The origin and behaviour of chiasmata, VII. *Zea mays*

by

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With 21 Text-figures

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Contents

1. Introduction: Material and Methods

Advances have recently been made in many different ways by the joint cytological and genetical study of maize. First, by the collaboration of many workers it has become possible to identify the linkage groups described in a later section. Secondly, each of the ten pairs of chromosomes has been identified at mitosis in the pollen grain of trisomics and at the pachytene stage in plants heterozygous for interchanges as well as for mendelian factors. A one-to-one correspondence has thus been established between the ten linkage groups and the ten chromosomes (McCLINTOCK 1930, 1931a and c, McCLINTOCK and HILL 1931, BRINK and COOPER 1931, 1932). The chromosomes are accordingly numbered from 1 to 10 in order of decreasing metaphase length.

Thirdly the inheritance of interchanges and their relation to sterility has been studied, as in *Oenothera* and *Campanula*, by numerous workers (BURNHAM 1930, 1932, COOPER and BRINK 1931b). Fourthly, the formation of new chromosome types by crossing-over has been shown in various ways (CREIGHTON and McCLINTOCK 1931, McCLINTOCK 1931b). Fifthly, the expected relationship between chiasmata and chromosome association and crossing-over has been shown in special segments of chromosomes in derivatives of a *Zea-Euchlaena* hybrid. Thus segments with 12 % of crossing-over were associated in a little

less than the 24 % of nuclei expected if association is conditioned by the formation of a chiasma and the chiasma is conditioned by crossing-over between two chromatids of partner chromosomes (BEADLE 1932b). Finally, a series of genetically controlled abnormalities of chromosome behaviour at meiosis, some of them of considerable theoretical importance, have been discovered and analysed from the genetical and cytological point of view by BEADLE (1930—1933).

These studies make maize an instrument of genetical research such as is found in no other organism save *Drosophila melanogaster*. But they also give it one advantage, found nowhere else. The chromosomes can be studied at the stages at which crossing-over occurs. The frequency and distribution of crossing-over is now becoming known with an increasing degree of accuracy and completeness. It is therefore important that the general properties of the chromosomes during the stages of meiosis providing the critical evidence of crossing-over should be studied in order that genetical predictions based on the assumptions, inferred from other evidence, that every chiasma results from a crossing-over, may be made and tested.

For this purpose the method of interpretation is of the first importance. The method used has been that which I have developed in a series of studies with the most favourable material available. (Cf. 1932a.) In these I have shown that the chromosomes only touch at points at which their constituent chromatids change partner — the chiasmata; further that the chromosomes always form loops between these chiasmata and that the successive loops come to lie at right angles to one another at later stages. In order to show the validity of such conclusions it has been necessary to demonstrate by comparison of statistically arranged data the one-to-one correspondence of the chiasmata observed at successive stages.

The resulting analysis has made it possible to define the mechanical principles on which the internal relationships of the bivalent chromosomes are determined. These principles are that the parts of chromosomes exert two kinds of force on one another, first, a specific attraction between homologuos parts of chromatids in pairs and secondly non-specific repulsion, between all parts of such paired chromatids. The attraction is effective laterally throughout the chromosome and terminally as well at the ends. The repulsion is uniform except at the spindle attachment, where it is stronger than elsewhere. Association between two chromosomes after division into four chromatids is therefore only preserved by exchanges of partner between the chromatids, the chiasmata. The original positions of these chiasmata are variable so that they divide the chromosomes into loops and open arms, into large loops and small loops, into loops including the spindle attachment and loops not containing it. The forces of repulsion are expected to be an inverse function of the distances of surfaces apart, and the forces of attraction are expected to be equal between any similar pair of chromatids. The chiasmata move accordingly towards equilibrium positions in which the repulsion-effect is equal on their two sides. All such movements of chiasmata, since they are in general away from the spindle-attachment and towards the ends, I have described as „terminalisation".

The evidence for these mechanical hypotheses consists, as I have said, in comparison of the observations of chiasma frequencies and positions ("terminal" or "interstitial") in all the chromosomes of a nucleus at successive stages from diplotene to metaphase. It is then found that every organism has a characteristic, and therefore genetic, property in regard to the behaviour of any chromosome pair of a given size, with a given position of spindle attachment, and a given original number of chiasmata at given positions. A change in any one of these four conditions changes the rate of movement and the type of equilibrium position reached. Yet the assumption of one independent variable — the strength of the special repulsion inferred at the spindle attachment — is adequate in explaining the variations in chromosome behaviour observed between diplotene and metaphase in all homozygous organisms. A high degree of terminalisation means strong attachment repulsion, a low degree of terminalisation means weak attachment repulsion. No doubt variations will be found later in the distribution of the generalised repulsion along the chromosome and as between different organisms. Indeed the special conditions of the *Drosophila* type of pairing seem to point in this direction (DARLINGTON 1933b). But in general no further assumption need yet be made.

We are not concerned here with the physical basis of the local and general repulsions. The simplest assumption is that they are due to the surface charges that a protein will acquire in a medium not at its isoelectric point. The different staining reactions of the attachment chromomere and the rest of the chromosome bear out this assumption. The analysis provides us with a fairly concrete basis on which to rest our interpretation of chromosome behaviour. The present discussion is merely a provisional outline of what may be done in the study of chromosome movements during prophase. I may note parenthetically that analysis of these movements is useful not only because of its bearing on the stages considered, but because it seems to provide the key to the understanding of chromosome mechanics in general.

The object of the present study is twofold: to interpret the behaviour of the paired chromosomes on the mechanical principles described, and to apply this interpretation to the prediction of genetical results.

Pollen mother-cells divisions were studied in the following plants: —

105b 5, 8—9 interchange heterozygote
105c 5, 1—7 „ „
17—1, 19—3, 19—5 } 1—2 „ „
31—1, trisomic for three chromosomes
31—2, „ „ two „
31—4, „ „ one chromosome (7 or 8).

The interchange heterozygotes are the progeny of X-rayed plants. 105b 5 was heterozygous for a single interchange, but reduction of pairing indicated that 105c 5 probably had a second unidentified change affecting a third pair of chro-

mosomes, while 17—1 probably had a second change affecting a chromosome in the ring of four. These plants are therefore less important for the present study.

The trisomic plants were the progeny of triploid females crossed by diploid males.

The methods I have used were those developed by McCLINTOCK and applied to these stages by BEADLE (l. c.). The inflorescence is fixed in acetic alcohol for 24 hours, smeared in acetocarmine, and heated under the cover-glass. In this way results are obtained which compare favourably in all respects essential for the present study with permanent FLEMMING smears which I find impracticable with maize.

2. Chiasma frequency

The chromosomes of maize are fairly uniformly graded in length from the longest, No. 1, to the shortest, No. 10, which is rather less than half as long. The spindle attachments are also graded in regard to position from dividing

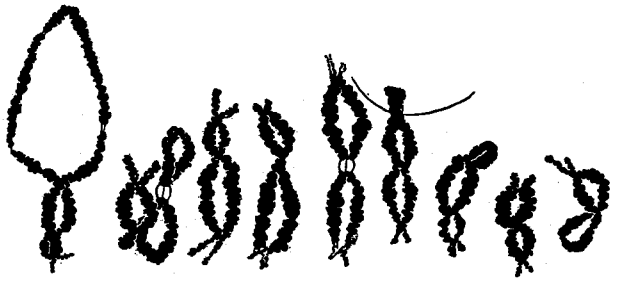

Fig. 1. The diplotene chromosomes of plant 105c 5: ring of four (7—1 interchange) and eight pairs. Some of the chromosomes are still held together at their spindle attachments. One pair (no. 6) is attached to the nucleolus. The distal ends of some chromosomes show less condensation than the proximal parts

Note. In this and succeeding illustrations of complete nuclei the bivalents are drawn separately; the total number of chiasmata and the number terminal are given under each bivalent, and their totals on the left hand side. An arrow indicates where a bivalent has been disturbed in smearing, a cross, where two bivalents have been interlocked. All drawings were made at a magnification of 4200 and reduced to 2800

the chromosome into two almost equal arms in no. 5 to dividing it into a long arm three times as long as the short in no. 8 and about six times as long in no. 6. A distinction between individual chromosomes is not therefore regularly possible after the pachytene stage except in regard to no. 6. The nucleolus is attached to a fixed position on the short arm of this chromosome, next to the satellite, until late diakinesis (cf. McCLINTOCK).

With regard to the first two of the conditions of chiasma behaviour, maize is not therefore a suitable object of study. Special means of inferring the individual chiasma frequency will be considered later, but for the present certain general distinctions, not quantitatively exact, may be pointed out between the larger and smaller chromosomes. Chiasmata are always formed in both arms of the longer chromosomes, but in the shorter ones, probably only in 6, 9 and 10,

just a single chiasma may be formed. In 6, with very unequal arms, two chiasmata may be formed, both in the long arm, or one only in the short or in the long arm. Thus the shortest chromosomes have two chiasmata as a rule, while the longest probably ranges from three to five.

At early diplotene the chromosomes are held together at their spindle attachments, which are still recognisable by their fainter staining, after the rest of the chromosomes have fallen apart (fig. 1). This stage however soon passes, and all the recorded observations (except fig. 1) are of nuclei in which the chromosomes are only associated at chiasmata. The number of diplotene observations is not sufficient in itself to give the average value for chiasma frequency (2·7 in 105b 5) a high accuracy, but the value agrees well with the later behaviour in the normal plants observed, which show a slight but consistent reduction in chiasma frequency as prophase advances.

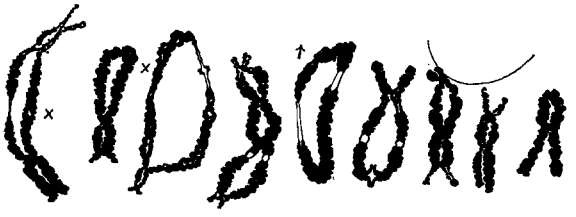

Fig. 2

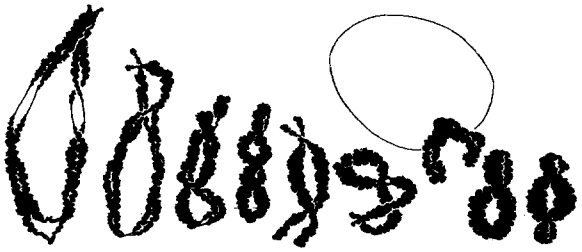

Fig. 3

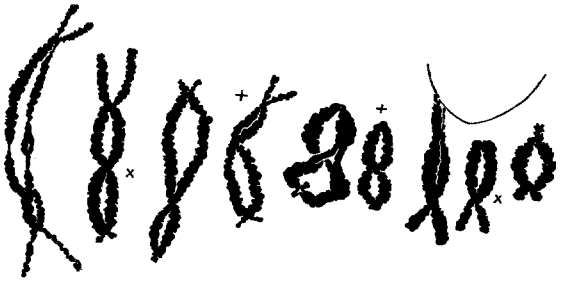

Fig. 4

Figs. 2—4. Successive stages of late diplotene in plant 105b 5. The ring of four is the 8—9 interchange group. The spindle attachment chromomeres are still distinguishable in the early stages

Plant 105b 5 I take to be normal, except in having the 8—9 interchange, for three reasons. It has regular pairing of all the chromosomes at metaphase; it agrees in chiasma frequency with the other diploid plants that also have regular pairing; it has a chiasma frequency of individual pairs which shows an expected relationship with length, as will be seen later.

Plant 105c 5 on the other hand has a slightly lower chiasma frequency (Tables III and IV), and failure of one pair has been observed at diakinesis. Also a second multiple configuration, a chain of four, was seen once. These observations suggest that a second unidentified change such as a small translocation is present in this plant.

Plant 17—1 departs from the normal behaviour only in the low chiasma frequency of its ring-of-four formed by the interchanged 1 and 2 chromosomes. This is associated with its appearance as two simple pairs and as a chain of three and univalent in some nuclei. It seems probable therefore that here also there is a second structural change, in this case affecting the ring chromosomes.

3. Chiasma movement (Terminalisation)

Since the reduction in the number of chiasmata between diplotene and metaphase is so slight, the only important change to be considered is chiasma movement. Although the chromosomes cannot be distinguished according to their size they can obviously be distinguished for the study of movement according to the number of their chiasmata. This analysis shows that the changes undergone are of the type described in *Tulipa* (Darlington and Janaki-Ammal 1932). Thus the proportion of terminal chiasmata increases as prophase advances wherever two or more chiasmata are formed. This means that loops grow at the expense of open arms (owing, according to the repulsion hypothesis, to repulsion being greater in a closed loop than between open arms). Single chiasmata do not move unless they lie in a short arm. The spindle attachment is then near to the end and its repulsion is capable of moving a chiasma to the end although it could not do so were it further from the end. Similarly in large chromosomes with two chiasmata, the chiasma in the shorter arm is more often terminalised than that in the larger arm (Fig. 8). The localised repulsion of the spindle attachments is less pronounced than in organisms with complete terminalisation, such as *Campanula persicifolia* and *Primula sinensis*. It shows itself in the enlargement of the spindle attachment loop at the expense of the distal loops to give the equilibrium positions seen at late diakinesis and metaphase, in which particular chromosomes with particular numbers of chiasmata have certain characteristic features. These are that the spindle attachment loop is larger than the distal loop or loops. Two distal loops, if on the same

Fig. 5

Fig. 6

Fig. 7

Figs. 5—7. Successive stages of diakinesis in the same plant, 105b 5, showing the opening out of the spindle attachment loop at the expense of the distal loops and the increasing proportion of terminal chiasmata

side of the spindle attachment, are the same size, while if on opposite sides the one in the shorter arm is smaller than that in the longer arm. It seems probable in view of the apparently random original positions of the chiasmata that these equilibrium positions are sometimes attained by movement of the chiasmata towards the spindle attachment, but evidence from more critical material is needed on this point. It also seems probable *ex hypothese* that, where occasionally two chiasmata are formed in a short arm of either a long or a short chromosome, equilibrium will not be reached except by the elimination of the distal loop and fusion of its two chiasmata as in organisms with complete terminalisation. This would account for the slight reduction in total chiasma frequency between diplotene and metaphase. If this is the case the difference between the minimum (*Tulipa* type) and maximum (*Campanula* type) terminalisation would depend on the relation of four conditions: degree of localised repulsion, degree of generalised repulsion, length of chromosome arms and existence of chiasmata in one or both of the arms.

Fig. 8

Fig. 9

Fig. 10

Figs. 8—10. Side views of first metaphase in the same plant, 105b 5. Three nuclei showing the ring of four, nondisjunctional in figs. 8 and 9, disjunctional in fig. 10. The connections between the chromatids and the usually invisible attachment chromomeres are often splayed out by pressure in smearing

Fusion of chiasmata only occurs at the ends of the chromosomes, as shown by the absence (as in *Tulipa*) of any increase in the proportion of single-chiasma bivalents. Fusion and reduction of number therefore only occur when there is more than one chiasma in one arm of a chromosome and when the spindle attachment is in a closed loop, i.e. from 4 to 3 or from 3 to 2, not from 2 to 1. The reduction, it should be noted, is so slight that for some purposes it is more convenient to consider the frequencies of successive stages together.

Observations on maize are thus in accordance with the earlier observations on *Tulipa* and *Stenobothrus* and with the electrical hypothesis of repulsion based on them. We may say that maize has a low degree of localised repulsion relative to the size of the chromosomes and the generalised repulsion, and hence a low degree of terminalisation.

Table I

Frequencies of bivalents with different numbers of total chiasmata and of terminal chiasmata in 23 nuclei at successive stages in 105b 5, excluding the ring of four

	Chiasmata per bivalent		1	2	3	4	5
Late Diplotene (3 nuclei)	Terminal chiasmata	0	—	5	9	1	—
		1	—	3	6	—	—
	Total bivalents		—	8	15	1	—
	Terminal chiasmata per bivalent		—	·38	·40	—	—
Early Diakinesis (7 nuclei)	Terminal chiasmata	0	2	7	1	—	—
		1	—	17	15	1	—
		2	—	1	6	5	1
	Total bivalents		2	25	22	6	1
	Terminal chiasmata per bivalent		·00	·86	1·23	1·83	2·00
Late Diakinesis (5 nuclei)	Terminal chiasmata	0	1	2	1	—	—
		1	—	14	6	—	—
		2	—	6	6	3	1
	Total bivalents		1	22	13	3	1
	Terminal chiasmata per bivalent		.00	1·18	1·38	2·00	2·00
Metaphase (8 nuclei)	Terminal chiasmata	0	1	4	—	—	—
		1	—	18	8	—	—
		2	—	12	14	7	—
	Total bivalents		1	34	22	7	—
	Terminal chiasmata per bivalent		·00	1·24	1·64	2·00	—
Total bivalents (23 nuclei)			4	89	72	17	2
Percentages (Fig. 11)			2·2	49·5	39·1	9·2	1·1

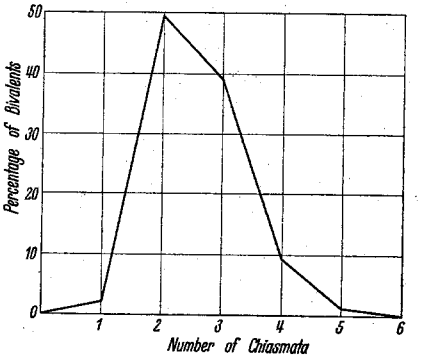

Fig. 11.
Polygon showing relative frequency of bivalents with different numbers of chiasmata, being the sum of all observations from diplotene to metaphase in 105b 5 (8—9 interchange ring excluded) (from Table I)

Note. The significance of the slight reduction in total chiasma frequency between early and late stages cannot be legitimately computed, since it may be derived from "systematic" errors of discrimination as well as from "accidental" errors of sampling.

Table II

Chiasma frequencies of the ring of four, the nucleolar chromosome and the seven others separately recorded from diplotene to metaphase in the same nuclei of 105b 5

Stage (Number of nuclei)	Seven bivalents		Nucleolar chromosome			8—9 Ring-of-Four				All chromosomes				
	Chiasmata	Terminal chiasmata	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Term. chias. per bivalent	Bivalents	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Term. chias. per bivalent
Late diplotene (3)	57	9	8	0	—	16	4	2·7	0·67	30	81	13	2·7	0·43
Early diakinesis (7)	133	54	14	6	—	28	15	2·0	1·07	70	175	75	2·5	1·07
Late diakinesis (5)	92	49	9	3	—	21	14	2·1	1·40	50	122	66	2·4	1·32
Metaphase (8)	163	92	—	—	—	32	25	2·0	1·56	80	195	117	2·4	1·46
Total (23)	445	—	31	—	2·1	97	—	2·1	—	230	573	—	2·5	—

Table III

Frequencies of bivalents with different numbers of total chiasmata and terminal chiasmata in 15 nuclei of 105c 5 at successive stages, excluding the 7—1 ring

	Chiasmata per bivalent	0	1	2	3	4
Late Diplotene (1 nucleus)	Terminal chiasmata 0	—	—	2	3	—
	Terminal chiasmata 1	—	—	—	2	1
	Terminal chiasmata 2	—	—	—	—	—
	Total bivalents	—	—	2	5	1
	Terminal chiasmata per bivalent	—	—	·00	·40	1·00
Early Diakinesis (6 nuclei)	Terminal chiasmata 0	—	1	8	—	—
	Terminal chiasmata 1	—	—	18	3	—
	Terminal chiasmata 2	—	—	10	7	1
	Total bivalents	—	1	36	10	1
	Terminal chiasmata per bivalent	—	·00	1·06	1·70	2·0
Late Diakinesis (8 nuclei)	Terminal chiasmata 0	1	1	4	—	—
	Terminal chiasmata 1	—	1	19	3	1
	Terminal chiasmata 2	—	—	14	18	2
	Total bivalents	1	2	37	21	3
	Terminal chiasmata per bivalent	—	—	1·27	1·86	1·67
Total bivalents (15 nuclei) (120)		1	3	75	35	6

Table IV

Comparison of chiasma frequencies of different chromosome types in 15 nuclei of 105c 5, including the 7—1 rings, and below, chiasma frequencies in ten nuclei of 17—1 with a 1—2ring

Stage (Number of nuclei)	Seven bivalents			Nucleolar chromosome			Ring-of-four				All chromosomes				
	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Terminal chiasmata per bivalent	Bivalents	Chiasmata	Terminal chiasmata	Chiasmata per bivalent	Terminal chiasmata per bivalent
Plant 105 c 5 (7-1 ring)															
Late diplotene (1)	21	3	—	2	0	2·0	6	2	3·0	1·00	10	29	6	2·9	0·60
Early diakinesis (6)	95	53	—	12	4	2·0	36	13	3·0	1·08	60	143	70	2·4	1·17
Late diakinesis (8)	135	87	—	16	6	2·0	42	27	2·6	1·69	80	193	120	2·4	1·50
Total (15)	251	—	2·4	30	—	2·0	84	—	2·8	—	150	365	—	2·4	—
Plant 17-1 (1-2 ring)															
Mid-diakinesis (10)	165	110	2·4	21	9	2·1	45	25	2·3	1·25	100	231	144	2·3	1·44

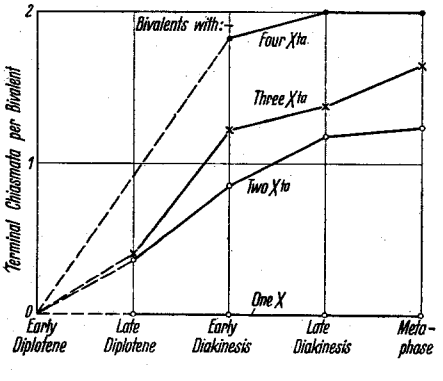

Fig. 12. Graph from Table I showing the different rates of increase in the number of terminal chiasmata in bivalents with different total numbers of chiasmata, from late diplotene to metaphase. By extrapolation it is concluded that no chiasmata are originally formed terminally, i.e. the number of terminal chiasmata is zero for all types of bivalents at an unrecorded "early diplotene" and further that terminal chiasmata arise more frequently in the early stages in bivalents with numerous chiasmata, and not at all in this plant in bivalents with only one chiasma

4. Rings of four in interchange heterozygotes

McClintock (1930) and others have shown that the four associated chromosomes in an interchange heterozygote come together to give the cross-figure expected from the operation of the specific attractions of their parts. At di-

plotene chiasmata appear in the homologous pairs of segments, as shown in *Campanula* (GAIRDNER and DARLINGTON 1932) and their terminalisation seems to follow the same rules as in single pairs (Tables II and IV). Like these they retain a proportion of interstitial chiasmata at metaphase.

Fig. 13. Types of chain and ring at first metaphase. 7 and 8 are from 105b 5 (7—1), and the rest from 19—1 and 19—5 (1—2 interchange possibly with other changes reducing the chiasma frequency to 3 or 4). 1 and 2 are disjunctional, the rest non-disjunctional; 2 and 3 have three chiasmata; 1, 4, 5, 6 and 7 have four chiasmata, 8 has five chiasmata

It is this retention of interstitial chiasmata, which as I have pointed out (1932a), distinguishes the *Zea* and *Pisum* rings from those of *Oenothera* and *Campanula*, and accounts for the higher proportion of non-disjunction which they show. The low spindle attachment repulsion probably influences the disjunction directly as well as indirectly. Not only does it leave a proportion of interstitial chiasmata at metaphase, which give a more rigid figure, but even where terminalisation is complete the terminal chiasma remains more rigid than where, as in *Oenothera*, the double connections between the pairs of chromatids are drawn out into fine threads by the repulsion, and a free orientation of the ring components on the metaphase plate is made possible.

5. Trivalents in trisomic plants

Plant 31—1 was trisomic for three chromosomes, each usually forming a trivalent (Fig. 14). Five nuclei at metaphase had the mean chiasma-frequency

Fig. 14. Metaphase of triple trisomic 31—1 with two trivalents and one univalent

per bivalent or trivalent of 2·94. This is higher than the value found for diploids and indicates that the trivalents have higher frequencies than corresponding bivalents would have had. This observation is consistent with the observations on *Tulipa* and with the crossing-over data in *Drosophila*. Triple (terminal)

chiasmata are frequently seen, indicating that terminalisation, leading to fusion, is stronger than in bivalents, at least in those trivalents with chiasmata on both sides of the spindle attachment in all three chromosomes.

Plant 31—2 was trisomic for two chromosomes (figs. 15—17). Its behaviour was abnormal in two respects. The chiasma frequency was lower and the terminalisation coefficient was higher in each type of bivalent than in any other trisomic or in any balanced plant. It was also exceptionally variable in both these respects. Doubtless the abnormality was genetically conditioned, perhaps by the particular type of genetical unbalance due to the particular two extra chromosomes. It is therefore interesting to notice the same relationship between terminalisation coefficient and chiasma frequency that is found in comparing with the parental species *Triticum-Aegilops* hybrids with reduced pairing. Their peculiarity is assumed to be due to structural differences between the pairing chromosomes and not to genetical conditions, yet it leads to more terminal association. The most likely explanation is that in both cases chiasmata are formed nearer the ends than usual owing to pairing being restricted to the distal regions. This restriction would be due to pachytene pairing beginning at the ends and being interrupted in the hybrid by a structural dissimilarity and in the unbalanced form by a cessation of attraction due to effective division of the chromosomes.

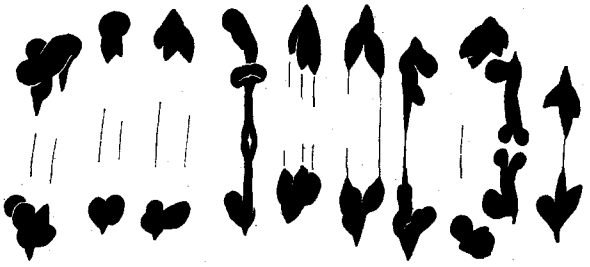

Fig. 15

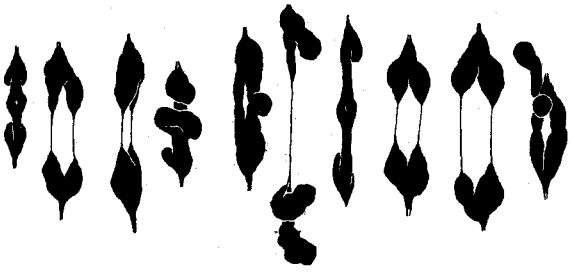

Fig. 16

Fig. 17

Figs. 15—17. Stages from metaphase to anaphase. Double trisomic, 31—2, with low chiasma frequency. Note the relatively high terminalisation

Plant 31—4 I have studied in detail because it is trisomic for a single chromosome. This was identified at pachytene as being either No. 7 or No. 8.

Table V

Frequencies of total and terminal chiasmata in three nuclei of the double trisomic with low chiasma frequency, 31—2, from metaphase only. A triple chiasma is counted as two terminal chiasmata since it is derived from terminalisation of two chiasmata

Chiasmata per bivalent or trivalent		1	2	3	4
Terminal chiasmata	0	—	1	—	—
	1	6	8	1	—
	2	—	9	2	1
	3	—	—	2	—
Total bivalents or trivalents		6	18	5	1
Total chiasmata		6	36	15	4
Terminal chiasmata per bivalent or trivalent		1·00	1·44	2·00	2·00

Mean No. of chiasmata per bivalent or trivalent 2·03.

Table VI

Analysis of 46 trivalents — 138 chromosomes — of type 7 or 8, having 120 chiasmata, in percentages, combined with the proportion of chromosomes with no chiasmata taken from the frequency of univalents (21 nuclei with univalents to 58 with trivalents)

Chiasmata per chromosome	0	1	2	3	4
Minimum variance	8·9	34·3	46·2	9·9	0·7
Maximum variance	8·9	42·9	31·0	14·6	2·6

At this stage the odd chromosome could be seen sometimes entirely unpaired, when it was folded back upon itself as described by McClintock (1932a). The three chromosomes were also seen to be associated in pairs at different points with exchange of partners as described in *Tulipa* and *Hyacinthus* (Newton and Darlington 1929). In either case it was possible to detect signs of division in the parts of the chromosomes that were unpaired, although no such signs were detectable in the paired parts. This might be due to any one of three causes: Difference in conditions of fixation of paired and unpaired threads, both of which are divided at this stage. Difference in intimacy of association of the split halves of paired and unpaired chromosomes. Difference in the time of division of paired and unpaired chromosomes. I see nothing in the first two possibilities which is incompatible with my mechanical theory, which supposes chromosome pairing to be due to attractions between effectively single threads, for the steps leading to division may, as I have pointed out, considerably anticipate its becoming mechanically effective. The explanation of the difference must at present remain doubtful.

At diakinesis and metaphase the extra chromosome was found unpaired in 21 out of 79 nuclei. In the remainder it formed trivalents (figs. 18 and 19). These were of the types described in *Tulipa* in regard to the numbers and disposition of the chiasmata (cf. DARLINGTON and MATHER 1932). Amongst others there was the type, illustrated in Fig. 16, bottom row, with „intercalary" chiasmata, which constitute a demonstration of genetic crossing-over (cf. CREIGHTON and MCCLINTOCK 1932). By fusion of chiasmata between one chromosome and each of two others a triple chiasma is formed. The chromosomes then separate at anaphase as shown in figs. 17 and 19.

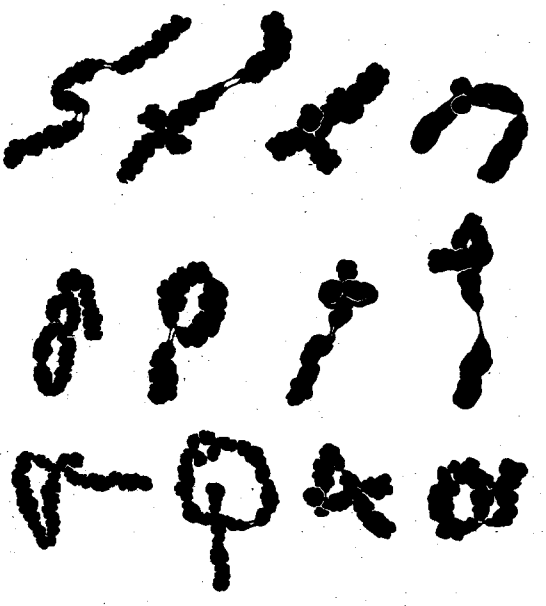

Fig. 18. Types of diakinesis trivalent found in 31—4, single trisomic of chromosome 7 or 8. In the bottom row are types with "intercalary" chiasmata demonstrating crossing-over

An analysis of 46 of these trivalents, according to the method used in *Tulipa*, gives the distribution shown in Table VI. The chiasma frequency from trivalents is not strictly combinable with the value of 8·9 per cent calculated for zero chiasmata from the proportion of univalents observed, since the bivalents corresponding to these would have a different frequency variation and a lower mean from the chromosomes entering into trivalents. Also slight discrimination, reducing the mean value, has been introduced in the choice of observations. The results are therefore imperfect, but I record them because they are the only ones available for single-type trivalents. They are similar to those found for the whole complement in *Tulipa*. They are therefore consistent with the view that three chromosomes pair at pachytene as though made up of a small number of blocks which pair at random, as was shown in *Tulipa*.

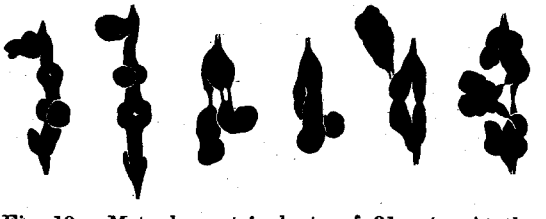

Fig. 19. Metaphase trivalents of 31—4. At the right are two with triple chiasmata

6. Chiasma frequency and crossing-over

I have concluded above that the "original" average chiasma frequency of the whole complement, that is to say the average number of chiasmata formed

at diplotene in all the paired chromosomes taken together, is 2·7. There is now evidence of several kinds making it possible to infer the approximate average frequency of the individual bivalents. The relative lengths of chromosomes have been determined from pachytene measurements by Dr. McCLINTOCK, who allows me to make use of her records (Table VIII and Figs. 20 and 21).

The two types of frequency-length relationship now known are the linear proportionality found in *Hyacinthus orientalis*, *Vicia Faba* and *Fritillaria imperialis* and the non-linear proportionality found in *Stenobothrus lineatus*, *Yucca flaccida* and *Hyacinthus amethystinus* (DARLINGTON and DARK 1932, DARLINGTON 1932a and b). The first type is found in organisms with high chiasma frequency, the second in organisms with low chiasma frequency especially where this is combined with high size variation. If we assume the *Vicia* relationship for

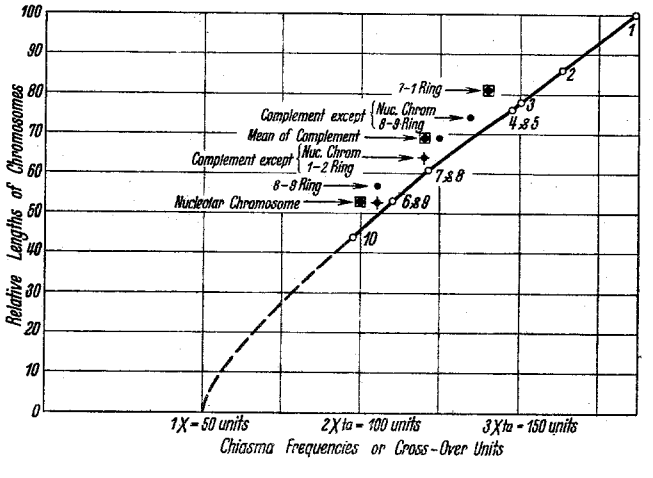

Fig. 20. The relation of chiasma frequency to chromosome length of the whole complement and of individual chromosomes, as given in Tables II and IV; a similar curve to that which these values represent, but corresponding to the mean original value of 2·7, is drawn for the derivation of hypothetical original values for individual chromosomes. From these values the cross-over lengths of the ten chromosomes are provisionally computed

maize we find the longest chromosome should have 3·9 chiasmata, the shortest 1·7. This would mean that the tenth chromosome alone would have at least 30 per cent of bivalents with one chiasma only, a value higher than that obtained for the whole complement (Table I). The *Stenobothrus* relationship, on the other hand, would give the lower frequency of this type of bivalent actually observed. The collective observations therefore favour the assumption of a *Stenobothrus* relationship.

Let us consider now the individual frequencies obtainable from the data (Table V and Fig. 20). We see that the nucleolar chromosome and the ring chromosomes in the two single-interchange heterozygotes studied show a length proportionality. The ring chromosomes show no reduction in total frequency of chiasmata on account of the interchange, although of course the original distribution may be changed. When frequency is plotted against length in these different chromosomes we see evidence of the *Stenobothrus* relationship (fig. 20). Taking a *Stenobothrus* type of curve through points which will give

Table VII. (From Tables II, IV and VIII)

Chromosome type	Mean length	Mean chiasma frequency		
		105b 5	105c 5	17-1
Nucleolar chromosome	53	2·1	2·0	2·1
8-9 Ring	57	2·1	—	—
Complement except 1-2 Ring and nucleolar chromosome	64	—	—	2·4
Whole complement	69	2·5	2·4	—
Complement except 8-9 Ring and nucleolar chromosome	74	2·7[1]	—	—
7-1 Ring	81	—	2·8	—

Table VIII

Number of chromosome	Pachytene length	Chiasma frequency	Cross-over length	
			calculated	observed
1	100	3·65	187	102
2	86	3·25	163	58
3	78	3·00	150	92
4	76	2·95	148	80
5	76	2·95	148	44
6	53	2·20	110	52
7	61	2·45	123	50
8	61	2·45	123	20
9	53	2·20	110	52
10	44	1·95	98	68

the mean value of 2·7 chiasmata for the ten chromosomes should therefore give the best approximation to the expected frequencies of the individual chromosomes.

Table VIII shows the results obtained by this method together with the expected corrected crossing-over lengths on the simplified chiasmatype hypothesis, according to which each chiasma follows crossing-over between two of the four chromatids and therefore corresponds to 50 corrected units of genetical crossing-over.

The sum of the expected total cross-over lengths is 1350, that of the cross-over distances so far observed genetically is 618. Allowing for double crossing-

[1] Pre-metaphase oberservations only and therefore not strictly comparable with the rest of this column.

over we have a mapped distance covering about half the calculated crossing-over in the chromosomes. Such a proportion would be expected, since of the 200 genes identified about 80 have been used in compiling the genetical maps. These are distributed at random in the ten chromosomes so that both ends of nearly all the chromosomes are still undetermined. Further development of linkage work however should soon provide a strict test of the one-to-one correspondence of chiasmata and cross-over in the individual chromosomes.

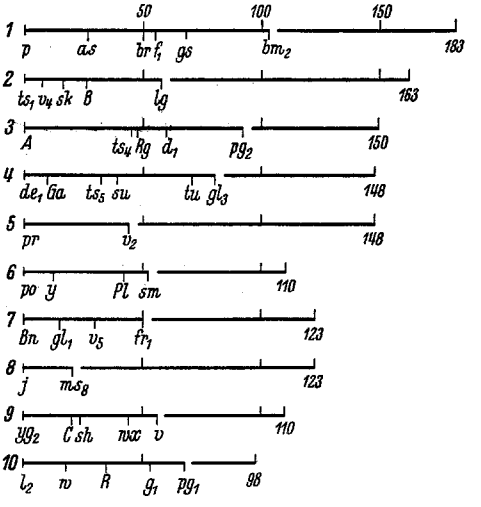

Fig. 21

Comparison of the cytologically computed cross-over lengths with those already obtained by genetical mapping. The lines represent the relative corrected total cross-over distances expected for the chromosomes on the basis of chiasma frequency (fig. 20). They are broken at the point at which genetic mapping, measured from the left, ceases. No allowance is made for double crossing over, the occurrence of which may be assumed to increase the uninterrupted distances of over 30 to an indefinite extent

The data are taken from LINDSTROM's summary (1931) with amendments from the following sources: 1. BURNHAM and BRINK (1932); 3. BRINK and SENN (1931); 4. BEADLE (unpublished); 6. BEADLE (1931); 8. BEADLE (unpubslished); MCCLINTOCK (unpublished); 9. BEADLE (1932e)

7. Summary

1. The ten paired chromosomes of *Zea Mays* form an average of 2·7 chiasmata at the prophase of meiosis in the pollen mother-cells. This number is only slightly reduced by fusion in terminalisation, which is of the *Tulipa* type.

2. The frequency of chiasmata in rings of four of known constitution and in the nucleolar chromosome indicates that the relation of chiasmata to length is a non-linear one as in *Stenobothrus*.

3. The inferred chiasma frequency of individual chromosomes makes it possible to predict on the simplified chiasmatype hypothesis what should be the total corrected crossing-over length of each chromosome. The length so far mapped genetically amounts to 46 per cent of this estimate.

4. Trivalents in trisomic plants were of the type found in *Tulipa*. They had a higher chiasma frequency than the coresponding bivalents. One trisomic plant had a specially low chiasma frequency in all chromosomes.

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