X-ray Breakage of Lily Chromosomes at First Meiotic Metaphase[1]

Helen V. Crouse

Department of Biology, Goucher College, Baltimore, Maryland

THE studies on lily chromosomes described in this article demonstrate that x-rays produce an effect on fully condensed meiotic (first metaphase, M I) chromosomes which is clearly detectable at the first meiotic division (first anaphase, A I), *not* in the form of chromosomal or chromatid aberrations, but in the form of bridges here interpreted as *half-chromatid bridges*. These studies clarify the anomaly of "potential x-ray breaks" (*1*) and simplify the interpretation of radiation effects on meiosis reached by Darlington and LaCour (*2*).

from 15 to 45 sec, depending on dose. Control and irradiated anthers were kept in Bonner's solution until they were fixed.

2) *X-ray treatment.* Unfiltered x-rays (10, 15, and 30 r) were given at 20 in. with a tube operated at 75 kv and 10 ma delivering 40 r/min.

3) *Fixation and cytological study.* Because preliminary study showed that microsporocytes at M I reached mid-A I after 2 hr at room temperature, anthers for A I analysis were rayed and fixed 2 hr later. For A II analysis following M I irradiation, the

TABLE 1. Microsporocytes rayed at M I and scored at Mid-A I. Frequency of normal cells and of cells showing acentric chromatid fragments and half-chromatid bridges. No chromatid bridges.

Dose	Slide	Total cells	Total chromosomes	Normal cells	Acentric F	Half-chromatid bridges					Bridges per cell
						1	2	3	4	5	
10	L21-1 = 1	71	852	49	2	16	4	0	0	0	24/ 71 = 0.33
15	L13-1 = 4	122	1464	74	0	36	10	2	0	0	62/122 = 0.50
15	L28-1 = 1	196	2352	107	1	73	13	2	0	0	105/196 = 0.53
30	L11-1 = 1	74	888	28	0	27	12	6	0	1	74/ 74 = 1.00
30	L28-2 = 1	276	3312	99	1	110	52	12	2	0	258/276 = 0.93
30	L28-2 = 2	132	1584	36	2 (2)	59	28	4	0	1	132/132 = 1.00

Lilium longiflorum (commercial variety "Croft") was chosen as material for study, not only because of the extraordinary size and structural clarity of its meiotic chromosomes ($n = 12$), but also because the excised anthers can be cultured artificially from pachytene through second meiotic anaphase (A II) without showing any microscopically visible adverse effects (*3*).

The following experimental procedure was used throughout:

1) *Selection and handling of buds.* Each bud was selected tentatively on the basis of its length. The bud was dissected and five of the anthers were floated in Bonner's solution (*3*). The tip of the sixth anther (all four locules) was smeared in aceto-orcein. If the smear showed meiotic stages no farther advanced than M I, the five remaining anthers were retained: four were irradiated immediately and the fifth was kept as a control. The entire operation of dissecting the bud, smearing an anther, and examining the smear usually took less than 10 min; irradiating the anthers took

[1] This work was done in the Laboratory of Prof. J. Herbert Taylor, Department of Botany, Columbia University, during the tenure of a Faculty Fellowship granted by The Fund for the Advancement of Education, 1952–53. I am indebted to Professor Taylor in countless ways.

anthers had to be cultured 8 to 9 hr. Anthers were fixed in 3 : 1 Carnoy and smeared in aceto-orcein. All slides were scored by the writer.

Although I have studied the response of various meiotic stages of lily to x-rays, the primary objective of this article is to present the results on sporocytes rayed at M I and examined at A I (Table 1). The data on cells rayed at pachytene (Table 2) are presented only by way of comparison: they provide a striking contrast to the data in Table 1, where no chromatid bridges, practically no acentric chromatid fragments, and numerous *half-chromatid bridges* are encountered.

That such bridges cannot be attributed to general chromosome stickiness induced by the irradiation seems evident from the following facts: (i) M I chromosomes fixed immediately after irradiation at 10, 15, and 30 r show no clumping or stickiness; (ii) within the same irradiated cell, certain chromosomes form bridges, while others undergo perfectly normal anaphase disjunction (Fig. 1); (iii) half-chromatid bridges are formed at A II fully 8 hr, after irradiation; and (iv) the frequency of half-chromatid bridges per cell at A I increases linearly with x-ray dose.

The photomicrograph in Fig. 1 and the camera lucida drawing in Fig. 2 show an A I bridge that is

TABLE 2. Microsporocytes rayed at early pachytene with 30 r and scored at mid-A I. Frequency of normal cells and of cells containing chromatid bridges (B) and acentric chromatid fragments (F). No half-chromatid bridges.

Slide	Total cells	Total chromosomes	Normal cells	1B and 1F	1B and 2FF	1B and 3FF	2BB and 2FF	No B, 1F	No B, 2FF	No B, 3FF
L10-1 = 1	86	1032	58	13	4	0	3	6	1	1
L10-1 = 2	88	1056	55	14	2	1	4	9	2	1

interpreted in this article as a half-chromatid bridge. The chromosome involved has a subterminal centromere and, hence, very unequal arms, labeled in Fig. 2. Eleven tetrads have undergone normal anaphase disjunction; the twelfth has formed a half-chromatid bridge, whose structural detail is shown in the drawing. This bridge is distinctly different from a chromatid bridge in that no acentric fragment is formed. Starting at the centromere, the short and long arms of the "free" monad, lying to the right at the upper pole and to the left at the lower pole, can be readily traced. The half-chromatid exchange has occurred in the other monad approximately midway of the long arm, and only at this point of exchange does the duplicate nature of the chromatid become evident (to show this duplicity the space between the half-chromatids is slightly exaggerated in the drawing). On this interpretation, meiotic tetrads at M I are really 8-parted—that is, made up of four chromatids, each composed of two half-chromatids. The half-chromatids belonging to different chromatids are capable of reciprocal exchange following x-ray breakage; such exchange leads to bridge formation when the two chromatids in question are taken to opposite poles (either at A I or A II), because the half-chromatids are not yet free to behave as separate mitotic entities.

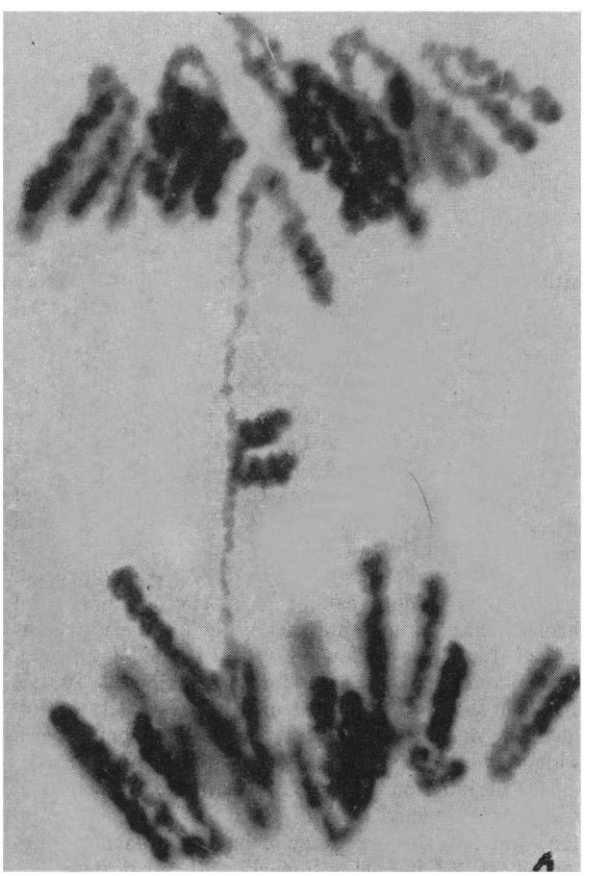

FIG. 1. Chromosomes from an irradiated cell of *Lilium longiflorum*.

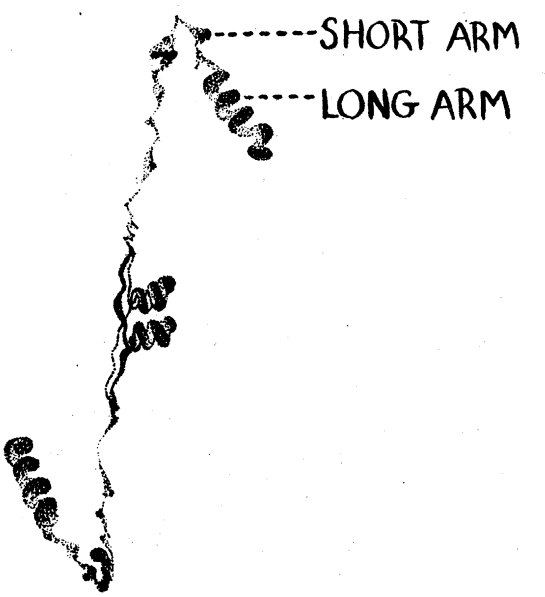

FIG. 2. Camera lucida drawing of half-chromatid bridge shown in the photomicrograph of Fig. 1.

If half-chromatids undergo such x-ray breakage and exchange, the question arises why *both* half-chromatids comprising the *same* chromatid are not broken more often, thereby producing an acentric *chromatid* fragment. On a basis of randomness, such fragments should occur 1/7 as often as half-chromatid bridges. Why they do not is a matter of conjecture. Two (and perhaps more) of the acentric fragments recorded in Table 1 (in parentheses) did *not* originate in this manner but were derived from breakage of a half-chromatid bridge at very early A I.

The behavior of the half-chromatid bridges subsequent to mid-A I has been determined by the author, and these studies will be published fully elsewhere. Suffice it to say first, that the half-chromatid bridges may either (i) persist until A II, or (ii) break at two

points, leaving the middle portion of the bridge as a fragment, or (iii) break at one point only, leaving no fragment. These three alternative modes of behavior depend on the location of the half-chromatid exchange in relation to both centromere position and the relative arm lengths of the bivalent. Second, the half-chromatids do not behave as separate mitotic entities at A I or A II. They should do so at the microspore division; and theoretically the sporocytes showing half-chromatid bridges at either A I or A II should give a large proportion of spores with fragments or bridges at the spore division.

References

1. A. H. Sparrow, *Proc. Natl. Acad. Sci.* **30**, 147 (1944).
2. C. D. Darlington and L. F. LaCour, *Heredity* (supplement) **6**, 41 (1953).
3. J. H. Taylor, *Am. J. Botany* **37**, 137 (1950). Also unpublished studies by Taylor and by Helen V. Crouse.

Significance of the Gordon Research Conferences

Emil Ott

Research Department, Hercules Powder Company, Wilmington, Delaware

SINCE this issue of *Science* contains the program of the 1954 Gordon Research Conferences and W. George Parks's editorial with its revealing statistics, a review of the significance of the Gordon Research Conferences to chemical science seems appropriate. The addition this year of a third conference site at Kimball Union Academy, Meriden, N. H., permits further expansion of the impressively rich and varied program. This growth gives pleasure to those who have seen the venture from its cautious beginnings around 1931 at Johns Hopkins University.

Growth of scientific meetings is, however, not always a sign of corresponding increasing value; to the contrary, size and value may not be favorably related from the point of view of scientific gains. In the case of the Gordon Research Conferences, their unique value on the American scientific scene has been maintained in spite of a substantial increase; moreover, the scope has been advantageously enlarged. It may be profitable to describe and analyze some of the factors that have contributed to this favorable picture.

I have been fortunate in being in a position to observe closely the beginning and the maturing of this venture. As my colleague at Johns Hopkins University, Neil E. Gordon revealed to me his hopes, plans, and visions. He felt that something worth while for American science could be accomplished by informal mingling of scientists working in certain fields. Particularly did he feel that the discussion should be concerned with the frontiers aspects. Hence, it would be important to have a recognized leader in the field as chairman for each particular conference. The group would also require discussion leaders or speakers who were concerned with actual advances of scientific work and who would be present by invitation. To these could be added others who, after publication of the program, felt the desire to attend for the purpose of learning and contributing. These additional members would be carefully screened to keep the group to a productive and creative size, to provide maximum quality and to insure representation of as many laboratories and institutions as possible. Representation from academic, governmental, and industrial institutions, Dr. Gordon felt, should be mixed in suitable manner. Size of a given conference would always have to be limited with emphasis on quality.

Although the main theme would be chemistry, Gordon realized from the beginning that the infusion of neighboring fields, such as physics, mathematics, and biology, would be vital.

With these points in mind, Dr. Gordon started the first experimental conferences on the Homewood Campus of Johns Hopkins University. He felt, however, that a location in the country would be far more ideal. There, the distractions of the city would not exist and the general meetings could be followed by small group discussions, possibly in connection with golf or swimming. He visualized the Gibson Island Club on Chesapeake Bay as a suitable place, and soon the conferences were transferred there.

When Dr. Gordon left Johns Hopkins, the University carried on for a while experimenting with two other locations. Later, Dr. Gordon took over again, establishing eventually a permanent house at Gibson Island. At that time, sponsorship was assumed by the American Association for the Advancement of Science, a logical arrangement which has continued to this day.

Interest in new fields for discussion developed continuously, and, from time to time, the program has been enlarged.

During the formative period, major parts of a mechanism for the perpetuation of the conferences in vital and creative form were developed. Dr. Gordon, as director, felt strongly that each conference should be as autonomous as possible. Although the chairman of a first conference had to be appointed, a system of election by the conference members was soon evolved.