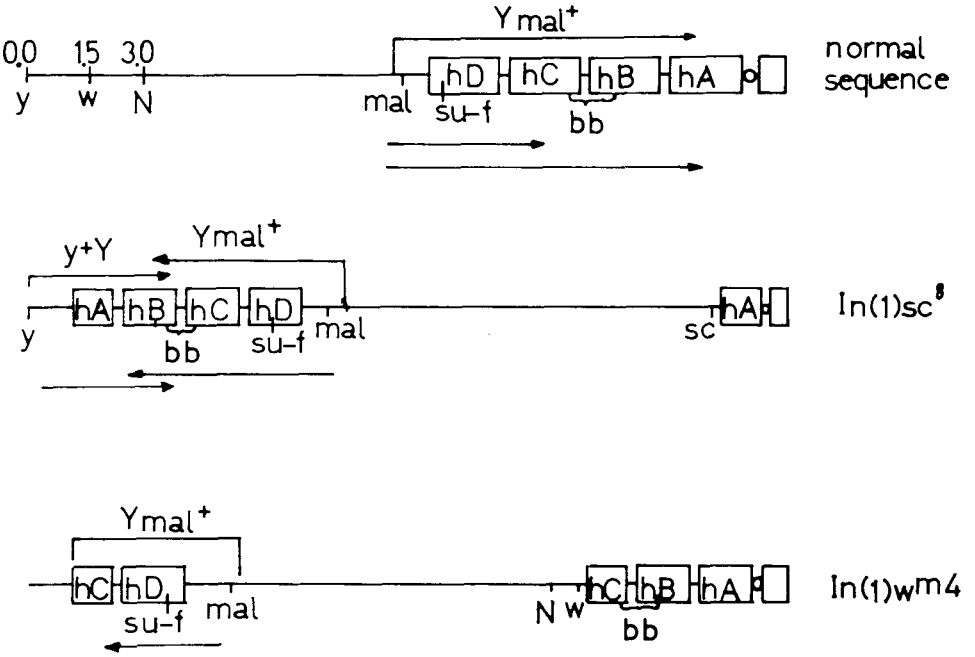

FIGURE 2.—Schematic representation of the normal and inverted chromosomes used in this study. Heterochromatic blocks are marked after COOPER (1959).

occur were defined by an array of recessive lethal gene mutations previously described (LIFSCHYTZ and FALK 1968; LIFSCHYTZ 1971) (see Figure 1). The fact that very likely not all possible intergenic intervals were studied does not affect the results, as will be discussed below. Some corrections of our map have been published (SCHALET and LEFEVRE 1973) while other left-right localizations, *e.g.*, lethal *B214* vs.*W4* or *X3* vs. *P19* are yet to be established by overlapping deficiencies. Important as they are, these uncertainties in no way affect the analysis or conclusions.

In practice, the mapping of a specific *su(f)* deficiency involved crossing females heterozygous for the *su(f)* deficiency to males bearing known lethal gene mutations covered by *Ymal+* and determining whether the lethal/deficiency heterozygote was lethal or viable.

Some of our earlier nonselected deficiencies such as *l(1)B12* (intervals 11–22 in Figure 2) or *l(1)B57* (intervals 16–22) were helpful in the analysis, as were the *w+Y* duplication, which covers intervals 1–5 and the *y+Ymal*^{126} duplication, which covers all lethals from *su(f)* to *E54*.

RESULTS

Distribution of breaks in a normal sequence chromosome

Among 47 *su(f)* deficiencies induced in normal sequence *X* chromosomes (see Table 1 and Figure 1A,B), 40 have their unselected left breakpoints in Section 20 (intervals 1–12). Of these, 21 or 52% of the total number of Section 20 breakpoints, occur in interval 11, the major "hot spot" for X-ray-induced breaks that had been identified previously (LIFSCHYTZ and FALK 1968, 1969; FALK 1973). Note, however, that in earlier experiments, deficiencies were not

preselected, but rather comprised a fraction of unselected lethals covered by the *Ymal*+ duplication. In addition to the 21 breaks in interval 11, 19 other deficiencies had their left breaks located in Section 20. Of the 10 possible intervals where breakage might have occurred, only four were realized. Intervals 7 and 9 could thus conceivably qualify as minor "hot spots" secondary to the major one in interval 11. No less important is the fact that certain intervals appear to be refractory to breakage, *e.g.*, 5, 8 and 10. Only seven deficiencies extended to the left of interval 11, and one of these mapping in interval 12, might yet be in Section 20. Of the remaining six, four were located in interval 18 and two have their ends distal to the mapped region. Although the number of deficiencies recovered in this part of Section 19 is insufficient for a firm conclusion, there is a definite suspicion that a "hot spot" exists in interval 18 (Figure 1).

Is there any kind of selection that tends to favor certain types of deficiencies? In a region where deficiencies exist for all subsegments, we cannot invoke haploinsufficient genes. Length of deficiencies will not serve either. For a deficiency with a nonselected end at unit 11, there were available 10 shorter locations to choose residency. For those ends residing in units 7 or 9, five other intervals closer to the fixed end were available, but obviously rejected.

Another source of error could be the unsaturated map we have employed. In its extreme, this argument bears the possibility that about 20 "hidden" genes are located within the limits of the main "hot spot" in unit 11. This is nearly impossible in the light of the extensive mapping (more than 200 mutations) that took place. Moreover, ascertaining the "hot spots" as being heterochromatic segments is more dependent on the lack of breaks in other sites than on their abundancy in one site. Thus, the arguments still hold if, say, one or more Mendelian genes are recovered at interval 10, or more complementation units will divide the other "hot spots" into two or three breakable sites. In this respect, it is not important whether between *E54* and *Q456* there are six complementation units or only two, since no breaks are located between *R9–13* and *Q464*.

Distribution of breaks in In(1)sc^{8} *chromosome*

Among 65 deficiencies induced in the *sc*^{8} chromosome, 51 have their unfixed, or left ends, in Section 20. Of the 51 Section 20 breaks, 38 (70%) occur in the major "hot spot" located at interval 11. The distribution of breaks among the other more proximal intervals of Section 20 appears to be different from that for deficiencies induced in the normal sequence chromosome (see legend for Figure 1). Breaks were found to occur in three more intervals, but no preferential breakage in intervals 7 and 9 is evident. More *sc*^{8} breaks were found in intervals 12 and 18, two breaks in intervals 14 to 17, but again none in the last four intervals. Four deficiencies extended to the left of the mapped region.

Distribution of breaks induced in the w^{m4} *chromosome*

Under the same experimental conditions, the proportion of induced *su(f)* deficiencies recovered in *w*^{m4} chromosome is only one-quarter of that recovered in the normal and *sc*^{8} chromosomes (see Table 1). Conceivably, this may be due

TABLE 1

Mutagenic data for the recovery of Df(1)su(f) *mutations after exposure of males to 3400r*

	Number of tested chromosomes	*Df(1)su(f)* mutations	*Df(1)su(f)* studied	Percent of *su(f)bb*⁻ deficiencies	Percent of breaks in Section 20
Normal	43,300	92*	47	20%	~90
In(1)sc⁸	50,230	103*	65	45%	~83
In(1)w^m4	30,544	21	18	—	100

* 15% of the F_1 *Df(1)su(f)/su(f)* females are sterile, about 10% died before eggs were laid, and some were discarded or not analyzed in this study.

to the shorter heterochromatic region to the left of *su(f)* available in w^{m4} for breakage. Moreover, all 18 *su(f)* deficiencies recovered in this chromosome fall within the limits of Section 20 and were thus "covered" by the y^+Ymal^{126} duplication (see Figure 1). However, the 18 unfixed "ends" distributed themselves equally among seven intervals. In contrast to the normal and sc^8 chromosomes, where at least 50% of the unselected breaks were found to occur in interval 11, only about 15% of w^{m4} deficiencies fall within this major "hot spot." Clearly, the distribution of breaks among the potential breakable intervals is strongly affected by the amount of heterochromatin available for the fixed end and may well depend on its "state."

Altogether 130 deficiencies having their "fixed" end in the centric heterochromatin and "free" ends in Sections 19 and 20 were studied. Most of the "free ends" reside in Section 20, although no selection against longer deficiencies is operative in this region. When mapping data for all 130 deficiencies are combined, the occurrence of "hot" and "cold" spots in Section 20 is evident. The incidence of sites with as many breaks, as in intervals 7, 9 and 11, or of sites with no breaks, as in intervals 5, 8 and 10, cannot be explained on the basis of chance alone (see legend for Figure 1). The occurrence of the "hot spot" in interval 18 of Section 19 is significant and most probably identical with the breakpoint for X-ray-induced fragments detected by FALK (1973) in this region. The lack of more breaks prevents the identification of other "cold" spots and minor "hot spots" in Section 19.

DISCUSSION

Susceptibility to breakage and specifically to X-ray-induced breakage and "ectopic pairing" or "nonspecific pairing" have long been considered characteristics of heterochromatin (HANNAH-ALAVA 1951; SHAH, LAKHOTIA and RAO 1973). The distribution of X-ray-induced breaks was correlated with ectopic-pairing regions, and as a result several major "hot spots" along the *X* chromosome were considered to be intercalary heterochromatin. LEE (1975) has recently reviewed the evidence that correlated KAUFMANN's (1946) "major" hot spots for chromosome breakage (and rejoining) with hot spots for hybridization of repetitious DNA. If KAUFMANN's major hot spots are envisaged as regions of inter-

calary heterochromatin, it is legitimate to ask whether shorter sequences of varied lengths are interspersed among all genes along the euchromatic portion of the *X* chromosome. This cannot be studied cytologically, since even the major hot spots are not resolvable in mitotic chromosomes as heterochromatin. One way to study the nature of intergenic intervals would be to perform analysis of X-ray-induced breaks on the fine-structure level.

The analysis of 130 deficiency ends presented here reveals a highly nonrandom distribution of breaks among genes in Section 19 and 20, thus uncovering several hot and cold spots that space single genes.

Since, however, all right-hand breaks reside in the proximal heterochromatin, the nonrandom distribution also indicates an interdependency between the region in which the "fixed" ends are anchored and the sites where the "free" ends preferentially reside. This interdependence is further illustrated by a comparison of the distribution of breaks in the normal and inverted chromosomes. The Section 20 breaks in sc^8 are evenly distributed among the potential breakpoints, if interval 11 is omitted from the calculation (see legend for Figure 1). If the w^{m4} breaks are compared with the normal, the proportion of *su(f)* deficiencies is drastically reduced in accord with the length of centric heterochromatin available to the left of *su(f)* (see Table 1 and scheme in Figure 1). In addition, and most important, a significant reduction in the fraction of breaks in interval 11, which disappears as a hot spot, is observed, and no "hot" spot is evident among the other potential breakpoints. "Cold" spots, however, remain as in the other chromosomes.

Interdependence of deficiency end breaks with a resultant nonrandom distribution could be inferred from data on *w-N* (white-Notch region, 3C1–2 to 3C7). Of *w* deficiencies induced in the w^{m4} chromosome, LEFEVRE, RATTY and HANKS (1953) observed that 60% were *w-N* and 40% *w* deletions only (see Figure 2). Here, only 3–4 euchromatic bands separate *w* and *N*, but more than 40 are available for the distal breaks to occur before deletion length reaches a critical size of 50. Euchromatic deficiencies longer than this are generally dominantly lethal. This highly nonrandom distribution indicates again an interdependence between a breakpoint in the centric heterochromatin and a second breakpoint between *w* and *N*. Indeed, when $In(1)z^{+64b9}$ was studied, comparatively few *w* deficiencies could be recovered, while *N* deficiencies were as frequent as in normal-sequence chromosomes (M. M. GREEN, personal communication). $In(1)z^{+64b9}$ has its left breakpoint immediately to the right of white and its proximal breakpoint in 12B (SORSA, GREEN and BEERMAN 1973). This, according to my interpretation, deprives the *w* gene of its flanking intercalary heterochromatin.

Thus, the most reasonable explanation of our results is that heterochromatic (ectopic pairing) segments with various breakability potentials flank small groups of genes (or every gene) in Sections 19 and 20. The intercalary segments interact with the heterochromatic blocks to the right of *su(f)* according to their length, thereby uncovering "hot" and "cold" spots. We wish to suggest, however, that such a distribution of hot and cold spots is not unique for Section 20. The

same pattern will be found all along the chromosome if other Sections are brought next to a large heterochromatic block to provide one "fixed" end region and if enough breaks are analyzed. Section 20 is unique only in possessing relatively long intercalary heterochromatic sequences and in being adjacent to a large heterochromatic block. As expected, analysis of deletions in Section 19 and 20 that have no breaks in the proximal heterochromatin or in the major hot spot has failed to reveal clustering of breakpoints (G. LEFEVRE, personal communication). For other Sections brought next to the proximal heterochromatin, identification of "hot" and "cold" spots will depend only on the resolving power. Conversely, were Section 20 located elsewhere, the spaced "minor" heterochromatic sequences (in the absence of "fixed" end selection) may well react with each other or with other flanking sequences, with resulting random or seemingly random distribution of length. This tendency can be observed in the w^{m4} chromosome, where the breaks occur, but no "hot spot" is evident.

Yet the possibility that a hot spot may be the consequence of a highly breakable sequence within a given gene cannot be eliminated. Moreover, since we define the breakpoints by complementation test, we do not have positive evidence that the breakpoint occurs exactly where it is mapped, *i.e.*, between genes rather than through them. It is possible to envisage a situation whereby the breakpoint is associated with a small inversion that also confers lethality so that the allelism test will be misleading. However, breaks in highly breakable unique sequences within genes, if they exist, should not be dependent on interaction with heterochromatin, and minute abberations associated with breakpoints would be hard to demonstrate.

The relations between sequences of different "hot spots" could not be easily deduced from the cytogenetic studies. In the present experiments the fixed ends could occur to the left or to the right of *bb*. About 20% of all the normal-chromosome *su(f)* deficiencies and 45% of the sc^8 of the *su(f)* deficiencies that are listed in Figure 2 (and Table I) include *bb*. They thus have their right breakpoint in the heterochromatic blocks *hA* and *hB*. Yet the distribution of their distal breakpoints is completely similar to that of *su(f)* non*bb* deletions (*i.e.*, those occurring in block hC and hD). No preference for a particular hot spot on the *X* chromosome by any of the autosomal breakpoints is indicated by KAUFMANN's study of *X-A* translocations nor by LINDSLEY's analysis of a nonselected class of established *X-A* (Lindsley 1965) or *X-Y* translocations (NICOLETTI and LINDSLEY 1960). In fact, hot spots on the *X* chromosome for *X-Y* and *X-A* translocations frequently overlap. Similarly, COOPER (1964) observed that blocks hB or hC and hD are nearly symmetrically paired with the *Y* chromosome during meiosis, despite the fact that heterochromatin has been shown not to be homogeneous in molecular composition (PEACOCK *et al.* 1973) or staining properties (HOLMQUIST 1975) and, moreover, to share families of repeated sequences with other euchromatic regions (WENSINK *et al.* 1974). HOLMQUIST (1975) rightly suggested that base composition alone is insufficient to account for all facets of heterochromatin behavior, and his analysis tacitly supports the notion that the

differences between the so-called heterochromatin and the so-called euchromatin are basically quantitative, a conclusion reached also by COOPER (1959). The difference could be due mostly to the organization (amount and mode of distribution) of middle repetitive sequences (of whatever base sequence) among the unique ones.

The heterochromatic nature of Section 20

In the light of what has been discussed thus far, it is justified to re-examine the conclusion drawn by SCHALET and LEFEVRE (1973) that Section 20 is euchromatic, that 20E is the approximate location for the eu-heterochromatic junction and that assignment of breaks by KAUFMANN (1946) or NICOLETTI and LINDSLEY (1960) to heterochromatin is incorrect. The argument that lethal genes occur in Section 20 is in itself no evidence for the euchromatic nature of the region, since *su(f)* and *bb*, which, according to these authors, are in heterochromatin, are not different from other genes, repetitive and otherwise, known to reside in euchromatic regions. Furthermore, Mendelian genes are known to occur in the centric heterochromatin of other chromosomes, *e.g.*, Y-fertility factors, *lt* in *D. melanogaster* and *pe* in *D. virilis* (see COOPER 1959 for review). Cytologically, SCHALET and LEFEVRE argue that in the case of y^{z5} (a sc^{8} chromosome that was broken in 20A where the *X* chromosome was capped by 2L), no centric heterochromatin is observed in orcein-stained chromosomes from ganglion mitoses. Block hD encompasses *ca.* 10% of the *X* chromosome DNA; Section 20 (maximum 19 bands) is expected, if heterochromatic, to occupy only the distal 10% of hD; and 20A is only 1/5 of Section 20. It is hard to see how one can positively exclude the existence of such a small segment if it were heterochromatic, especially in a rearranged chromosome. The same criticism applies to their analysis of y^{z15}, which the authors themselves note that the evidence for lack of heterochromatin is not compelling. Contrary to COOPER (1959), who used the same technique, SCHALET and LEFEVRE failed to detect any loss of heterochromatin in *Dp(1;f)3* and correctly pointed out (see also LIFSCHYTZ 1971) that the duplication covers most genes in Section 20. The crucial point, however, is that if Section 20 is euchromatic, as they argue, they should have presented positive evidence for the occurrence of the euchromatic portion of this duplication. Thus, positive proof or disproof of the cytological nature of such a small segment is in my judgment, beyond the resolving power of the orcein-squash method.

In my view, there is no single hetero-euchromatic junction in Section 20. Long heterochromatic sequencies of various molecular natures are only seldom separated by Mendelian genes (*bb*, *su(f)*, etc.) in the proximal part of the *X* chromosome. Moving distally, more genes intermingle with the heterochromatic sequences, some of which replicate to give rise to the cytological picture of Section 20 (or β heterochromatin if the reader wishes; GALL 1973). The breaks assigned by KAUFMANN (1946) and NICOLETTI and LINDSLEY (1960) as heterochromatic were probably so, no matter if Section 20 is not a formal part of the central heterochromatin that is not replicated in polytene chromosomes.

Being loaded with heterochromatin, Section 20 is a transitional region endowed with distinctive features of recombination (LIFSCHYTZ 1975), breakability and band organization.

I wish to express my appreciation to PROF. M. M. GREEN for his valuable discussions and helpful comments on the manuscript, and to DR. A. BAILES for his invaluable help in the statistical analysis.

LITERATURE CITED

COOPER, K. W., 1959 Cytogenetic analysis of the major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin." Chromosoma (Berl.) **10**: 535–588. ——, 1964 Meiotic conjunctive elements not involving chaiasmata. Proc. Natl. Acad. Sci. U.S. **52**: 1248–1255.

FALK, R., 1973 Breakage and rejoining in a short segment of the X-chromosome of *Drosophila melanogaster*. Chromosomes Today **4**: 283–296. (Edited by J. WAHRMAN and K. R. LEWIS).

GALL, J., 1973 Repetitive DNA in Drosophila. Pp. 59–74. In: *Molecular Cytogenetics*. Edited by B. A. HAMKALO and J. PAPACONSTANTINOU. Plenum Press, New York.

HANNAH-ALAVA, A., 1951 Localization and function of heterochromatin in *Drosophila melanogaster*. Advan. Genet. **4**: 87–125.

HOLMQUIST, G., 1975 Hoechst 33258 fluorescent staining of Drosophila chromosomes. Chromosoma (Berl.) **49**: 333–356.

KAUFMANN, B. P., 1946 Organization of the chromosome I. Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exp. Zool. **102**: 293–320.

LEE, C. S., 1975 A possible role of repetitious DNA in recombinatory joining during chromosome rearrangement in *Drosophila melanogaster*. Genetics **79**: 467–470.

LEFEVRE, G. JR., F. J. RATTY JR. and G. D. HANKS, 1953 Frequency of Notch mutations induced in normal, duplicated and inverted *X* chromosomes of *Drosophila melanogaster*. Genetics **38**: 345–359.

LIFSCHYTZ, E., 1971 Fine structure analysis of the chromosome. Recombinational patterns at the base of the *X* chromosome of *Drosophila melanogaster*. Mutation Res. **13**: 35–47. ——, 1975 Differential sensitivities and the target of heat-induced recombination at the base of the *X* chromosome of *Drosophila melanogaster*. Genetics. **79**: 283–294.

LIFSCHYTZ, E. and R. FALK, 1968 Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of X-ray induced lethals. Mutation Res. **6**: 235–244. ——, 1969 Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of ethyl methanesulphonate induced lethals. Mutation Res. **8**: 147–155.

LINDSLEY, D. L., 1965 In: *Genes and chromosomes structure and function*. Edited by J. I. VALENCIA and R. F. GRELL. National Cancer Institute Monograph **18**, pp. 275–290.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627**.

NICOLETTI, B. and D. L. LINDSLEY, 1960 Translocations between the *X* and *Y* chromosomes of *Drosophila melanogaster*. Genetics **45**: 1705–1722.

PEACOCK, W. J., D. BRUTLAG, E. GOLDRING, R. APPELS, C. W. HINTON and D. L. LINDSLEY, 1973 The organization of highly repeated DNA sequences in *Drosophila melanogaster* chromosomes. Cold Spring Harbor Symp. Quant. Biol. **38**: 405–416.

SCHALET, A. and G. LEFEVRE, 1973 The localization of "ordinary" sex-linked genes in Section 20 of the polytene *X* chromosome of *Drosophila melanogaster*. Chromosoma (Berl.) **44**: 182–202.

SHAH, V. C., S. C. LAKHOTIA and S. R. V. RAO, 1973 Nature of heterochromatin. J. Sci. Indust. Res. **32**: 467–480.

SORSA, V., M. M. GREEN and W. BEERMAN, 1973 Cytogenetic fine structure and chromosomal localization of the *white* gene in *Drosophila melanogaster*. Nature New Biol. **245**: 34–37.

WENSINCK, P. C., D. J. FINNEGAN, J. E. DONELSON and D. S. HOGNESS, 1974 A system for mapping DNA sequences in the chromosomes of *Drosophila melanogaster*. Cell **3**: 315–325.

Corresponding editor: G. LEFEVRE