THE RELATIONSHIP OF CHIASMATA AND CROSSING OVER IN LILIUM FORMOSANUM

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THE origin of chiasmata has been a subject for controversy since JANSSENS (1909, 1924) proposed that they result from crossing over. Although the partial chiasmatype hypothesis has been widely adopted at various times and places, its general validity has recently either been questioned or not accepted in research reports (COOPER 1949; RHOADES 1946; STEINITZ-SEARS and SEARS 1953), in review articles (STURTEVANT 1951; HUGHES-SCHRADER 1952), and in an introductory text-book (SRB and OWEN 1952). The bearing of some of these demonstrations on the chiasmatype hypothesis will be considered in the discussion, as well as the alternative hypotheses of SAX and MATSUURA.

Evidence in favor of the chiasmatype hypothesis has been presented by many different workers and from a wide variety of different organisms. Some demonstrations point to a parallelism between genetic and cytological phenomena such as the similar effect of temperature differences on frequency of crossing over and of chiasma-formation; these indirect demonstrations have been summarized by MATHER (1938) and will not be considered in detail in this report.

A correspondence between the frequencies of chromosome associations and recombination of genetic markers has been shown in only one case, that of BEADLE'S (1932) Zea-Euchlaena hybrid in which 20 percent associations in a specific chromosome region led to the expectation of 10 percent crossing over, a value very close to the observed 12 percent crossing over. However, as emphasized again by COOPER (1949, p. 112) BEADLE was careful to point out that he did not have direct cytological evidence that the observed associations were really chiasmata.

Double interlocking of two bivalents has been considered an excellent demonstration of the chiasmatype hypothesis (MATHER 1938). However, only three examples have been offered in support of the hypothesis (two in Lilium, MATHER 1933; BEAL 1936, and one in Eremerus, UPCOTT 1936). Furthermore, as pointed out quite clearly by MATSUURA (1944), the critical chiasma has apparently been identified only by analogy. A single twist of the two homologues prior to pairing could result in a similar node. Until an accurate discrimination between these two possibilities has been made, this sort of demonstration can carry no weight as evidence.

Other types of cytological evidence are based on studies of multivalent configurations or structural heterozygotes. Certain single, intercalary chiasmata in quadrivalents and trivalents have been shown by DARLINGTON (1930) and DARLINGTON and MATHER (1932) to be inexplicable except as the consequence of genetic crossing over unless one assumes multi-strand, rather than 2-by-2 pairing at zygotene. A

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similar conclusion was reached by MATHER (1935) from observations of association of small fragments with normal bivalents in Lilium. In neither of the first two examples was it apparently possible for the workers to validate their interpretations through identification of exchange types during the subsequent anaphase, and their deductive conclusions have thus remained in need of such substantiation. In the case of the fragment chromosomes, however, such a validation should have been possible from a study of anaphase separations and the fact that the expected exchange types were not reported casts doubt on the interpretation of the observed associations as chiasmata.

In rings formed by translocation heterozygotes of 6 or more members, a chiasma between interstitial homologous sections of two otherwise non-homologous chromosomes will result in a "figure-of-eight" rather than an open ring configuration. According to DARLINGTON (1931), such an interstitial chiasma could result only from genetic exchange, and would provide an explanation for the origin of new types of rings in Oenothera. Numerous examples of interstitial chiasmata were later found by SANSOME (1932) in a translocation heterozygote of *Pisum sativum*, and she was able to identify the chromatids in several of them. A "figure-of-eight" configuration was found also in a complex translocation heterozygote of Datura by BERGNER and BLAKESLEE (1932) who were able to recover an expected crossover type. For Datura, no quantitative data were offered to enable a comparison of the frequency of interstitial chiasmata with that of certain crossover types among the progeny.

Heteromorphic bivalents have been observed in many different plants and animals but have been little used in support of the chiasmatype hypothesis. KOLLER (1938) reported a consistent relationship between chiasma frequency and equational separations in the heteromorphic sex chromosomes of the golden hamster. HAGA's (1944) analysis of a small terminal deficiency in Paris showed a significantly higher proportion of equational separations at anaphase than would have been expected from the observed number of chiasmata. More recently, BARTON (1951) found neither chiasmata nor crossing over in a heteromorphic example of the nucleolus-organizer arm of chromosome 2 of tomato. Evidence obtained from heteromorphic bivalents must be considered also with regard to the possibility of equational separation of the centromere, and this problem will be given further attention in the discussion.

Critical analyses of inversion heterozygotes have not been provided. DARLINGTON (1937) has diagrammed several of the theoretical expectations of crossing over in inversion heterozygotes. On the basis of the chiasmatype hypothesis, various types of asymmetric bivalents and characteristic "inversion" chiasmata would be expected at diakinesis and metaphase, but actual demonstrations of such configurations have been meagre.

In summation, the evidence in favor of the chiasmatype hypothesis is fragmentary and incomplete. It seems worthwhile, therefore, to re-examine the relation between chiasma formation and crossing over in an organism, such as Lilium, which readily permits the identification both of chiasmata and of the exchange types at anaphase. The present report provides information obtained from two terminal deficiencies and a paracentric inversion.

MATERIALS AND METHODS

Seed of *Lilium formosanum* was obtained from a commercial source, and the plants were grown in the greenhouse and field at Berkeley. The cultures show variation in easily noted vegetative characteristics such as vigor and anthocyanin pigmentation; a partially male-sterile condition, presumably due to a recessive gene, is appearing in some populations. Meiotic irregularities were found in two of the aberration types to be described later. However, routine examination of the pollen-mother cells of several hundred other plants has revealed only the usual meiotic sequence.

Structural heterozygotes were found among the progeny obtained after use of pollen treated with a 1000r dose of X-rays. Tetraploid individuals were produced by means of EMSWELLER and LUMSDEN'S (1943) method of treating bulb-scales with colchicine.

Anthers were fixed for 24 hours or longer in the 3:1 alcohol-acetic mixture and their contents were examined in temporary aceto-carmine mounts. Because the anthers were quite large, they were cut into nine or ten sections before smearing. Usually only a few such sections were needed to give an adequate number of cells. The cells at the edge of the slide were obscured by the sealing compound and large clumps of cells were routinely excluded from the tallies; the totals for each anther section were much larger, therefore, than is apparent from the tabular entries. In a few cases the preparations were exceptionally good and only 3 or 4 unanalyzable cells occurred on each slide; these few cells were not tallied and are not listed in the tables. In several cases, when only two slides from adjacent anther sections were available, individual slide tallies were not made during the collection of the data.

STEWART'S (1947) karyotype of the closely related species, *L. philippinense*, was used for identification of the chromosomes.

TERMINAL DEFICIENCIES

Chromosome A

This deficiency included about two thirds of the short arm and was apparently terminal since no chiasmata were observed which united the ends of the deficient and the normal short arms. The heteromorphic homologues always formed a bivalent with at least one chiasma in the long, normal arm. For the deficient arm, no configurations were observed which could not be explained as due either to the absence of a chiasma (fig. 1, 2) or to the presence of a single chiasma (fig. 3–5). At first anaphase, separations of the deficient arm could be readily classified as equational (fig. 7, 8) or reductional (fig. 6).

Table 1 presents the results of tallies made with the original X_1 plant and with an X_2 plant obtained by selfing the original plant. In both cases, it was possible to compare metaphase and first anaphase stages obtained from the same part of a single anther, and, in both cases, there was an excellent correspondence between the occurrence of chiasmata at metaphase I and equational separations at anaphase I. Three of the four heterogeneity tests showed good agreement of frequencies among the several slides examined. With respect to the significantly heterogeneous set (anaphase, table 1b), an observation made frequently during the course of this

TABLE 1

Chiasmata at metaphase I and segregation at anaphase I for a deficient short arm of chromosome A

a. Field grown X_1 plant (018-33), collected Sept. 7, 1951; five slides from the upper half of one anther

Slide no.	Metaphase		Anaphase		Not analyzable	
	Xma	No Xma	Eq.	Red.	Meta.	Ana.
1	100	40	33	11	2	6
2	51	26	35	11	15	8
3	74	28	30	13	20	7
4	74	35	73	33	7	18
5	126	54	1	1	5	0
Totals	452	183	172	69		
Heterogeneity						
χ^2	1.2		1.6			
df	4		4			
P	>0.80		>0.80			
χ^2, metaphase vs. anaphase totals	0.17					
df	1					
P	>0.50					

b. Field grown X_2 plant (018-33 selfed), collected Sept. 8, 1953; five slides from the upper half of one anther

1	34	37	9	4	3	1
2*	—	—	18	20	—	5
3	27	22	6	7	4	0
4	29	24	9	17	2	2
5	30	31	39	19	2	4
Totals	120	114	81	67		
Heterogeneity						
χ^2	1.0		10.2			
df	3		4			
P	>0.80		>0.02			
χ^2, metaphase vs. anaphase totals	0.42					
df	1					
P	>0.50					

* This slide was slightly overstained, and metaphase tallies were not made.

work is pertinent. It has repeatedly been noted that cells of one class; i.e., with or without a chiasma in a specific region, tend to occur in groups or relatively restricted regions on a slide. This sort of clumping effect would lead to a lack of homogeneity among several slides made from one anther if only a few cells of a specific stage were present on each slide. It is believed to be responsible for the high heterogeneity chi-square just mentioned. Since tallies for single slides with more cells of a given stage would already include heterogeneous groups of cells, it seemed reasonable to add together the anaphase tallies from these slides. If, however, a significant discrepancy between the totals for anaphase and metaphase had thereby resulted, the heterogeneity would have weakened the conclusion that a correspondence did not exist.

In the X_1 plant, the frequencies of chiasmata at metaphase I and of equational separations at anaphase I for the deficient arm were 70 and 71 percent, respectively, while in the X_2 plant, the respective values were 51 and 55 percent. When the frequencies of chiasmata at metaphase in the two plants were compared statistically, a χ^2 of 25.7 was obtained, with df = 1, and $P < 0.01$. The comparison for anaphase between the two plants was also highly significant, $\chi^2 = 11.2$, df = 1, and $P < 0.01$. It is thus of interest to note that the correspondence between frequency of chiasmata and equational separation is maintained for a specific chromosome region even though the frequency of both events is markedly different between the two plants. Since the two plants presumably differed genetically and were grown during different years, any or all of several factors may have been responsible for the difference in frequency.

Chromosome I

This deficiency included about two thirds of the long arm and was apparently terminal. The first collections available for comparison came from two field-grown X_1 subdivisions, and were obtained within 8 days at the peak of the summer growing season (table 2a). The second collection was made the following year from another X_1 subdivision but early in the growing season and shortly after the plants had been transplanted from the greenhouse to the field. Meiosis was normal in the material collected in 1952 but that collected in 1953 showed partial desynapsis with as many as six univalents present at late diakinesis or metaphase.

From the collections made in both years, the presence or absence of chiasmata in the deficient chromosome (fig. 9, 10) and the type of separation at first anaphase (fig. 11, 12) could be determined. For the 1952 material, only the early diakinesis stage was available for the investigation of chiasmata. At this stage it was not possible to identify the centromere so that the arm in which a single chiasma occurred had to be determined by the distance of the chiasma from either end of the chromosome. Measurements made from mitotic division figures gave a ratio of 4:1 for the length of the deficient long arm to that of the normal short arm. This ratio was then used as a basis for classifying the chiasmata according to the arm in which they occurred. In this fashion, all but 16 of 168 chiasmata (table 2a) were accurately located with the majority occurring at or near the end of either arm. In every case in which two chiasmata were observed, each was clearly in a different arm. The

TABLE 2

Chiasmata at diakinesis and segregation at anaphase I for a deficient long arm of chromosome I

a. Field grown X_1 subdivisions (018-118); diakinesis from subdiv. no. 6, collected Aug. 19, 1952; anaphase from subdiv. no. 4, collected Aug. 11, 1952

Tallies at early diakinesis (one slide)		Chiasma in deficient arm:	
		Present	Absent
One chiasma in deficient arm	121	121	
One chiasma in short arm	10		10
One chiasma in each arm	21	21	
No chiasma	3		3
One chiasma, location not determinable	16	*Corr. 8*	*8*
Unanalyzable cells	27		
Totals: Uncorr.	155	142	13
Corr.	*171*	*150*	*21*

*Tallies of type of anaphase I segregations from anaphase I and II (two slides each from different anthers of one flower)**

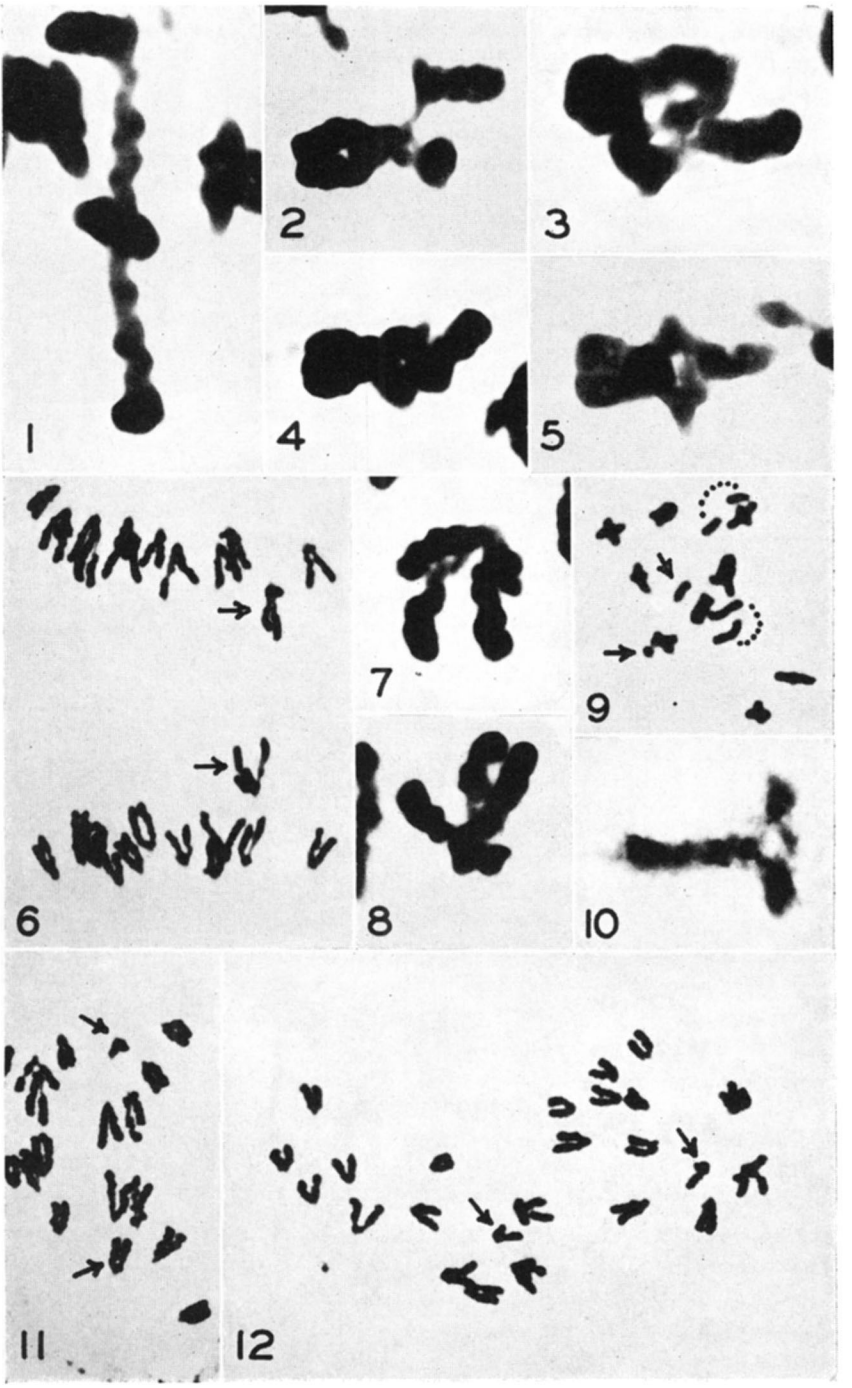
	Equational	Reductional
Anaphase I	90	10
Anaphase II (whole meiocytes)	33	5
Totals	123	15

χ^2, totals of diakinesis uncorr. vs. anaphase, = 0.27, df = 1, P > 0.50.
χ^2, totals of diakinesis corr. vs. anaphase, = 0.043, df = 1, P > 0.80.

*b. Field grown X_1 subdivision (018-118), collected June 26, 1953 (four slides from the lower third of one anther)**

Slide no.	Late diakinesis		Anaphase I	
	Xma	No Xma	Eq.	Red.
1	193	52	27	14
2	80	20	28	9
3	0	0	73	16
4	0	0	21	4
Totals	273	72	149	43
Heterogeneity				
χ^2	0.014		4.9	
df	1		3	
P	>0.90		>0.10	
χ^2, late diakinesis vs. anaphase totals	0.1			
df	1			
P	>0.70			

* Unanalyzable cells not tallied, see text.



FIGS. 1–12

16 chiasmata whose location could not be determined with sufficient accuracy to assign them to a specific arm were assumed to occur with equal frequency on either side of the centromere, and were thus apportioned as the corrected values in table 2a. Their inclusion in the totals obviously makes very little difference for the statistical comparison.

In the 1953 material, counts of chiasmata were made at late diakinesis, at a time when the centromere could be recognized, and only the chiasmata occurring in the deficient arm were tallied (table 2b). Observations on desynapsis in this material will be summarized in the next section.

For the 1952 collection, a single chiasma in the deficient arm was found in 90 percent of the meiocytes while equational separations occurred in 89 percent of the anaphases. For the 1953 collection, the corresponding values were 79 percent for diakinesis and 78 percent for anaphase. Comparisons of the 1952 results with those of 1953 give statistically significant differences: for the diakinesis stages (corrected values), $\chi^2 = 5.1$, df = 1, $P < 0.05$; for the anaphase stages, $\chi^2 = 6.6$, df = 1, $P < 0.02$. As with the heteromorphic A bivalent, the heteromorphic I bivalent gave the same correspondence between frequency of chiasmata and equational separations under two different conditions. Because both sets of examples of the heteromorphic I bivalent were taken from original X_1 subdivisions, environmental factors alone are to be considered responsible for the observed differences.

Desynapsis in the heteromorphic I material of 1953

The meiocytes from the 1952 collection showed regular associations of the normal bivalents. The heteromorphic homologues appeared as univalents in only a small percentage of cases and no laggards were observed at anaphase. In the 1953 material, the heteromorphic homologues, as well as the normal homologues, frequently appeared as univalents at diakinesis (fig. 7) and later stages. The failures in bivalent formation in the 1953 material should be attributed to environmental factors because the collection was obtained from a subdivision of the original X_1 plant which had previously shown normal meiosis. The presence of univalents at diakinesis and metaphase seemed to be the result of desynapsis rather than asynapsis. Examination of pachynema, which is very clear in this material, showed little or no failure in pairing, and, at diakinesis, many of the homologous univalents were lying close together and oriented in a parallel fashion. Both of the heteromorphic homologues frequently lagged at first anaphase and also showed precocious division in a small percentage of meiocytes. Table 3 summarizes the observations of univalents from

FIGURE 1–12.—Diakinesis, metaphase, and anaphase I of heteromorphic bivalents, various enlargements from ca. 500× to ca. 2350×. Figure 1, 2. Heteromorphic A bivalent at metaphase I with no chiasma in deficient arm. Figure 3–5. Heteromorphic A bivalent at metaphase I with one chiasma in deficient arm. Figure 6. Heteromorphic A homologues (arrows) separating reductionally at anaphase I. Figure 7, 8. Heteromorphic A homologues separating equationally at anaphase I, the two dyads from opposite poles of one cell. Figure 9. Heteromorphic I homologues (arrows) unpaired at late diakinesis in material showing desynapsis; note the fairly close approximation of the other two pairs of univalents (connected by dotted lines). Figure 10. Heteromorphic I bivalent at diakinesis with chiasma in deficient arm. Figure 11. Heteromorphic I homologues (arrows) separating reductionally at anaphase I. Figure 12. Heteromorphic I homologues (arrows) separating equationally at anaphase I.

TABLE 3

Univalents at diakinesis in the heteromorphic chromosome I clone, 1953 collection

Configurations of		No. of meiocytes
Normal homologues	Heteromorphic I	
11 II	1 II	185*
	2 I	46
10 II, 2 I	1 II	75
	2 I	12
9 II, 4 I	1 II	20
	2 I	5
8 II, 6 I	1 II	2
	2 I	0

Normal homologues:	Heteromorphic homologues:	
	as bivalent	as univalents
no univalents	185	46
2–6 univalents	97	17

$\chi^2 = 0.96$ df = 1 $P > 0.30$

* In 9 cases, the two homologues were associated by the short arm only; i.e., no chiasma in the heteromorphic arm.

the same two slides which provided the data on chiasmata in table 2b. It is of interest to note that there is no statistically significant difference in frequency of heteromorphic univalents on comparison of the meiocytes which otherwise lack univalents with those which have 2 to 6 univalents. There is thus no evidence here for the sort of competition for chiasmata described by MATHER and LAM (1935) and MATHER (1936). However, among the 345 cells examined, the heteromorphic pair appeared as univalents in 63 while all other homologues gave only 121 pairs of univalents. A negative correlation of chiasma frequencies may, therefore, be masked in this instance by the relatively much greater probability of univalent formation by the heteromorphic rather than the normal pairs.

A PARACENTRIC INVERSION

An X_1 plant showing approximately 50 percent pollen abortion proved on further examination to be heterozygous for a long paracentric inversion. A majority of meiocytes at anaphase I or II had acentric fragments and bridges or loops each of uniform size (table 4c, fig. 24–27). Observations at anaphase II, when the short arms can be easily recognized, enabled an identification of chromosome H as the altered member. Because the inversion chromosome can not be recognized as such during mitosis, it has not, however, been possible to discriminate with complete certainty between chromosome H and the very similar chromosome I.

In *L. formosanum* pachynema is unfavorable for the identification of inversions because of the numerous intertwinings of the long threads. At anaphase I, a comparison of the length of the fragment with that of the loop and noncrossover chromatids

TABLE 4

Chiasmata and crossing over in two X_2 plants heterozygous for a paracentric inversion

*a. Chiasmata within the inverted region at diakinesis; Plant No. 221-11, collected August 30, 1954, Plant No. 221-19, collected September 3, 1954; three slides each from one anther**

	Configuration type †	Plant No. 221-11		Plant No. 221-19	
	Two chiasmata				
1	Symmetric	(7, 9, 9)	25	(8, 9, 8)	25
2	Asymmetric	(14, 6, 16)	36	(16, 12, 16)	44
	Sub-total		61		69
	One chiasma				
	Symmetric				
3	Open	(4, 3, 6)	13	(7, 6, 5)	18
4	Not distinguishable	(6, 5, 8)	19	(6, 7, 3)	16
	Asymmetric				
5	B-type	(7, 3, 8)	18	(4, 3, 4)	11
	$-type				
6	Open	(1, 2, 4)	7	(2, 2, 2)	6
7	Not distinguishable	(2, 3, 5)	10	(1, 1, 2)	4
	Sub-total		67		55
8	*No chiasma*	(7, 3, 7)	17	(5, 1, 3)	9
	Total		145		133
	Non-analyzable	(3, 2, 4)	9	(4, 2, 2)	8

b. Chiasmata exterior to the inverted region at diakinesis; collections and slides as in part a, above

Inversion chiasmata	Plant No. 221-11	Plant No. 221-19	Combined totals
With chiasma(ta) in inverted region			
1 proximal, 1 distal	127	124	
1 proximal	1	0	
Totals	128	124	(252)
With no chiasma in inverted region			
1 proximal, 1 distal	9	7	
2 proximal, 1 distal	0	1	
1 proximal, 2 distal	2	0	
1, proximal or distal	6	1	
Totals	17	9	(26)

* Frequencies for the individual slides are listed in order in parenthesis.

† Types of bivalents are numbered for convenience of reference.

TABLE 4--*Continued*

c. Configurations at anaphase I, collection dates as cited in part a, above, one slide each from a different anther of the same flowers used for diakinesis

Number of fragments	Plant No. 221-11	Plant No. 221-19
2 fragments		
double bridge	11	16
two loops	9	13
Sub-total	20	29
1 fragment		
bridge	39	65
loop	41	58
Sub-total	80	123
0 fragment	21	45
Total	121	197

d. Statistical comparison of frequencies of chiasma formation within inverted region, and crossover products at anaphase I

Plant No. 221-11					Plant No. 221-19				
Frequencies of observed configurations at diakinesis		Expected crossover products at anaphase I			Frequencies of observed configurations at diakinesis		Expected crossover products at anaphase I		
		0 frag.	1 f.	2 f.			0 frag.	1 f.	2 f.
2 chiasmata	61	15.25	30.50	15.25	2 chiasmata	69	17.25	34.5	17.25
1 chiasma	67		67		1 chiasma	55		55	
0 chiasma	17	17			0 chiasma	9	9		
Expected totals		32.25	97.50	15.25	Expected totals		26.25	89.5	17.25
Observed frequencies at anaphase I		21	80	20	Observed frequencies at anaphase I		45	123	29
$\chi^2 = 2.36$ df = 2, P > 0.30					$\chi^2 = 0.81$ df = 2, P > 0.50				

e. Statistical comparison of frequencies of chiasma-formation proximal to the inverted region and types of separations at anaphase I

Plant No. 221-11				Plant No. 221-19			
Frequencies of observed configurations at diakinesis (with 1 or 2 chiasmata in the inverted region)		Expected separation products		Frequencies of observed configurations at diakinesis (with 1 or 2 chiasmata in the inverted region)		Expected separation products	
		Bridge	Loop			Bridge	Loop
1 proximal chiasma	128	64	64	1 proximal chiasma	124	62	62
0 proximal chiasma	0	0	0	0 proximal chiasma	0	0	0
Expected totals		64	64	Expected totals		62	62
Observed frequencies at anaphase I		50	50	Observed frequencies at anaphase I		81	71
$\chi^2 = 0.00$ df = 1, P = 1.00				$\chi^2 = 0.31$ df = 1, P > 0.50			

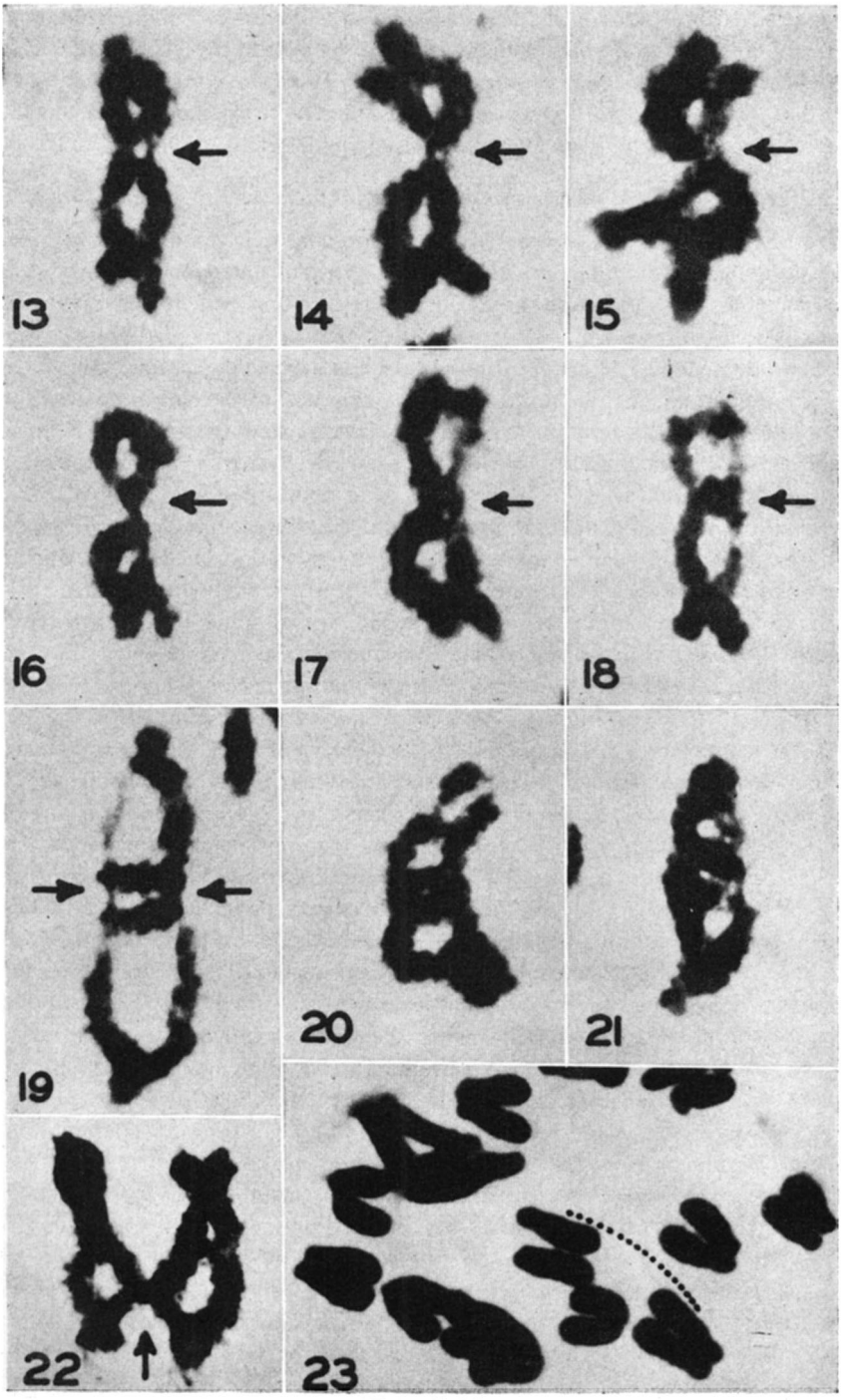
showed the center of the inverted region to be slightly closer to the centromere than to the end of the chromosome. At diakinesis, bivalents with two chiasmata within the inverted segment enabled an estimate of the length of the inversion as at least one third that of the arm. On the basis of these estimates, it was possible to construct a model of the probable pachytene configuration (fig. 28a).

Inversion configurations at diakinesis

According to the chiasmatype hypothesis crossing over within a long inversion should lead to the formation of characteristic types of inversion bivalents at diakinesis. Some of these configurations, if found, would offer evidence in support of the chiasmatype hypothesis while others could be explained just as readily in accordance with a two-plane model. The appearance of the expected configurations will, of course, be determined in part by the morphology and behavior of the chromosomes in which they occur; they will, therefore, be described here as we have observed them in lily chromosomes. The configurations have been observed only in two X_1 plants, each heterozygous for a paracentric inversion, and in only one of the 12 bivalents; they occurred infrequently for the first inversion which had little double crossing over and was not studied further and commonly for the inversion described above. The results of a quantitative study of the latter will be given in the following sections.

A single crossover within the inverted region will produce one acentric and one dicentric chromatid. The single chiasma resulting from such a crossover will therefore be a *reversed chiasma* where two of the four chromatids become changed in direction. Such chiasmata have previously been termed inversion chiasmata by DARLINGTON (1937) and elsewhere; his terminology becomes confusing when two chiasmata occur in the inverted segment. On the assumption that diplotene opening out occurs at random, the reversed chiasma will be either *open* or *interlocked* in a one-to-one ratio (fig. 29). Because only two, instead of four chromatids are continuous through the region of the chiasma, the open reversed chiasma would be expected to form a region of mechanical weakness. The open chiasmata have been readily identified according to the criterion that only two chromatids connect the two parts of the bivalent at this point, and, in contrast to ordinary chiasmata, many were considerably stretched on smearing. In the first few examples observed, the "yoke bivalents" reported by BROWN and ZOHARY (1953), the two continuous chromatids were so badly stretched that they were no longer identifiable as such; configurations of this type were not analyzed with certainty until after unstretched examples and others with intermediate degrees of distortion had been found. Examples of open, reversed chiasmata are shown in fig. 13, 14, 16, 17.

If a single crossover occurs near either end of the inverted region, an asymmetric bivalent (fig. 14–18, 28b) will result which has either a B- or $-shape depending on whether the two long segments lie on the same or opposite sides of the bivalent. Presumably the difference between the B- and the $-bivalents is determined by chance during the smearing process. The external chiasmata usually found in the inversion bivalent have without doubt been partially responsible for the internal stretching which gives a very striking appearance to many of the asymmetric bivalents. Several hundred clear examples of asymmetric bivalents have been



FIGS. 13–23

observed in preparations from different collections. In general, the open reversed chiasmata have been much more readily identified in the $-$ than in the B-bivalents.

If double crossing over occurs within the inverted region, the part of the inversion loop between the points of crossing over will be arrested between the two resultant chiasmata in the diakinetic bivalent (fig. 19–21, 28c). Both of the chiasmata will be measured whether from 2-, 3-, or 4-strand crossing over. If the two points of crossing over are equidistant from the center of the inversion, the bivalent will appear symmetrical. If one is farther removed from the center than the other, the bivalent will be asymmetric, and the asymmetry will always be of the B-type because both long segments will be attached to the same side of the arrested loop segment. Numerous examples of double crossing over within the inverted region have been identified; because of the compact nature of these bivalents, open reversed chiasmata have not been readily identified although frequently one of the two chiasmata will show the mechanical weakness expected with the open form.

Scoring of inversion configurations

Material for the two quantitative comparisons each came from a single flower one anther of which provided material for one or two slides of anaphase I, another, for three slides of diakinesis. For both stages, the cells were examined first under low power (150×). The anaphase I examinations were restricted to isolated cells at mid-anaphase and all were analyzable. For diakinesis, only those cells which showed twelve clearly separated bivalents were chosen for further study. This stringent selection was necessary to assure favorable material for the quantitative classification of the inversion configurations; they are only rarely recognizable under low power so that little or no bias was to be expected from this method of selection.

Of the eight types of diakinetic configurations listed in table 4a, all but two, types 4 and 8, could be readily recognized under oil immersion (1350×). With types 4 and 8, the inversion bivalent had to be distinguished from one other bivalent very similar to it in size. If both of these bivalents either had or lacked a chiasma in the proper region, no further discrimination was necessary. In a few instances, only one of the two bivalents had a chiasma in the appropriate region and in these cases the identification of the inversion bivalent was based on the fact that its external chiasmata are usually sharply localized with the distal one being about twice as far as the

FIGURE 13–21.—Inversion bivalents at diakinesis (ca. 2100×). Figure 13. Symmetric bivalent with open reversed chiasma (arrow). Figure 14. Asymmetric, $-shaped bivalent with open reversed chiasma (arrow). Figure 15. Asymmetric, $-shaped bivalent with reversed chiasma (arrow) in which the chromatid arrangement is not distinguishable. Figure 16. Asymmetric, B-shaped bivalent with open reversed chiasma (arrow) in which the two continuous chromatids are superimposed. Figure 17. Asymmetric, B-shaped bivalent with open reversed chiasma (arrow). Figure 18. Asymmetric, B-shaped bivalent with reversed chiasma (arrow) in which the chromatid arrangement is not distinguishable and the two longer segments are overlapping. Figure 19. Asymmetric bivalent with two chiasmata in the inverted region (arrows); the axis of the arrested loop segment is at right angles to the bivalent; the chiasma indicated by the left arrow is apparently an open reversed chiasma. Figure 20, 21. Asymmetric bivalents with two chiasmata in the inverted region. Figure 22. Quadrivalent from tetraploid material demonstrating interstitial chiasma (arrow); (ca. 2100×). Figure 23. Precocious separation of centromeres in anaphase I (ca. 1700×); dotted lines connect homologous dyads; the two daughter centromeres of the upper dyad are widely separated.

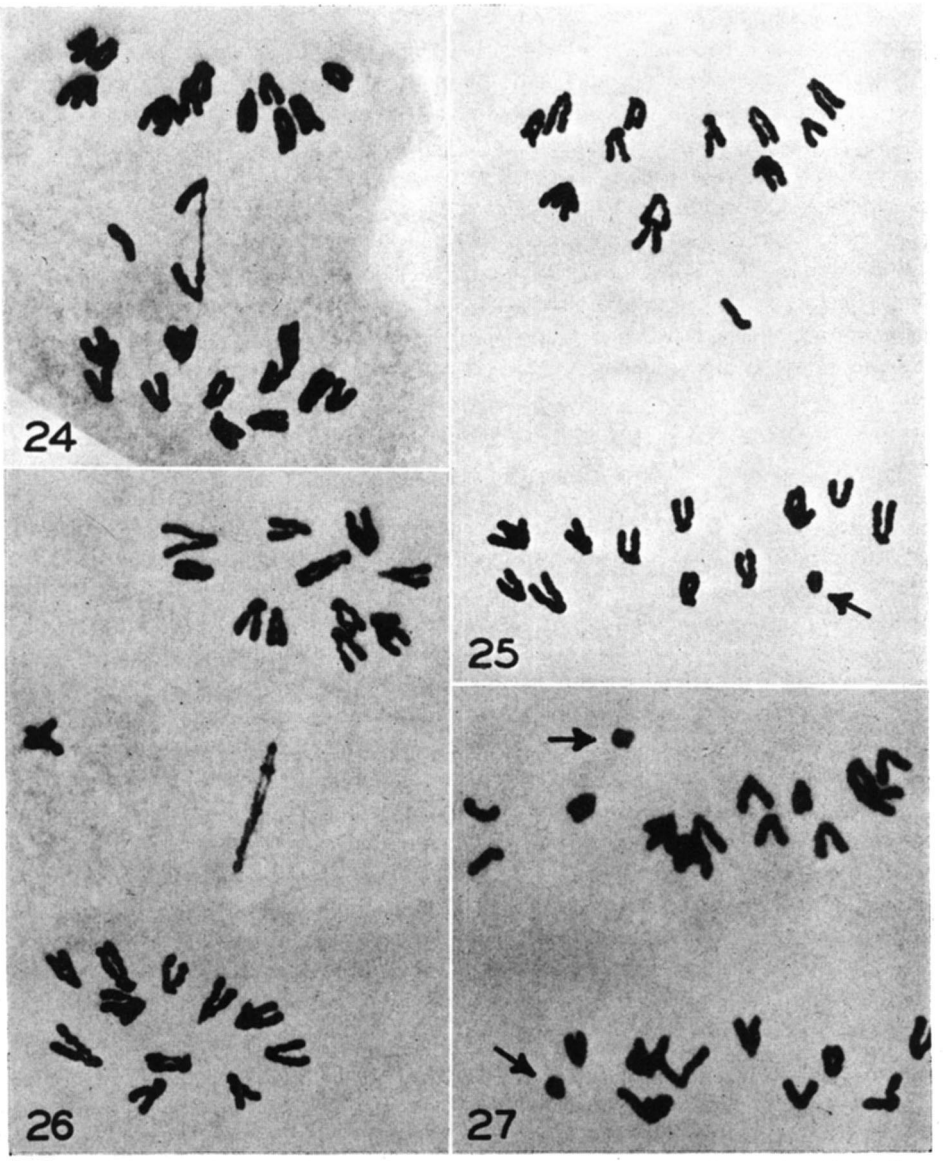


FIGURE 24–27.—Products of crossing over in the inverted region at anaphase I (ca. 650×). Note uniform size of fragments and loops. Figure 24. Single chromatid bridge and single fragment. Figure 25. Single loop chromatid (arrow) and single fragment. Figure 26. Double chromatid bridge and two fragments adhering to each other. Figure 27. Two loop chromatids (arrows) and two fragments.

proximal from the adjacent chromosome end (fig. 13–21). It seems likely that not more than two or three of the bivalents tallied in table 4a could have been misclassified, and these would be, and with apparently equal probability in each case, either type 4 or type 8 mistaken for the other.

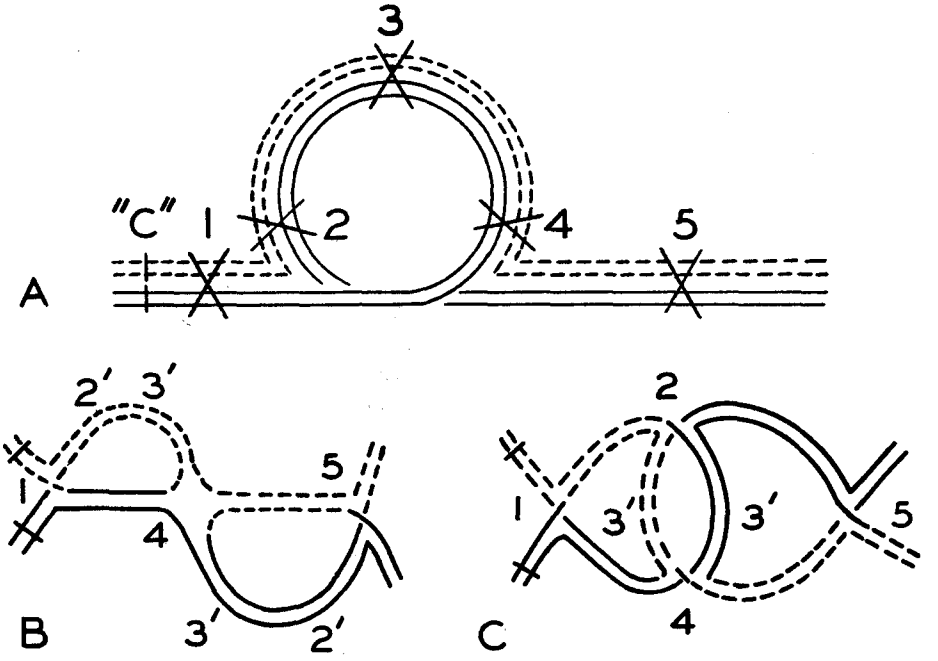


FIGURE 28.—A. Model of probable pachytene configuration of the inversion bivalent. "C" marks centromere position. The numbers, 1–5, refer to regions in which crossing over may occur. Regions 1, 5 are exterior: 1, proximal, 5, distal. Regions 2–4 are within the inverted segment. Single exchanges at 3 produce symmetric bivalents, at 2 or 4, asymmetric. Double exchanges at 2 and 4 produce symmetric bivalents, at 2 and 3 or 3 and 4, asymmetric.

B. Diagram of the chromatid relationships in a diakinetic bivalent expected according to the partial chiasmatype hypothesis following crossing over in regions 1, 4, and 5. Regions in which crossing over did not occur are indicated by primes. This diagram of an asymmetric $-shaped bivalent shows only one possibility; the same general relationships would exist in the B-type of asymmetry, and the reversed chiasma (4) could be either open, as shown, or interlocked (see fig. 29).

C. Diagram of the chromatid relationships in a diakinetic bivalent expected according to the partial chiasmatype hypothesis following crossing over in regions 1, 2, 4, and 5. Following double crossing over within the inverted segment the portion between the two exchanges, between regions 2 and 4, becomes arrested and will appear to be held at right angles to the main axis of the bivalent.

The unanalyzable cases at diakinesis were those in which the orientation of the bivalent prevented analysis of the chiasmata although the inversion bivalent was sometimes recognizable as such. Because of its more compact nature, the inversion bivalent with two chiasmata might be expected to contribute disproportionately to the unanalyzable class, and, as will be shown later, this class seems to be deficient.

Quantitative comparisons, internal chiasmata

Table 4a lists the various types of configurations for chiasmata in the inverted segment and the frequencies in which they were observed at mid-diakinesis. The open reversed chiasma (fig. 29) may be readily identified at this stage when a single chiasma only is present and the asymmetry is not of the B-type. However, some clear examples of open chiasmata have been seen also in B-configurations. The interlocked

reversed chiasmata have not been critically demonstrated although in some cases where the configurations are stretched, and overlapping eliminated, interlocking seems the most likely interpretation. If there is a random relationship among the chromatids when the homologues open out, then a 1:1 ratio between the open and interlocked types of reversed chiasmata is to be expected. This ratio is closely approximated by the proportion of those identifiable as open (table 4a, types 3, 6) to those not thus identifiable (types 4, 7). Considering both plants together, there is thus a total of 44 open to 49 not distinguishable or an excellent agreement with the expected 1:1 ratio. Although this quantitative comparison does not prove the existence of the interlocked type, it does give indirect evidence for its occurrence.

A single chiasma laterally placed in the inverted region will yield an asymmetric configuration (table 4a, types 5–7); a centrally placed chiasma will yield a symmetric bivalent (types 3, 4). With two chiasmata, the configuration will be symmetric if both

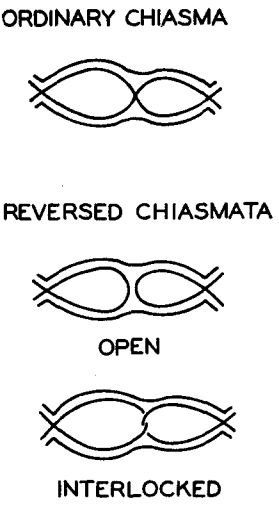


FIGURE 29.—Diagrams showing the chromatid relationships in open and interlocked reversed chiasmata (see text for further explanations) and of an ordinary chiasma for comparison.

are lateral (type 1), and asymmetric if one is lateral, the other central (type 2). The ease with which the symmetric types could be distinguished from the asymmetric testifies again to the pronounced localization of chiasmata in this material. A lateral chiasma may occur in either of two positions while a central chiasma may occur in only one (fig. 28a). Our material possessed no sort of cytological marker, such as a large knob, which would aid in distinguishing the two types of lateral chiasmata; however, an assumption of equal or other relative frequency for the two types is unnecessary for the considerations to follow. It becomes apparent from inspection of the data that there is a much higher frequency of lateral chiasmata when two chiasmata, rather than one, are present in the inverted region. For the following estimate, the frequencies for the two plants summarized in table 4a will be combined. For the single chiasmata, there are 66 central (types 3, 4) to 56 lateral (types 5–7). For the types with two chiasmata, there are 50 with two lateral (type 1), and 80 with

one lateral, one central (type 2) or a total of 80 central to 180 lateral chiasmata. The marked difference in the ratios, 66:56- and 80:180-central:lateral, respectively, may be a reflection of chromosomal interference but may also be partially the result of pairing complications in the region near the ends of the inverted segment. This difference may also be considered in light of the equal expectation, one central to two lateral chiasmata, for both one and two chiasmata on the assumption of equal probability for chiasma formation in each of the three regions.

On the assumption of random relationship among the four chromatids involved in two crossovers, it is possible to calculate from the diakinesis data the frequencies of the expected crossover types and to compare these with those observed at the subsequent anaphase. Table 4d shows the calculations and the comparisons; because the expected values were derived from a sample, a 2 × 3 table was used in making the chi-square test. In general, there is good agreement between the observed and expected frequencies; the major discrepancy is to be found in the larger expectation for the 0-fragment and the smaller expectation for the 2-fragment crossover types than were actually observed. This discrepancy, if not merely a sampling error, may in part be attributed to the somewhat greater difficulty of identifying bivalents with two chiasmata (types 1, 2) because of their more compact nature.

Quantitative comparisons, external chiasmata

As may be seen from table 4b, all but one of the 252 bivalents with chiasmata in the inverted region also have two external chiasmata. Among the 26 bivalents without chiasmata in the inversion, 7 had only one external chiasma. This observation conforms to cytological demonstrations of asynapsis and non-homologous associations in chromosomes with various sorts of rearrangements (McCLINTOCK 1933).

On the assumption of a random relationship among the four chromatids involved in the various exchanges, anaphase I bridges, whether single or double, will be changed into loops in half the cases in which a single proximal chiasma is formed. The almost constant occurrence of a proximal chiasma leads to the expectancy of a 1:1 ratio between loops and bridges at anaphase I. That this expectation is borne out very closely is demonstrated by the anaphase observations reported in table 4c and the statistical comparisons of table 4e; because the expected values were themselves derived from observed samples, 2 × 2 tables were used for the calculation of chi-square. As is the case with the chiasmata in the terminal deficiencies, the evidence from the proximal chiasmata must be considered in relation to the possibility of equational separation of the centromere.

It would seem reasonable to expect the two loop chromatids resulting from certain triple crossovers (2 within, 1 proximal to the inverted region) to be not infrequently interlocked and to appear so at anaphase I. UPCOTT (1937) explained the occurrence of an example of interlocking of bridges at anaphase II by the prior interlocking of two loops at anaphase I but did not directly demonstrate the latter. Among the anaphase I cells tallied in table 4c we found no cases of interlocking of two loops and only one example during a careful search among a considerable number of other cells with two fragments. Although the expected percentage of interlocking would depend on the mode of crossing over envisaged, all those we have considered lead to a

higher expectation than that actually found. This interesting problem can not be further discussed in this report but its study is being continued.

For the work with the inversion, two different X_2 plants were used, and these presumably would differ genetically. The samples, however, were collected at about the same time under good growing conditions. If the frequencies at diakinesis of the 0, 1, and 2-chiasma types are compared, there is no significant difference between the two plants, with $\chi^2 = 3.58$, df $= 2$, $P > 0.10$.

OTHER OBSERVATIONS

Two other observations made during the course of this study are of interest in connection with the relationship of chiasmata to crossing over, and will be briefly described.

The first of these concerns the precocious equational division of the centromere. In some of the material collected from an inversion heterozygote during the poor climatic conditions early in the growing season, clumps of cells were found, among the normal meiocytes, in which some or all of the centromeres had separated equationally during anaphase I (fig. 23). In all such precocious separations, both sister centromeres retained the orientation toward the original pole; among the scores of cells observed, no examples were found in which the two sister centromeres had become oriented toward opposite poles. The significance of this observation will be considered further in the discussion.

In tetraploid material, it was possible to find good examples of quadrivalents with interstitial chiasmata. Such chiasmata have been considered by DARLINGTON (1930) and DARLINGTON and MATHER (1932) to provide evidence in favor of the chiasmatype hypothesis. A photograph is offered of one example in which the change of chromatid partners is evident at the interstitial chiasma (fig. 22). This observation thus provides another confirmatory piece of evidence from the species under consideration.

DISCUSSION

Heteromorphic homologues

In only a few cases have heteromorphic bivalents been used for analyzing the quantitative relationship between chiasmata at diakinesis and type of separation at anaphase. Of the studies on this subject already cited in the introductory section, only that of HAGA (1944) has given results at variance from those expected on the basis of the chiasmatype hypothesis. In Paris HAGA found a significantly higher proportion of equational separations at anaphase I than of chiasmata in the deficient arm at metaphase I. However, the centric regions of the Paris chromosomes remain paired until anaphase separation begins so that chiasmata immediately adjacent to the centric region would be difficult to identify. Thus, the excess of equational separations reported by HAGA is not unexpected in view of the complication imposed by such special centric association.

In Lilium, the present analysis of the heteromorphic homologues and of the proximal chiasmata in the inversion bivalent was simplified by the fact that not more than one chiasma was present. In use of this information as evidence in support of the partial chiasmatype hypothesis one also should consider, however, the possibility

that the centromere may separate equationally as well as reductionally. There are three reasons for believing that equational centric separations occur rarely, if at all, in Lilium. In the first place, an equational separation would yield two chiasmata, one on either side of the centromere. To explain why such paired chiasmata do not occur the further assumption must be made that a chiasma in the short arm may slip off, that is, terminalize and then disappear completely. We have, however, no evidence that chiasmata terminalize in Lilium; our counts at early diakinesis and metaphase agree well with those of MATHER (1935) in showing little or no terminalization between these stages. Secondly, the frequency of chiasma formation in those regions where centromere separations need to be considered is of the same general order as that for the inversion region where none of the chiasmata could have been formed by equational centric separations. Lastly, Lilium has a very typical meiotic sequence and there seems, therefore, no reason for not extending to this genus a generalization of the many observations, from diverse organisms, of the reductional separation of the centromeres at the first anaphase: Drosophila, (BRIDGES and ANDERSON 1925); Zea, (RHOADES 1931; MCCLINTOCK 1933); Neurospora, (LINDEGREN 1933); Lycopersicon, (BARTON 1951); Triticum, (HUSKINS and SPIER 1934); the mole, Talpa, (KOLLER 1936a); several marsupials, (KOLLER 1936b); several Orthopterans (CAROTHERS 1913). For these three reasons the information obtained from the heteromorphic homologues and from the proximal chiasmata of the inversion bivalents would seem to be acceptable for demonstration of the relationship of crossing over to chiasma formation.

A parallelism between the influence of temperature on chiasma formation and crossing over has been noted by WHITE (1934) in comparing his cytological observations on three species of Acridiidae with the older genetic data gathered by PLOUGH (1917) from Drosophila. Although the specific environmental factors responsible for differences have not been identified in the present study, a similar parallelism between crossing over and chiasmata is apparent on comparing the frequencies for the two collections of each of the two deficiencies.

Inversion configurations

Evidence in support of the partial chiasmatype hypothesis is provided by the analysis of the inversion heterozygotes. The open reversed chiasmata and the asymmetric configurations seen at diakinesis are inexplicable on a two-plane model unless the unlikely assumptions are made of pairing and chiasma formation in non-homologous regions. MATSUURA (1950) has very clearly outlined the types of configurations which are to be expected on the basis of a two-plane model and none of these have been observed at all. Furthermore, the frequency of chiasma formation at diakinesis is not at variance with the frequency of crossover types which appear at anaphase I. The regularity of the occurrence of the various inversion configurations at diakinesis also supports the partial chiasmatype hypothesis. From this point of view the extreme rarity of critical diakinetic configurations in earlier examinations of inversion bivalents is certainly unfortunate. The combined efforts of SMITH (1935), RICHARDSON (1936), DARLINGTON (1936), DARK (1936) and UPCOTT (1937) resulted only in the observation of a few examples. UPCOTT (p. 349) found two asymmetric

bivalents in Tulipa. A single case of an open reversed, or "inversion" chiasma was reported by DARLINGTON in Chorthippus. Five others were seen by SMITH in Trillium and DARK reported two more for Paeonia. It seems most likely that these workers were dealing with small inversions in which chiasma formation was rather rare. The small size of an inverted region would be sufficient explanation for the failure of formation of detectably asymmetric bivalents. The smearing techniques employed earlier did not result in much flattening of the chromosomes and therefore would make the detection of open reversed chiasmata rather difficult.

Conflicting evidence and hypotheses

In a dicentric chromosome in wheat a chiasma formed between the primary and secondary centromeres would be expected to produce a double bridge at anaphase. In their study of this chromosome STEINITZ-SEARS and SEARS (1953) found only 9.1% bridges in striking contrast to 28.0% chiasmata between the two centromeres; they concluded that these data could not be satisfactorily reconciled with the partial chiasmatype hypothesis. However, other explanations were not entirely excluded and among these was that of premature separation of secondary sister centromeres. DARLINGTON (1939) has summarized and interpreted various observations of a transverse stretching of the centric region under stress at anaphase I. It seems plausible that the small secondary centromeres of the wheat dicentric might simply split rather than stretch transversely when under stress. The observations reported above on the precocious equational separation of sister centromeres in *L. formosanum* demonstrate that such separations may not be detectably associated with any change in activity or orientation. Thus the failure of the wheat secondary centromere to show activity at anaphase I cannot safely be used as a criterion for judging the ease with which the sister secondary centromeres might be pulled apart.

COOPER (1949) has successfully demonstrated that chiasmata or chiasmata-like configurations may be formed in meiosis in male *Drosophila melanogaster*, yet few or no crossovers appear. Likewise, the frequency of chiasmata in paired somatic chromosomes (KAUFMANN 1934; COOPER 1949) is seemingly much higher than the amount of somatic crossing over, although the two processes have not been studied in the same tissue. It thus seems apparent that a chiasma cannot safely be assumed to represent a prior crossing over in all organisms. One of the future tasks of comparative cytology will be to determine when such an assumption may be made with reasonable assurance of its validity. In addition, the presence of such unusual features as somatic pairing in the fruit fly may help to provide valuable clues as to the nature of the chiasmata under consideration.

SAX (1930, 1932) suggested the idea that crossing over occurs through breakage and union at chiasmata originally formed by alternate equational and reductional openings at diplotene. Although later abandoned by SAX (1936), it should be noted that his idea becomes almost identical with the partial chiasmatype hypothesis if the equational separations are assumed to extend over only very small regions and one of each pair of chiasmata then always becomes resolved in early diplotene. If SAX's scheme thus modified were applied in the present instance, two chiasmata would have originally been present in the very short deficient arm of the heteromorphic A chromosome. As pointed out by DARLINGTON and MATHER (1932) the regular

occurrence of pairs of chiasmata so close together would be unlikely if judged by the distribution of chiasmata apparent at diakinesis; however, if crossing over regularly follows the formation of pairs of closely juxtaposed chiasmata, these pairs would not be distributed in the pattern perceptible at later stages. SAX's hypothesis as so modified may thus receive confirmation or disproof only when the mechanism of crossing over becomes understood.

In recent years MATSUURA (1937, 1938, 1950) and HAGA (1944, 1953) have been the outstanding defenders of the two-plane hypothesis. In 1950 MATSUURA very clearly described the diplotene or diakinetic configurations to be expected in inversion bivalents according to his neo-two-plane hypothesis and according to the partial chiasmatype hypothesis. He recognized that asymmetric inversion configurations (with single chiasmata) are to be expected only on the basis of the chiasmatype hypothesis. As previously mentioned, the types of configurations to be expected on the basis of the neo-two-plane hypothesis (MATSUURA 1950, p. 53) have not been observed at all in our material although they would have been expected to provide some very striking configurations. Thus, our observations on Lilium conform to MATSUURA's requirements for the partial chiasmatype hypothesis rather than to those for the neo-two-plane hypothesis.

Time of crossing over

In 1932 CREIGHTON and MCCLINTOCK presented a demonstration of four strand crossing over in which the crossover products were identifiable at diakinesis. Much of the work summarized in the introduction would also point to stages prior to diakinesis or mid-diplotene as the time of occurrence. In the Lilium material reversed chiasmata have been clearly observed at late diplotene or early diakinesis. It thus seems most probable that meiotic crossing over usually occurs sometime between the onset of pairing and the completion of the diplotene opening of the bivalents.

SUMMARY

Two terminal deficiencies and a paracentric inversion, resulting from X-ray treatment of pollen, were used for a study of the relationship of chiasmata and crossing over in *Lilium formosanum*.

In plants heterozygous for either terminal deficiency, there was a close correspondence between the frequency of a single chiasma in the deficient arm and of equational separations at anaphase. This correspondence proved true for two different environmental circumstances for each deficiency. In plants heterozygous for the inversion, the regular occurrence of a chiasma between the centromere and the inverted region led to an expectation of a 1:1 ratio for dicentric bridge and loop chromatids at anaphase I; this expectation was closely borne out by the anaphase observations. Reasons are given in the discussion for believing these observations are not to be explained by equational centric separations.

Chiasmata in the inversion region are frequently directly recognizable because of the formation of "reversed" chiasmata, asymmetric configurations, and the characteristic configuration when two chiasmata are present. The occurrence of reversed chiasmata and of asymmetry afford evidence in support of the partial chiasmatype hypothesis. On the assumption of a random relationship among the chromosomes

involved, the frequency of crossing over observable at anaphase I was not at variance from that expected on the basis of chiasma formation at diakinesis.

The discussion includes a brief consideration of earlier work with heteromorphic and inversion configurations, of observations at variance to the partial chiasmatype hypothesis, and of the time of crossing over.

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