LACK OF SPONTANEOUS SISTER CHROMATID EXCHANGES IN SOMATIC CELLS OF *DROSOPHILA MELANOGASTER*

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Manuscript received February 20, 1978
Revised copy received April 28, 1978

ABSTRACT

Neural ganglia of wild type third-instar larvae of *Drosophila melanogaster* were incubated for 13 hours at various concentrations of BUdR (1, 3, 9, 27 μg/ml). Metaphases were collected with colchicine, stained with Hoechst 33258, and scored under a fluorescence microscope. Metaphases in which the sister chromatids were clearly differentiated were scored for the presence of sister-chromatid exchanges (SCEs). At the lowest concentration of BUdR (1 μg/ml), no SCEs were observed in either male or female neuroblasts. The SCEs were found at the higher concentrations of BUdR (3, 9 and 27 μg/ml) and with a greater frequency in females than in males. Therefore SCEs are not a spontaneous phenomenon in *D. melanogaster*, but are induced by BUdR incorporated in the DNA. A striking nonrandomness was found in the distribution of SCEs along the chromosomes. More than a third of the SCEs were clustered in the junctions between euchromatin and heterochromatin. The remaining SCEs were preferentially localized within the heterochromatic regions of the *X* chromosome and the autosomes and primarily on the entirely heterochromatic *Y* chromosome.—In order to find an alternative way of measuring the frequency of SCEs in Drosophila neuroblasts, the occurrence of double dicentric rings was studied in two stocks carrying monocentric ring-*X* chromosomes. One ring chromosome, *C(1)TR 94-2*, shows a rate of dicentric ring formation corresponding to the frequency of SCEs observed in the BUdR-labelled rod chromosomes. The other ring studied, *R(1)2*, exhibits a frequency of SCEs higher than that observed with both *C(1)TR 94-2* and rod chromosomes.

SISTER-chromatid exchanges (SCEs) were first observed 20 years ago by TAYLOR, WOODS and HUGHES (1957) in cells of *Vicia faba*. Differentiating sister chromatid: (SC) with tritiated thymidine and autoradiography, SCEs were visualized as label switches between a chromatid and its sister at the same locus. Further studies on SCEs led TAYLOR (1958, 1959) to conclude that: (a) the chromatid is composed of two subunits with opposite polarity and therefore probably of the two strands of a DNA double helix; (b) that SCEs involve both subunits of the chromatid; (c) that SCEs occur spontaneously. Subsequent autoradiographic investigations confirmed the occurrence of SCEs in many plant and animal materials and opened up a persistent controversy as to whether SCEs

are spontaneous events or are induced by the tritium incorporated in the DNA (WOLFF 1964; PRESCOTT 1970).

The introduction of 5-bromodeoxyuridine (BUdR)-Hoechst 33258 and related Giemsa procedures for the differential staining of SCs (LATT 1973; PERRY and WOLFF 1974; KIM 1974; KORENBERG and FREEDLENDER 1974) has recently given a new impetus to the study of the origin of SCEs. These techniques have made it possible to obtain a greatly improved differentiation of the sister chromatids and to detect SCEs with higher resolution.

A reinvestigation of the properties of SCEs using BUdR-labelling procedures has largely confirmed the original conclusions of TAYLOR (for a review see KATO 1977; WOLFF 1977). However, it is still unclear whether SCEs are spontaneous events or whether they are induced by BUdR which, like tritiated thymidine, can damage the DNA and induce chromosomal aberrations (HSU and SOMERS 1961; DEWEY and HUMPHREY 1965). To answer this question, the yield of SCEs at increasing concentrations of BUdR has been studied. The dose-response curves obtained by KATO (1974, 1977) in both DON Chinese hamster cell lines and in human fibroblasts showed a plateau at low concentrations of BUdR, followed by a linear increase in the frequency of SCEs at higher concentrations. A similar study carried out by WOLFF and PERRY (1974) in Chinese hamster ovary (CHO) cells gave completely different results. They obtained a rapid rise in SCE frequency at low concentrations of BUdR, followed by a plateau at the higher concentrations. Since their dose-response curve could be extrapolated to zero, WOLFF and PERRY (1974) concluded that there was insufficient evidence for a background level of spontaneous SCEs.

In the present study, we have investigated the occurrence of SCEs in somatic cells of *Drosophila melanogaster* with two different approaches: (a) differentiation of the sister chromatids with BUdR labelling and fluorescence staining; (b) scoring dicentric rings in two stocks carrying monocentric ring chromosomes (SCEs in rings can produce double sized dicentric rings). Taken as a whole, the results show that in *D. melanogaster* SCEs are not spontaneous events, but are induced by the BUdR used to reveal them.

MATERIALS AND METHODS

Stocks: The following stocks were used: Oregon-R wild type; *R(1)2,yf/FM7*, carrying a single ring-*X* chromosome (SCHULTZ and CATCHESIDE 1938) balanced with the multiply inverted rod-*X* chromosome, *FM7* (MERRIAM 1968); *C(1)TR 94-2/0*, carrying a stabilized derivative of a compound-ring chromosome synthesized from two tandemly attached-*X* chromosomes (SANDLER and LINDSLEY 1967) and no free-*Y* chromosome. All stocks were grown on standard medium at 25 ± 1°.

Differentiation of sister chromatids: Neural ganglia obtained by dissection of third-instar larvae were incubated in the dark at 25° for 13 hr in saline (0.7% NaCl) supplemented with 20% foetal calf serum and containing various concentrations of BUdR (1, 3, 9 and 27 μg/ml). Metaphases were collected with colchicine (final concentration 10^{-5} M) for 1.5 hr. The ganglia were then squashed in 45% acetic acid under a siliconized coverslip (GATTI, PIMPINELLI and SANTINI 1976). The coverslips were removed after freezing on dry ice and the slides were stained for ten min with 10 μg/ml Hoechst 33258 dissolved in a solution of 0.15 M NaCl, 0.03 M

KCl, and 0.01 M phosphate (pH 7). The slides were then mounted in 0.16 M sodium phosphate, 0.04 M sodium citrate (pH 7) and exposed to sunlight for 30 min to improve the differential staining of sister chromatids (PERRY and WOLFF 1974; GOTO *et al.* 1975). Chromosome fluorescence was observed under a Zeiss fluorescence microscope equipped with a 200 W mercury light source. The Zeiss filters used were 2 × BG 12, FT 510 and LP 515.

Induction of C-anaphases: Dicentric rings produced by sister-chromatid exchange in monocentric rings were scored in neural ganglia of larvae from the stocks carrying ring chromosomes. In order to increase the yield of C-anaphase figures for scoring, the following procedure was developed. After dissection, the neural ganglia were incubated for two hr in saline (0.7% NaCl) containing 10^{-5} M colchicine. They were then treated with a hypotonic solution of 0.5% sodium citrate for 25 min at 30°, then fixed and squashed in acetic orcein according to our usual procedure (GATTI, TANZARELLA and OLIVIERI 1974b).

RESULTS

Differentiation of sister chromatids: Previous studies have shown that the cell cycle of the larval neuroblasts of *D. melanogaster* at 25° takes about eight hours (GATTI, TANZARELLA and OLIVIERI 1974b; PIMPINELLI *et al.*, 1976). Therefore a 13-hr BUdR treatment was chosen to obtain metaphases that had incorporated BUdR for two successive S phases. Indeed, after 13 hr of treatment, most of the metaphases showed a clear second mitosis (M2) sister-chromatid labelling pattern. A direct correlation was observed between the degree of differentiation of the SCs and the concentration of BUdR used. At a concentration of 1 μg/ml, although a low degree of SC differentiation appeared to be present in most of the metaphases, only 15% of these were unequivocally differentiated. The fraction of well-differentiated metaphases went up to about 40% after treatment with 3 μg/ml of BUdR. At concentrations of 9 and 27 μg/ml the vast majority of metaphases showed a clear differentiation of the SCs (Figure 1). At these two higher concentrations of BUdR, some cells also showed a slight decondensation of the heterochromatic material. However, no specific pattern of decondensation of the heterochromatin, such as that produced by Hoechst 33258 (PIMPINELLI, GATTI and DE MARCO 1975; GATTI, PIMPINELLI and SANTINI 1976), was observed.

In the experimental conditions used, the fluorescence of the preparations proved to be rather stable and the absence of a sensitive fading image permitted the scoring of the preparations under the fluorescence microscope. It was also possible to obtain good FPG (fluorescence plus Giemsa) preparations simply by following Hoechst 33258 staining and exposure to light by staining with 4% Giemsa (Merck) for 15 min (Figure 1). The degree of differentiation of the preparations stained with Giemsa always corresponded to that observed in fluorescence.

Effects of BUdR concentration on SCE yield: In the cells incubated with 1 or 3 μg/ml of BUdR, the SCEs were scored in only those metaphases with an unequivocal differentiation of SCs. This selection was not made for metaphases labelled with 9 and 27 μg/ml since, at these concentrations of BUdR, SCs consistently exhibited a high degree of differentiation. In Figure 2 are presented the results of two independent experiments to determine the relationship between BUdR concentration and SCE frequency. Since the results of the two experiments

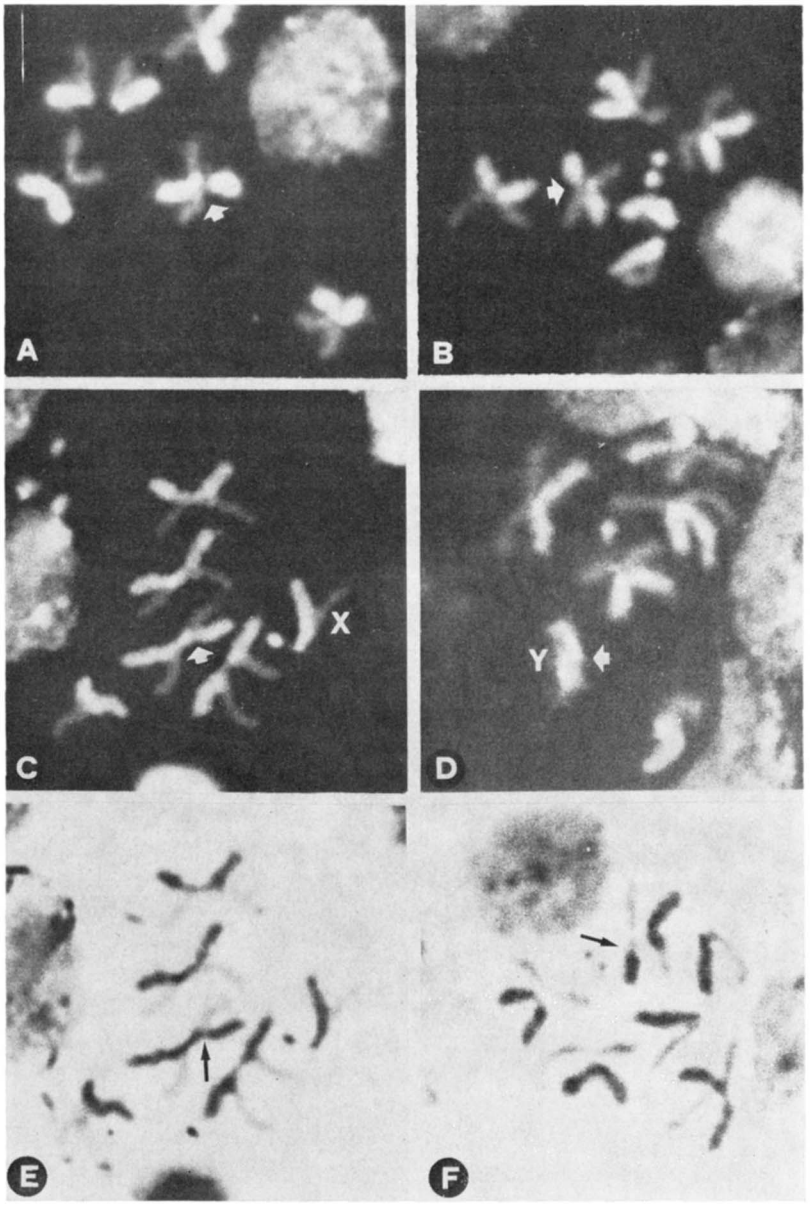

FIGURE 1.—Examples of SCEs (arrows) in *D. melanogaster*. C and E were sequentially stained with Hoechst 33258 and Giemsa.

are rather similar, they have been pooled in Table 1. These data clearly show that the neuroblast metaphases of both sexes treated with 1 μg/ml of BUdR do not exhibit spontaneous SCEs. The SCEs occur only at higher concentrations of BUdR and with a higher frequency in females than in males. This implies that in wild-type *D. melanogaster* the background level of spontaneous SCEs is

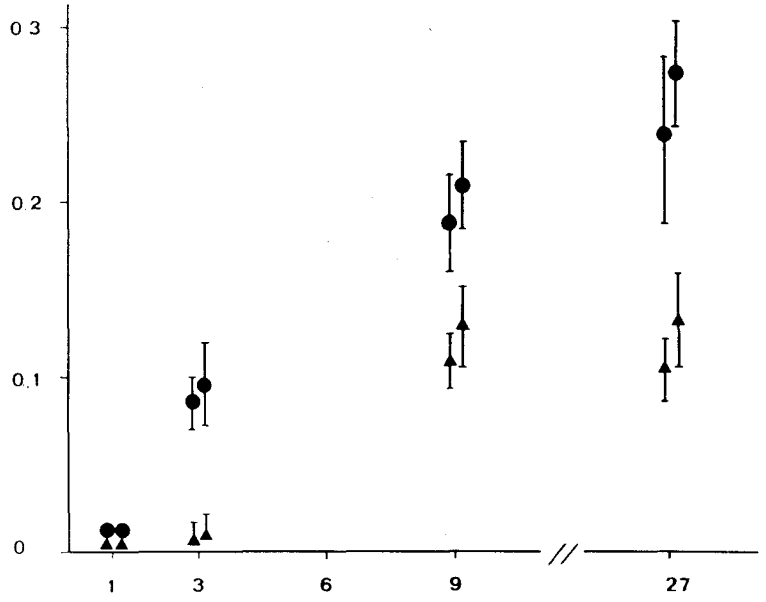

FIGURE 2.—Effect of BUdR concentration on the frequency (± s.e.m.) of sister-chromatid exchanges in somatic cells of *D. melanogaster*. (●) females; (▲) males. Abscissa: BUdR concentration μg/ml. Ordinate: SCEs per cell.

zero, and they are induced by BUdR with a different frequency in the two sexes. The dose-response curves of both males and females rise steeply but reach two different plateau levels. Thus, there is a saturation effect of SCEs in both sexes, but the frequency of SCEs at which females saturate is about twice that of males.

We should like to emphasize that the selective scoring of cells treated with the two lowest concentrations of BUdR should not have affected the validity of these conclusions. Indeed, the selected cells must have incorporated more than the average amount of BUdR and would be expected to show more SCEs than poorly differentiated metaphases.

TABLE 1

Frequency of sister-chromatid exchanges in neuroblast chromosomes of Drosophila melanogaster

BUdR concentration μg/ml	Sex	Number of cells scored	Number of SCEs scored	SCEs per cell
1	♀	183	—	—
	♂	178	—	—
3	♀	512	46	0.09
	♂	338	4	0.01
9	♀	464	94	0.20
	♂	672	76	0.11
27	♀	304	82	0.27
	♂	424	48	0.11

No more than 25 metaphases per larva were scored.

TABLE 2

Distribution of SCEs among chromosomes of D. melanogaster

Sex	Total number SCEs	Number SCEs in the autosomes Obs.	Exp.	Number SCEs in *X* chromosome(s) Obs.	Exp.	Number SCEs in *Y* chromosome Obs.	Exp.	χ^2	*df*	*P*
♀	222	182	177.6	40	44.4	—	—	0.55	1	0.46
♂	128	80	102.4	10	12.8	38	12.8	55.12	2	<0.001

The expected values were calculated assuming that SCEs were distributed among chromosomes in proportion to their length.

Distribution of SCEs along chromosomes: The karyotype of *D. melanogaster* is composed of two pairs of metacentric chromosomes, a pair of dot chromosomes, and, in males, an acrocentric *X* chromosome and a submetacentric *Y* chromosome. Not considering the dot chromosomes (which include 1–2% of the genome), the two pairs of autosomes comprise 80% of the genome and each of the sex chromosomes approximately 10%. The entire *Y* chromosome, the proximal 40% of the *X* chromosome and the proximal 20% of the autosomes are heterochromatic.

In scoring the SCEs, it was not possible routinely to discriminate between the two pairs of metacentric chromosomes (second and third chromosomes) or evaluate the presence of SCEs in the dot chromosomes. Within the chromosomes, SCEs could be assigned to euchromatin or heterochromatin regions since the heterochromatic regions are easily recognized as they have the SCs closely apposed.

The distributions of SCEs between and within chromosomes are given in Tables 2 and 3, respectively. In females SCEs are distributed among chromosomes in proportion to their length, while in males the *Y* chromosome has a significant excess. Within chromosomes, the SCEs are preferentially localized in heterochromatin and exhibit a striking clustering in the junction between eu-

TABLE 3

Distribution of SCEs within chromosomes of D. melanogaster *and rates* (in parenthesis) of SCEs per unit of length in different chromosomal regions*

Sex	Total number SCEs	Autosomes Eu.	Jun.	Het.	*X* chromosome(s) Eu.	Jun.	Het.	*Y* chromosome
♀	222	46	70	67	8	17	14	—
		(0.32)		(1.89)	(0.30)		(0.79)	
♂	128	20	33	27	4	2	4	38
		(0.24)		(1.32)	(0.52)		(0.78)	(2.97)

Eu = euchromatin; Jun = junction between euchromatin and heterochromatin; Het = heterochromatin.

* To obtain the rates of SCEs per unit of length, the frequencies of SCEs occurring in the different chromosomal regions were calculated. These percents were then divided by the number of units of length of each region. The genome of *D. melanogaster* males was estimated to be made up of 100 length units (l.u.) so distributed: autosomal euchromatin (2 pairs) = 64 l.u.; autosomal heterochromatin = 16 l.u.; *X* euchromatin = 6 l.u.; *X* heterochromatin = 4 l.u.; *Y* chromosome = 10 l.u.

and heterochromatin (Figure 1a,b,c) In addition, the heterochromatin of the *Y* chromosome appears to be more susceptible to the SCEs than that of the *X* chromosome or the autosomes.

Experiments on ring chromosomes: Before the cytological observation of SCEs, McCLINTOCK (1938) and SCHWARTZ (1953) postulated their occurrence to explain ring instability in maize. Since a SCE within a ring chromosome can produce a double sized dicentric ring, BREWEN and PEACOCK (1969) studied the occurrence of dicentric rings in the leucocytes of a human male heterozygous for a ring chromosome, in both the presence and the absence of tritiated thymidine. They observed that the frequency of ring dicentrics induced by tritium approximated the frequency of SCEs determined autoradiographically in rod chromosomes. They therefore suggested that the scoring of dicentric rings could be an alternative way of measuring the frequency of SCEs.

In *D. melanogaster* several ring chromosomes have been synthesized and the behavior of some of them has been analyzed in detail (for review, see LEIGH 1976). We therefore considered it useful to study the frequency with which dicentric rings are produced from monocentric ring-*X* chromosomes, both spontaneously and after treatment with BUdR. The main interest in carrying out these experiments was to determine whether the frequency of SCEs, inferred from the rate of dicentric ring formation corresponds to that directly observed in M2 rod chromosomes. The demonstration of such a correspondence would provide further support for our finding that spontaneous SCEs do not occur in *D. melanogaster*, and in addition might provide a quick and easy way of measuring the frequency of SCEs in *D. melanogaster* neuroblasts.

Before presenting the data, let us list briefly the events that can produce double dicentric ring chromosomes: (1) An SCE, whatever its localization along the ring, will produce a double dicentric ring with equal intercentromere distances (See Figure 3-a and *cf.* UHL 1965; WOLFF, LINDSLEY and PEACOCK 1976). Two SCEs within a ring will produce either two free monocentric rings or, alternatively, two interlocked rings if the second SCE does not counteract the first. (This case is not depicted in Figure 3). (2) An isochromatid deletion with sister union

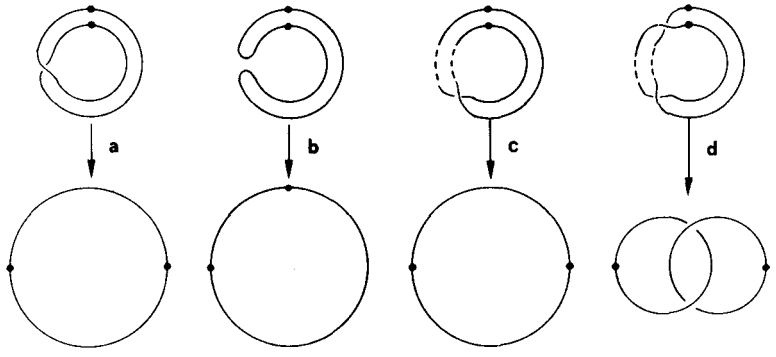

FIGURE 3.—Origin of dicentric rings. (See text for explanation.)

will produce a double dicentric ring with variable intercentromere distances (Figure 3b). (3) A dicentric ring can originate if two chromatids broken at the same locus undergo a restitution of the break after a torsion (BAUER 1942). A twisting through 180° will produce a symmetrical dicentric ring (Figure 3c), while a twisting through 360° will produce two interlocked single rings (Figure 3d). (4) Dicentric rings might be generated by the rotation of the plane of split (Moebius strip phenomenon) after errors occurring during replication of DNA (MCCLINTOCK 1938).

Figure 3c gives an up-to-date version of this hypothesis if we assume that the twisting units are chromatid subunits instead of entire chromatids. With an unineme chromosome model, this hypothesis would imply that switches in polarity of chromosomal DNA can occur that would produce dicentric rings after the replication of DNA. It is interesting to note in this respect that there is evidence that such switches in polarity occur in mammalian chromosomes (WOLFF LINDSLEY and PEACOCK 1976). The two latter events are from a working point of view equal to the first. In fact, they produce double dicentric rings with equal intercentromere distances and, if they occurred in BUdR labelled rod chromosomes, they would produce cytologically detectable SCEs. Therefore they can be considered as hypothetical mechanisms of formation of SCEs.

Whatever the origin, the dicentric rings are not transmittable from one cell generation to the next. They form a double chromatid bridge at anaphase and will most likely be eliminated (for review see HINTON 1959; LEIGH 1976). Thus the dicentric rings scorable at metaphase must have been formed during that mitotic cycle and could not be formed in previous mitotic cell cycles.

The rings used in the experiments described below were *R(1)2* formed by a single *X* chromosome (SCHULTZ and CATCHESIDE 1938) and *C(1)TR 94–2*, which is a stabilized derivative of a compound-ring chromosome synthesized from a tandem attached-*X* chromosome (SANDLER and LINDSLEY 1967). It should be noted, however, that the rings used in the present experiments are probably no longer equivalent to the original ones. It has been in fact reported that in Drosophila ring chromosomes tend to "evolve" into series of new chromosomes often having a greater stability (LEIGH 1976). For this reason, both in the presentation of the results and in the related discussion, we have avoided comparison of the present data with those previously obtained on the same chromosomes.

The *R(1)2* chromosome was studied in females heterozygous for an *FM7* rod chromosome, and *C(1)TR 94–2* in females without a free *Y* chromosome. Since dicentric rings are eliminated during mitosis, in the experiments with BUdR the ganglia were exposed in the dark to 6 μg/ml of BUdR for only seven hours (approximately one mitotic cycle). Only colchicine-induced C-anaphases of the type shown in Figure 4 were scored, and interlocked rings were never observed. All the dicentric rings in which it was possible to recognize the centromeres showed equal intercentromere distances. Table 4 gives the frequency of spontaneous and BUdR-induced dicentric rings in both *R(1)2/FM7* and *C(1)TR 94–2/0* females.

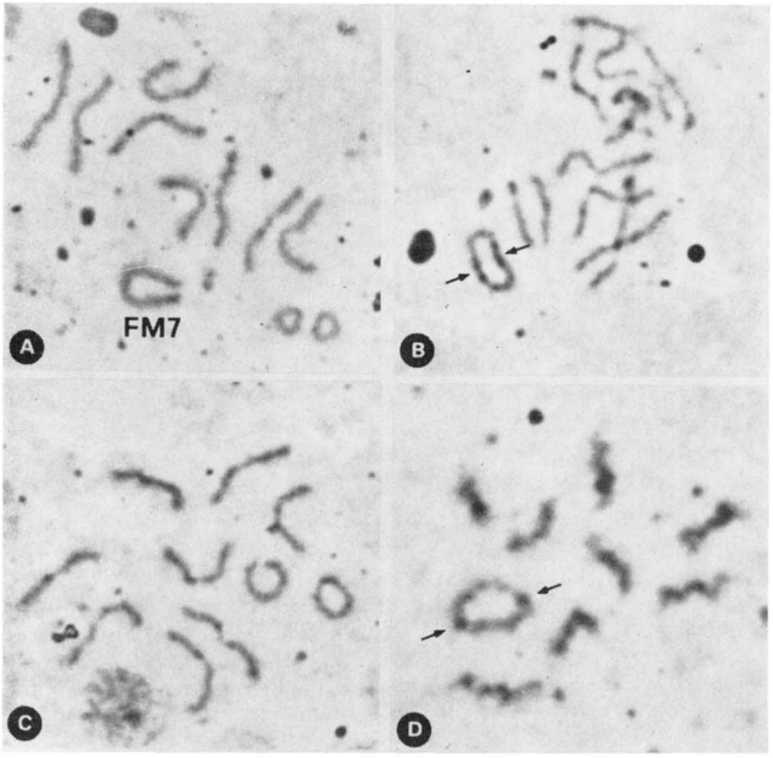

FIGURE 4.—C-anaphases showing monocentric and dicentric ring chromosomes. (A) and (B), *R(1)2/FM7*; (C) and (D) *C(1)TR 94–2/0*. The arrows indicate the positions of centromeres.

As can be seen, *R(1)2* has a significantly higher frequency of spontaneous dicentric formation than *C(1)TR 94–2*. Treatment with BUdR considerably increases the frequency of dicentric formation in both rings; however, in this case they exhibit almost the same rate of dicentric formation. Since *C(1)TR 94–2* is double the size of *R(1)2* and contains almost the same proportion of hetero-

TABLE 4

Frequency of spontaneous and BUdR-induced dicentric rings in two Drosophila stocks carrying ring-X chromosomes

Stock/treatment	Number of ganglia scored	Number of cells scored	Number of dicentric rings	Frequency of dicentric rings (%)
R(1) 2/FM7 No treatment	35	3,282	19	0.58
C(1)TR 94–2/0 No treatment	31	2,820	5	0.18
R(1) 2/FM7 6 μg/ml BUdR, 7 hr	28	1,329	26	1.96
C(1)TR 94–2/0 6 μg/ml BUdR, 7 hr	29	1,317	23	1.74

* Significantly different in χ^2 test: $\chi^2 = 6.26$; $P < 0.02$.

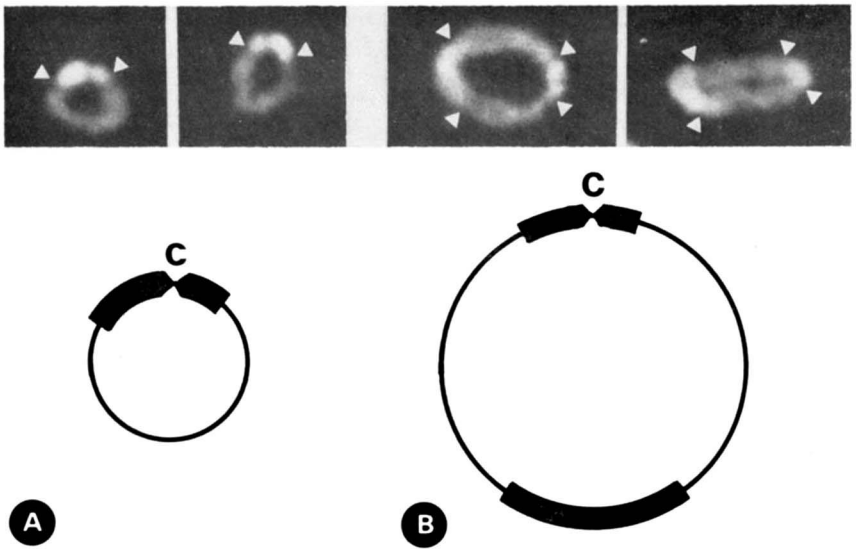

FIGURE 5.—Cytological characterization of *R(1)2*(A) and *C(1) TR 94–2*(B) using Hoechst 33258 banding techniques (GATTI, PIMPINELLI and SANTINI 1976). The arrows indicate the heterochromatin, which appears brighter than euchromatin. The heavy lines represent heterochromatin in the schematic drawings of the ring chromosomes. (C = centromere.)

chromatin as *R(1)2* (Figure 5), it should be about twice as susceptible to any event that converts it into a dicentric ring. Thus, it appears correct to adjust the rate of dicentric formation for the dimension of the chromosomes. With this criterion, *R(1)2* has a spontaneous rate of dicentric formation 5.6 times higher than *C(1)TR 94–2*, while it appears only twice as susceptible as *C(1)TR 94–2* to the induction of dicentrics by BUdR.

The different behavior of the two ring chromosomes cannot be the consequence of a different frequency of SCEs in the two stocks *R(1)2/FM7* and *C(1)TR 94–2/0*. In fact, as shown in Table 5, they do not differ significantly with regard to the frequency of SCEs scored in *M2*-labelled chromosomes after treatment for 13 hours with 6 μg/ml BUdR.

TABLE 5

Frequency of SCEs in BUdR-labelled (6 μg/ml for 13 hr) M2 metaphases of two Drosophila stocks carrying ring-X chromosomes

Stock	Number of ganglia scored	Number of cells scored	Number of SCEs	Frequency of SCEs (%)
R(1) 2/FM7	10	311	35	11.3
C(1)TR 94–2/0	12	339	51	15.0

* Not significantly different in χ^2 test: $\chi^2 = 2.03$; $P = 0.16$.

TABLE 6

Comparison of the observed frequency of SCEs with that inferred from the rate of dicentric ring formation*

Stock	Inferred frequency of spontaneous SCEs (%)	Inferred frequency of BUdR-induced SCEs (%)	Observed frequency of BUdR-induced SCEs (%)
R(1) 2/FM7	11.6	38.2	11.3
C(1)TR 94-2/0	1.8	17.4	15.0

* The frequency of SCEs per genome for two cell cycles was calculated by multiplying the rate of dicentric rings $\times$ 2 $\times$ the ratio of the dimension of the entire genome to that of the ring chromosome. Thus the inferred frequency of spontaneous SCEs in *R(1)2* is $0.58 \times 2 \times 10 = 11.6$. In *C(1)TR 94-2*, it is $0.18 \times 2 \times 5 = 1.8$.

Assuming that the dicentric rings are produced by SCEs, from the frequency of dicentric rings it is possible to infer the frequency SCEs per genome per two cell cycles and compare it with that directly observed in *M2* chromosomes. This comparison, given in Table 6, shows that while in *C(1)TR 94-2* the inferred and the observed frequencies of SCEs are almost the same, in *R(1)2* the inferred frequency of BUdR-induced SCEs is about three times the observed frequency.

One interpretation of these results is that the frequency of SCEs in a given ring chromosome is determined by factors, in addition to the size, intrinsic to its structure. With respect to these ring chromosomes, three possibilities can be considered: (a) if ring chromosomes are structurally less sensitive than rod chromosomes to the SCEs, they should exhibit a frequency of SCEs less than that of the rod chromosomes, in both the absence and the presence of BUdR; (b) if ring chromosomes have the same sensitivity as rod chromosomes to SCEs, they should consistently show the same rate of SCEs as the rod chromosomes; (c) if ring chromosomes are structurally more sensitive than rod chromosomes to SCEs, they should show a greater frequency of both spontaneous and BUdR-induced SCEs. However, it should be pointed out that at concentrations of BUdR at which a saturation effect occurs, the differences among the three types of rings in the rate of SCEs could be greatly reduced. According to this rationale, the *C(1)TR 94-2* chromosome seems to have the same susceptibility to SCEs as rod chromosomes. *R(1)2*, on the other hand, has a spontaneous rate of dicentric formation much higher than that of *C(1)TR 94-2*. It exhibits also a higher rate of BUdR-induced dicentric rings. However, in this case, probably because of an effect of saturation of the SCEs, the difference between the two ring chromosomes appears reduced. It can therefore be concluded that some unknown factors intrinsic to *R(1)2* make it more susceptible to the SCEs than either *C(1)TR 94-2* or the rod chromosomes.

DISCUSSION

Lack of spontaneous SCEs: The present data have clearly demonstrated that in *D. melanogaster* SCEs are not a spontaneous phenomenon. Not a single SCE was found in 183 female and 178 male metaphases showing an unequivocal dif-

ferentiation of SCs after treatment with 1 μg/ml of BUdR. Therefore the SCEs observed at the highest concentrations of BUdR must have been induced by the analog incorporated in the DNA.

The comparative examination of the frequency of SCEs in various animal and plant species has revealed a good correlation between DNA content per cell and rate of SCEs (for review, see KATO 1977). However, the absence of spontaneous SCEs at the lowest concentration of BUdR cannot be explained on the basis of the low DNA content per diploid nucleus in *D. melanogaster* (0.72 pg/4C nucleus, see Table 7). The 361 (183 + 178) metaphases examined contain a total of 260 pg of DNA; therefore, even if they had the lowest rate of SCEs per pg of DNA so far reported (0.14 SCEs/pg DNA, see Table 7), they would have shown about 36 exchanges. In addition, as shown in Table 7, at the higher concentration of BUdR the Drosophila neuroblasts also have a SCE rate per pg of DNA that is considerably lower than that so far reported in various *in vivo* and *in vitro* systems. Recently, a very low rate of SCEs in *D. melanogaster* neuroblasts has been reported by WIENBERG (1977), who found a frequency of about 0.005 SCEs/chromosome in the euchromatin after exposing ganglia for 15 hr to 15 μg/ml of BUdR. These findings, which are consistent with the observation that SCEs do not occur in Drosophila meiosis (BEADLE and EMERSON 1935), suggest two possibilities: (1) the absence of spontaneous SCEs could be a common feature of all the organisms listed in Table 7. The neuroblasts of *D. melanogaster* would, however, differ from the other systems in their peculiar resistance to BUdR. Therefore in Drosophila—but not in the other organisms—it was possible to find a concentration of BUdR that gives a discernible differentiation of the SCs without inducing SCEs. (2) The absence of spontaneous SCEs in *D. melanogaster* could be a characteristic in which this organism differs from the others species that may have a low rate of spontaneous SCEs.

Unfortunately it is not possible at present to discriminate between these two possibilities, primarily because the rate of incorporation of BUdR into the chromosomes of the various organisms so far studied is not known. The study of the rate of dicentric ring formation could provide a way of measuring the frequency of SCEs in the absence of BUdR and therefore permit discrimination between the above possibilities. From the rate of dicentric ring formation, BREWEN and PEACOCK (1969) inferred a spontaneous rate of 0.12 SCEs per chromosome per cell cycle in human leucocytes. This frequency is comparable to that found in the same material at low concentrations of BUdR (see authors listed in Table 7). However the present data indicate that different ring chromosomes can show different spontaneous levels of dicentric formation and, therefore, most likely different rates of SCEs. Moreover there is evidence that the mitotic stability of different human ring chromosomes varies markedly independently of ring size (KISTENMACHER and PUNNET 1970). It is possible that the ring chromosome studied by BREWEN and PEACOCK (1969) was structurally more susceptible than the corresponding rod chromosome to SCEs and did not provide a correct measurement of the rate of SCEs in man. Nevertheless, we feel that once tested for their

sensitivity to SCEs (according to the rationale put forth in the RESULTS), some of the numerous human ring chromosomes could be advantageously used to establish whether or not SCEs occur spontaneously in man.

The *C(1)TR 94–2* ring chromosome, in both the presence and the absence of BUdR, has shown a rate of dicentric ring formation that corresponds to the SCE level observed in the rod chromosomes. From its spontaneous rate of dicentric ring formation, a rate of 0.009 SCEs per cell per cell cycle can be inferred. Such a frequency of SCEs is compatible with the absence of SCEs found in 183 female metaphases labeled with 1 μg/ml of BUdR and is very similar to the frequency of spontaneous chromosomal aberrations, which in Drosophila females is 0.006 per cell (GATTI, TANZARELLA and OLIVIERI 1974b). In this respect it should be stressed that in mammals SCE rates about a hundred times greater than the rate of spontaneous chromosomal aberrations were found (for review see KATO 1977). Therefore, the data on *TR 94–2* constitute further support for considering SCEs a phenomenon that does not occur spontaneously in *D. melanogaster*, but that must be considered, like chromosomal aberrations, to be the cytological manifestation of errors occurring during DNA metabolism.

Finally, the ring chromosome *C(1)TR 94–2* constitutes a particularly favorable system for the experimental induction of SCEs. It offers the possibility of studying the induction of SCEs in the absence of a significant spontaneous background of exchanges and in the absence of the possible interactions of the mutagenic agent with BUdR incorporated into the DNA. The simple squashing techniques and the speed of scoring rings also make *C(1)TR 94–2* particularly useful in evaluating the chromosomal effects of environmental mutagens.

Variations with sex of BUdR-induced SCEs: The present data have clearly shown that *D. melanogaster* females consistently exhibit a higher level of SCEs than that of males. Previous studies have shown that females have a greater frequency of both spontaneous aberrations (GATTI, TANZARELLA and OLIVIERI 1974b) and those induced by X rays (GATTI, TANZARELLA and OLIVIERI 1974a,b) and by methyl methanesulphonate (GATTI *et al.* 1975). Moreover, a greater frequency of aberrations in females than in males has recently been found in four out of five mutants that produce spontaneous chromosomal aberrations in Drosophila (GATTI, submitted) This suggests that the same factors are involved in determining the greater sensitivity of females to chromosomal aberrations and to SCEs. Thus, at least some steps may be common to the generation of these two types of chromosomal rearrangements.

It has been suggested that in Drosophila females there could be present in somatic cells some enzymes involved in crossing over that, in agreement with the hypothesis of misrepair (EVANS 1967), could determine a more efficient transformation of lesions present in DNA into chromosomal aberrations (GATTI, TANZARELLA and OLIVIERI 1974a,b; GATTI *et al.* 1975). We feel that this hypothesis can also explain the greater sensitivity of females to BUdR-induced SCEs. It is interesting to note that it has recently been found that in *D. melanogaster* the same loci are utilized in meiotic recombination and in the control of mitotic

TABLE 7

*Rates of SCEs per pg of DNA in various animal and plant systems** in vivo *and* in vitro *after two rounds of DNA replication in the presence of different concentrations of BUdR* (Reference numbers† in parentheses)

Species and systems	DNA content per metaphase (4C)	Concentrations of BUdR used ≤1 μg/ml SCE/cell	SCE/pg DNA	2.5 to 3.5 μg/ml SCE/cell	SCE/pg DNA	8 to 12 μg/ml SCE/cell	SCE/pg DNA	25 to 35 μg/ml SCE/cell	SCE/pg DNA
Human lymphocytes *in vitro*	11.68 (24)	10.5 (7)	0.90	4.1 (3)	0.35	12.5 (1)	1.07	17.5 (1)	1.50
				7.1 (4)	0.61	6.4 (8)	0.55	6.9 (2)	0.59
				7.9–11.2 (5)	0.68–0.96	7.9 (10)	0.68	22.8 (9)	1.95
				8.0 (6)	0.69				
Various lines of human fibroblasts *in vitro*	11.68 (24)	3.2–4.2 (12)	0.28–0.36	9.0–11.8 (11)	0.77–1.01	5.2–7.3 (12)	0.45–0.63		
				9.3 (4)	0.80				
				3.1–4.2 (12)	0.27–0.36				
Chinese hamster ovary cells *in vitro*	10.75 (25)	7.9–11.7 (13)	0.73–1.09	15.0 (13)	1.40				
				12.2 (14)	1.13				
Chinese hamster cell line D6 *in vitro*	10.75 (25)	2.3 (15)	0.21	2.8 (15)	0.26	4.9 (15)	0.46		
Primary cell cultures *in vitro* of 23 mammalian species belonging to 11 orders	16.5–6.8 (25)	2.9–8.6 (16)	0.32–0.82						
Rat bone marrow cells *in vivo*	10.63 (25)	1.5 (17)	0.14						
Vicia faba root tips	86.23 (26)							20.6‡(18)	0.24
Allium cepa root tips	105.64 (26)							88.0 (19)	0.83
								44.8‡(19)	0.42

D. melanogaster ganglion cells	♀ ♀	0.72	(27)	0–0.018§	(20)	0–0.025	0.09	(20)	0.13	0.19	(20)	0.26	0.25 (20) 0.35
	♂ ♂			0	(20)	0	0.02	(20)	0.028	0.12	(20)	0.17	0.13 (20) 0.18
Mouse bone marrow cells *in vivo*		10.75	(25)	4.0	(21)	0.37	multiple injections of BUdR						
Mouse spermatogonia *in vivo*		10.75	(25)	1.8	(22)	0.17	multiple injections of BUdR						
Chicken embryonic cells *in vivo*		5.00	(28)	0.75	(23)	0.15	25 μg BUdR/embryo						

* In all systems in the upper part of the table except one (rat bone marrow cells) the concentration of BUdR to which the cells were exposed is known. However, in this system the SCEs were scored at the minimum BUdR concentration that permitted a discernible differentiation of SCs (Tice, Chaillet and Schneider 1976). It has been assumed that this concentration is less than 1 μg/ml as in the *in vitro* systems. In the three systems listed in the bottom of the table the final concentration of BUdR to which the cells were exposed is not known.

† (1) Latt 1974; (2) Chaganti, Schonberg and German 1974; (3) Beeck and Obe 1975; (4) Hayashi and Schmid 1975; (5) Latt *et al.* 1975); (6) Sperling *et al.* 1975; (7) Tice, Chaillet and Schneider 1976; (8) Crossen *et al.* 1976; (9) Lambert *et al.* 1976; (10) Morgan and Crossen 1977; (11) Wolff *et al.* 1975; (12) Kato and Stich 1976; (13) Wolff and Perry 1974; (14) Perry and Evans 1975; (15) Kato 1974; (16) Kato 1977; (17) Tice, Chaillet and Schneider 1976; (18) Kihlman and Kronborg 1975; (19) Schvartzman and Cortes 1977; (20) present data; (21) Vogel and Bauknecht 1976; (22) Allen and Laff 1976; (23) Bloom and Hsu 1975; (24) Mirsky and Ris 1951; (25) the DNA content per metaphase has been calculated from the densitometric measurements relative to human cells reported by Kato 1977; (26) the DNA contents of *Vicia faba* and *Allium cepa* metaphases were calculated from the average amount of DNA per root tip cell (McLeish and Sunderland 1961) and from the relative proportions of 2C, 4C and 8C nuclei in the same material (Sunderland and McLeish 1961; (27) Rasch *et al.* 1971; (28) Ris and Mirsky 1949.

‡ Cell exposed to BUdR for only one round of DNA replication.

§ Frequency of SCEs inferred from the rate of dicentric ring formation of *C(1)TR 94–2*.

chromosome stability (BAKER *et al.*, 1976a,b; BAKER, CARPENTER and RIPOLL 1978; GATTI, submitted).

Lastly, we note that in females the SCEs saturate at a level about twice that of the males. Since the two sexes have a similar genome organization, it is possible that the limiting factor that leads to the saturation of BUdR-induced SCEs is of an enzymatic nature.

Distribution of SCEs along chromosomes: A series of studies carried out by various research workers on several animal and plant systems have shown a nonrandom distribution of SCEs along the chromosomes. A preferential localization of the SCEs in the constitutive heterochromatin was found in human chromosomes (KIM 1974; TICE, CHAILLET and SCHNEIDER 1975; LAMBERT *et al.* 1976) and in those of *Microtus agrestis* (NATARAJAN and KLASTERSKA 1975; PERA and MATTIAS 1976). On the contrary, in the constitutive heterochromatin of Chinese hamsters and *Microtus montanus* (HSU and PATHAK 1976), the Indian Muntjac (CARRANO and WOLFF 1975), *Dipodomys ordii* (BOSTOCK and CHRISTI 1976) and *Allium cepa* (SCHVARTZMAN and CORTÉS 1977) SCEs occur at a significantly lower rate than in euchromatin. However, in both the Indian muntjac and *Dipodomys ordii* a dramatic increase in the SCE rate was found in the junctions between eu- and heterochromatin. Besides, in *Dipodomys ordii*, where the C-banded regions can be divided into blocks on the basis of their lateral asymmetry pattern, the few exchanges present on the constitutive heterochromatin were localized at the interfaces of these substructures (BOSTOCK and CHRISTIE 1976).

The present data have shown that also in *D. melanogaster* the SCEs exhibit a striking clustering in the junctions between eu- and heterochromatin. More than a third of the SCEs scored were localized in these regions. The remaining SCEs were mainly localized in the heterochromatic regions of the *X* chromosome and the autosomes and primarily on the entirely heterochromatic *Y* chromosome. The clustering of the SCEs on the junction between eu- and heterochromatin agrees well with the findings of CARRANO and WOLFF (1975) in the Indian muntjak and with those of BOSTOCK and CHRISTIE (1976) in *D. ordii*. However, contrary to these organisms, *D. melanogaster* exhibits a high rate of SCEs within the constitutive heterochromatin. A possible explanation of this discrepancy could lie in the peculiar organization of the heterochromatin of *D. melanogaster*, which is constituted by several subunits that can be discriminated by Quinacrine-, Hoechst- and N-banding (GATTI, PIMPINELLI and SANTINI 1976; PIMPINELLI, SANTINI and GATTI, 1976). If, as in *D. ordii*, the junction areas between these subunits were particularly susceptible to SCEs, then the heterochromatin as a whole could present a high rate of exchanges.

Another factor that could make the heterochromatin of *D. melanogaster* more susceptible to SCEs is its richness in AT bases. A large proportion of Drosophila heterochromatin is composed of three AT-rich satellite DNAs (PEACOCK *et al.* 1973). It might, therefore, incorporate more BUdR than euchromatin, thereby receiving more effect. In addition, there is cytochemical evidence that the heterochromatin of the *Y* chromosome is on the average more AT-rich than that of the *X* chromosome and the autosomes (PIMPINELLI, GATTI and DE MARCO 1975;

GATTI, PIMPINELLI and SANTINI 1976; PIMPINELLI, SANTINI and GATTI 1978). It has also been demonstrated that the *Y* chromosome is particularly resistant to chromosomal aberrations induced by methyl methansulphonate, which preferentially attacks guanine (GATTI *et al.* 1975); however, it is highly sensitive to UV, which produces chromosomal damage through the formation of thymine dimers (PIMPINELLI *et al.* 1977). We suggest that the higher susceptibility of the *Y* chromosome as compared to the other heterochromatic regions is due to a higher rate of BUdR incorporation. Nevertheless, factors other than base composition are probably involved in determining the nonrandom distribution of SCEs between and within chromosomes. Of these factors, considerable importance could be given to a nonrandom distribution among chromosomes of "hot spots" like those of the junctions between euchromatin and heterochromatin or between different heterochromatic blocks.

We are grateful to B. S. BAKER and C. H. HINTON for their critical reading of the manuscript. This work was supported in part by the Association between Euratom and CNR, contract no. 136–74–7 BIOI.

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