THE ACTIVITY OF INERT CHROMOSOMES IN *ZEA MAYS*

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(With Plate 9 and Eleven Text-figures)

CONTENTS

1. KINDS OF CHROMOSOMES

IN all sexually reproducing plants and animals there are a certain number of chromosomes whose regular division at mitosis and segregation at meiosis is a condition of regular growth and reproduction. Such chromosomes are therefore constant in number within species or related groups of uniform character. Now these chromosomes, being necessary, are assumed to be active, and rightly so, for any chromosome which is not doing something in the stock that is carrying it will sooner or later be lost by that stock. It will succumb to the hazards of mitosis.

Other chromosomes of a second kind are those which are variable in number, or at least in form, in many species of plants and animals. Some of these are of a very general type and well understood, such as the sex chromosomes; others are of a very special type and little understood, like the sex-limited chromosomes of Sciara. In plants there is a vaguely defined group of chromosomes known by the term *supernumerary fragment*. Chromosomes of this kind, if indeed it is a kind, have been found in natural populations of some forty species of flowering plants (Langlet, 1927; Darlington, 1937, Table 16).

Observations of these fragment chromosomes have been for the most part too accidental for inference of any general properties in them. Their origin has been ascribed merely to breakage or deletion of an ordinary active member of the set. Yet enough common properties may already be inferred in them to encourage a further enquiry.

In *Tradescantia, Fritillaria, Lilium, Tulipa* and *Ranunculus* the fragments are small. They vary in number somatically and are capable of indefinite reduplication without any definable effect on the plant. They pair at meiosis by chiasmata with a frequency proportionate to their length and when multiple form multivalents. In the first three examples they show a relationship with the major chromosomes by pairing with them. In *Secale* the fragments are larger and they are, it seems, a constant type in the stocks of three continents. They do not pair with the major chromosomes. They must therefore be regarded as old-established accessories of the regular complement; so old-established indeed that they have lost their original relationship with it. All these fragments are what would generally be described as inert although, as we shall see, this provisional description conceals an analytical error.
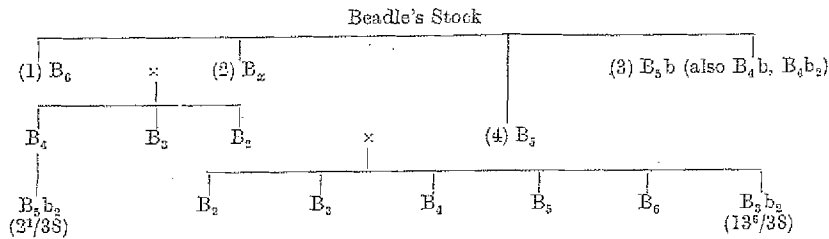
In maize there are extra chromosomes of which we know something in all these respects. They are said to be of two recognizable types, B chromosomes somewhat smaller than the smallest standard or A chromosomes, and C chromosomes smaller still (Randolph, 1928 *a, b*). As many as twenty-five B chromosomes have been accumulated in one plant by selection without seeming to alter it in general or particular ways. These chromosomes are heterochromatic in the prophase of meiosis and sometimes at metaphase of mitosis (McClintock, 1933). They are present in plants of about a quarter of commercial varieties and genetic cultures. In each stock they have what appears to be an equilibrium distribution. Supernumeraries thus show, as in *Secale*, a contradiction between our failure to detect their genetic activity in individual plants and our ability to infer their genetic function from the population as a whole. The same situation has arisen with the extra X chromosomes of *Cimex lectularius*, which float in the population—here, however, with different equilibria in nature and in genetic cultures. The extra Y chromosomes described by Wilson in species of *Metapodius* seem to be in a like case (Darlington, 1939*b*).

The B chromosome of maize therefore offers us the means of attacking several problems. Mechanically its indefinite reduplication allows of the exact comparison of crossing-over in bivalents and multivalents. Chemically its nucleic acid activity has to be compared with that of such chromosomes as the Y in *Drosophila*. Physiologically its indirect effects remain to be discovered. Evolutionarily the chances of its loss have to be equated against the advantages of its presence under different kinds of selection equilibrium. We will take the problems in this order.

2. The B chromosome stock

We are indebted to Prof. G. W. Beadle for seeds of a genetic culture containing B chromosomes. Their inheritance is shown in outline in Table 1 to illustrate the origin of deficient types. Three plants appeared independently with one or two smaller B's which we shall designate b chromosomes. This high frequency of visible change is of general im-

Table 1. *Origin of b chromosomes from B's by deficiency, showing numbers in each plant*

Beadle's Stock

(1) B_6 × (2) B_2 (3) $B_5 b$ (also $B_4 b$, $B_6 b_2$)

B_4 B_3 B_2 ×   (4) B_5

$B_5 b_2$ B_2 B_3 B_4 B_5 B_6 $B_5 b_2$
$(3^1/38)$ $(13^5/38)$

portance for the population genetics of B chromosomes. For the moment, however, it warns us that the classification of B and b chromosomes by their visible structure, which we necessarily use, is generic rather than specific. Our b chromosomes are presumably of three types and our B chromosomes may be equally diverse. We can, however, for our present purposes treat them as two groups.
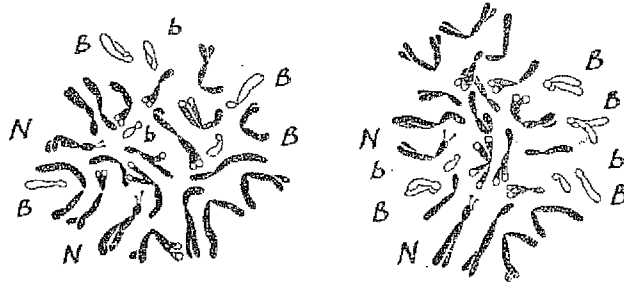

Text-fig. 1. Mitoses in root tips, 3/36: La Cour's 2 BE and gentian violet. $2n = 20 + 4B + 2b$. N, nucleolar chromosome, No. 6, with trabant. Note precocity of centromere in some B's. × 3000.

3. The conduct of mitosis

The differential staining of B chromosomes has been described at various stages, at metaphase of mitosis by Darlington (1937, fig. 103), at pachytene by McClintock (1933), at diakinesis and first metaphase by Randolph (1928 a). Now La Cour (unpublished) finds that they show the differential Feulgen reaction during the mitotic resting stage which is

characteristic of heterochromatin. Dark-staining masses are seen in the nucleus equal in number to the B·chromosomes seen at metaphase. Now, if B chromosomes are to be regarded as heterochromatic, it is of interest to recall that McClintock found a variable staining capacity of B chromosomes at pachytene just as with variable temperatures there is a variable nucleic acid content in the metaphase heterochromatin of
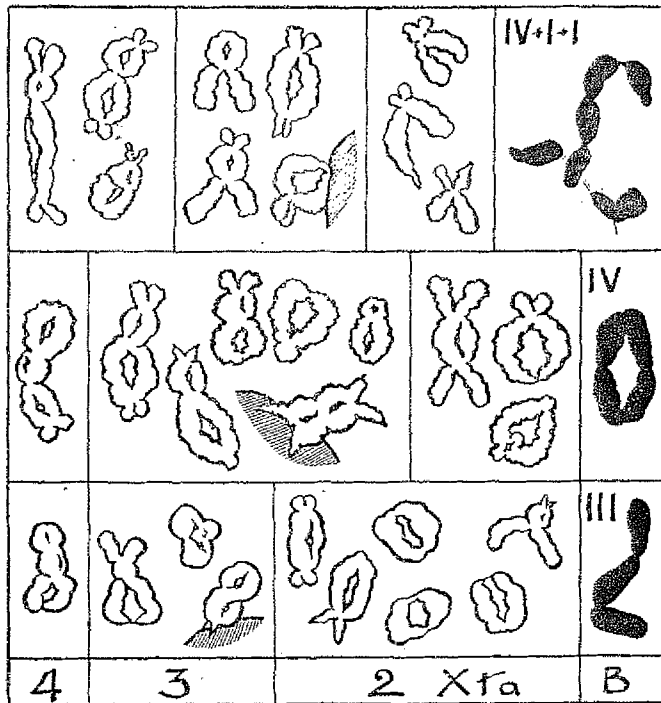
Text-fig. 2. Diakinesis in plants with 3, 4 and 6 B's. showing the chiasma distribution of A and B chromosomes and sticking of two unpaired B's. Acetic alcohol and iron acetocarmine preparation (as also following figures). ×2000.

Trillium (cf. Darlington & La Cour, 1940). These observations relate the B chromosomes of maize with an extra chromosome described by Fernandes (1939) in *Narcissus juncifolius*.

The difference in nucleic acid charge must not be confused with a difference in spiralization. As in *Trillium* this is the same for the two sorts of chromosome. At metaphase of mitosis both are contracted to about one fifteenth of their pachytene length.

Correlated with the excessive nucleic acid charge of B's is their stickiness and more even outline during prophase of meiosis (Text-fig. 2).

This property they share, as Longley (1937) points out, with the knobs scattered on the larger A chromosomes.

There is a second abnormality of mitosis which B chromosomes share in a lower degree with the extra fragments of *Tulipa galatica* (Darlington, 1937, fig. 17). That is the common habit of lying at the edge of the plate and of dividing at the centromere either before or after the major chromosomes (Text-fig. 1). These properties we are inclined to put down to the B chromosome having a weaker centromere than the rest, a centromere which, although usually sufficient for its smaller size, sometimes fails to work in concert with the larger ones. The same evidence of weakness has been found in the *Secale* supernumerary (Hasegawa, 1934) and in *Uvularia* (Barber, 1940).

Hence we find occasional mitotic lapses in the B chromosome. From one of these we obtained material for a useful experiment. Plant 3/36 had some flowers with four and others with five B chromosomes in addition to one small b, as well as root-tips with two b's (Table 1).

Both B and b chromosomes in our plants are seen to have subterminal not terminal centromeres like those described by McClintock and Avdulov. We are inclined to suppose that this again means variation in the structure of the B chromosomes in the population, rather than misinterpretation on one side or the other.

The terminal centromeres, as well as the errors of mitosis shown by some B's in common with many other supernumeraries, they may well owe to their origin. Misdivision of the centromere is a common means of fragmentation and gives rise to terminal centromeres themselves fragmented and of weaker activity in various degrees (Darlington, 1940 a). On this view it is significant that the smallest fragments are liable to show the most frequent errors, while proportionately larger fragments like the B's seem to be relatively stable.

4. CHIASMA FORMATION IN B CHROMOSOMES

We already know from McClintock's work (1933) that B chromosomes pair at mid-prophase of meiosis but frequently fall apart at diplotene and are then unpaired at metaphase. Or, as we should say, after having paired at pachytene they do not always cross-over and form chiasmata.

The chiasma formation of B's has to be considered in the light of the three respects in which they differ from the ten A's: shorter length, nearness of the centromere to one end, and heterochromatic properties.

Chiasmata are formed in three positions: S, short arm; P, proximally in long arm and usually very close to the centromere; and D, near the
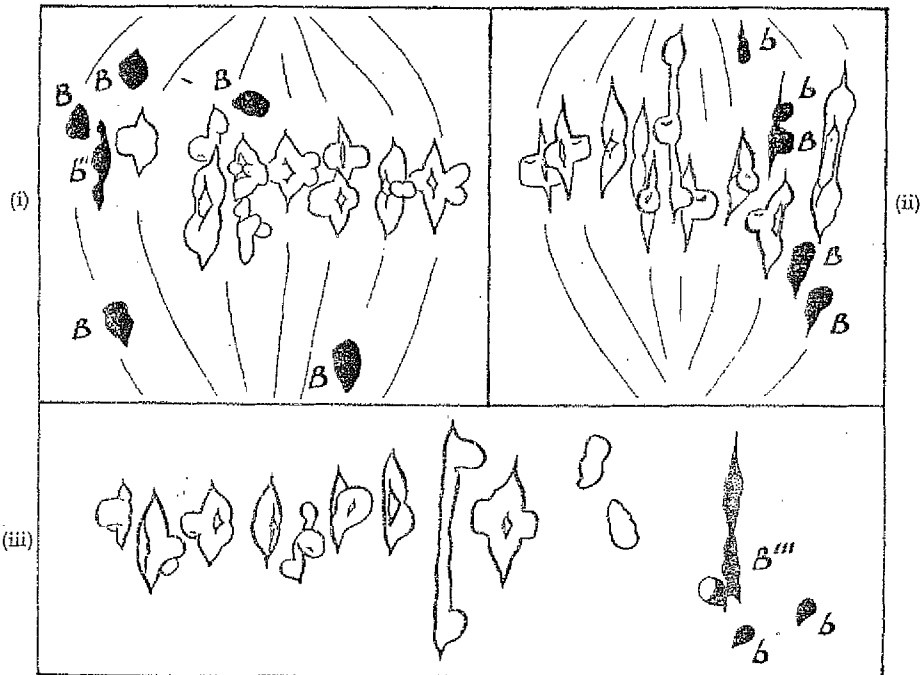
Text-fig. 3. First metaphases in plants with several B and b chromosomes, showing the position and orientation of univalents and multivalents. ×2000.
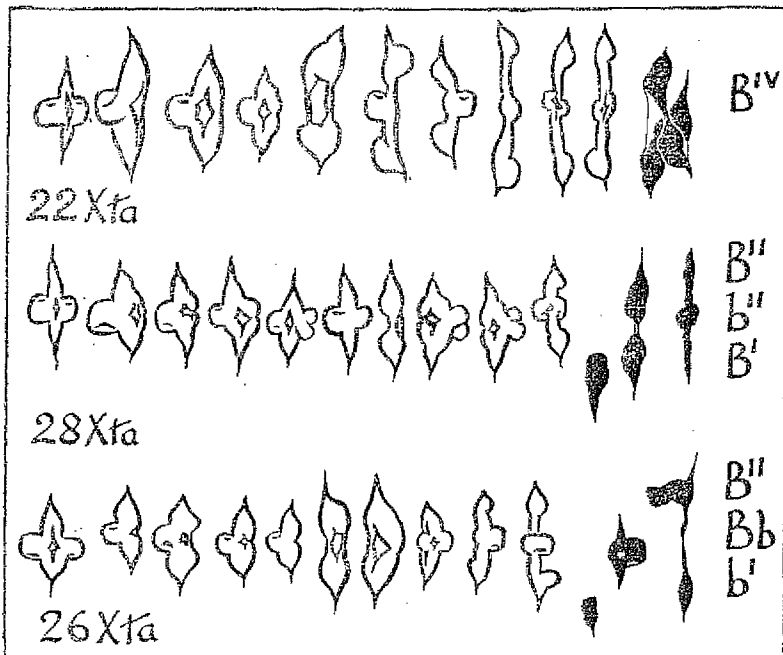
Text-fig. 4. First metaphases. Above with 22 A chiasmata, a ring quadrivalent of B's. Below with 28 and 26 chiasmata, two different arrangements of B's in $13^5/38$ with 3 B's and 2 b's. ×2000.

distal end of the long arm (Text-fig. 5 and Table 2). The formation of the S and P chiasmata distinguishes both B and b chromosomes from the A
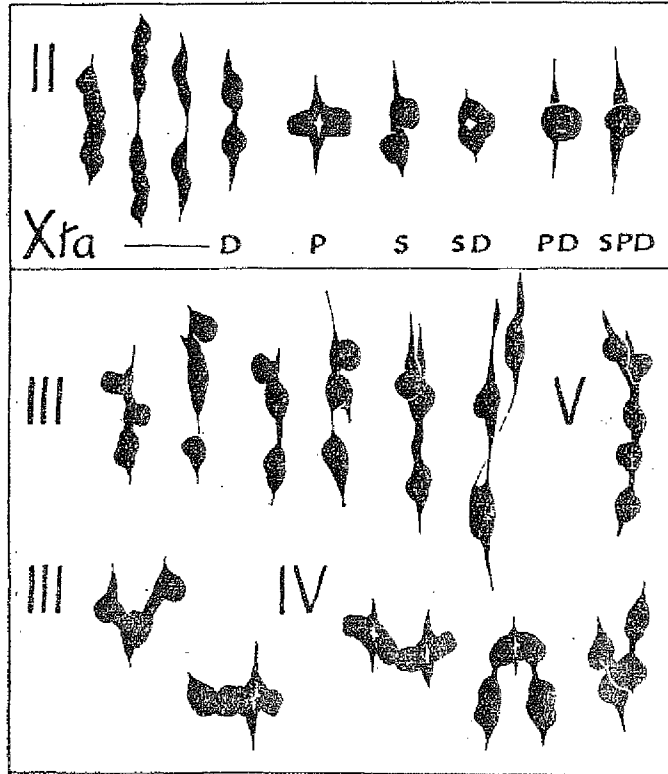
Text-fig. 5. Bivalent and multivalent types of E showing the distribution of chiasmata in S, P and D segments and different co-orientations. × 2000

Table 2. *Positions of chiasmata (Xta) in BB, bb and Bb bivalents*

Segments	S	P*	D	PD	SD	SPD	Bivalents	Xta	Xta/ bivalent
Symbols	100	010	001	011	101	111			
BB	1	46	13	0†	8	1	69	79	1·15
bb	0	0‡	5	3	4	0	12	19	1·51
Bb‡	·3	1	2	—	—	—	6	6	1·00

* Or SP.
† Illustrated, but not recorded in this sample.
‡ All illustrated and from 13°/38.

N.B. Sample of Bb and bb is selected, since these combinations do not occur without extra B's as well. In all groups bivalents alone are considered, without regard to the frequency of univalents.

chromosomes, for in these no chiasma is ever formed so close to the centromere as is possible and in fact most frequent in the B type. It

seems most likely that this change in position is due to the pairing being regularly proterminal, or beginning at the ends, in maize and to the centromere of the B being near to one end, so that it lies in the region of the highest crossing-over potential (Darlington, 1940a). Whatever the mechanism, the B's show what is meant by the position determination of chiasmata (Mather, 1940).
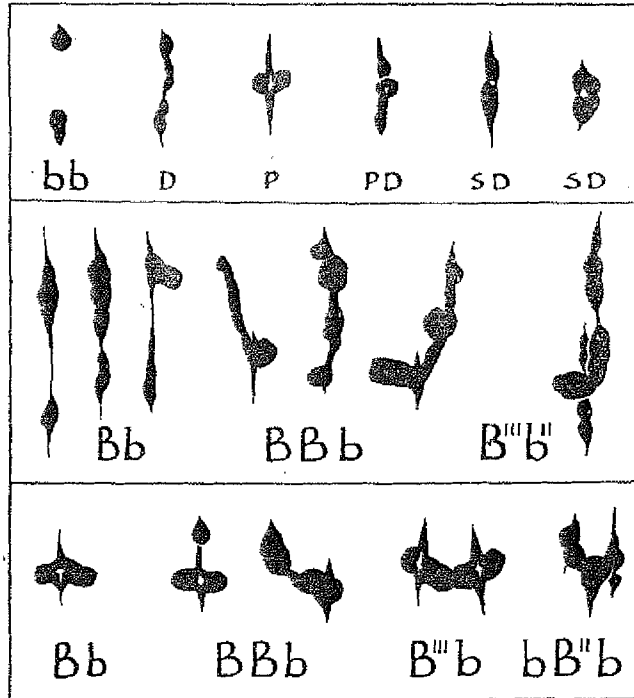
Text-fig. 6. Bivalent b chromosomes and pairing of b with B to give Bb bivalents with S, P and D chiasmata as well as multivalents. Cf. Text-fig. 7 for a diagrammatic representation. ×2000.

These chiasmata near to the centromere, together with the stickiness of the heterochromatin, lead to an unusual problem of interpretation. All bivalents with P-chiasmata appear also to have S-chiasmata, although simple S-chiasma bivalents are rare. Evidently the P-chiasma is so close to the centromere as to pull back the short arm on the other side of the centromere in the same way as happens in the separation of first division inversion bridges (Darlington, 1939a). That there is not necessarily an S-chiasma is shown by multivalents of the BBb type of Text-fig. 6 (lower left-hand figure) where one B has an S-chiasma with a third chromosome, the b: the short arm without a chiasma is still con-

cealed behind the P chiasma. We have therefore made the minimum assumption that, in the absence of contrary evidence, bivalents with the P-chiasma have no S-chiasma.

B and b chromosomes pair with one another and form chiasmata in the three positions comparable to those shown by simple BB and bb bivalents (Text-figs. 3, 4 and 6). This means that the difference between the two types of chromosome lies in the longer segment between the P and D chiasmata: it consists in an intercalary deletion. Such a deletion

Origin of b from B chromosomes and their comparative chiasma formation
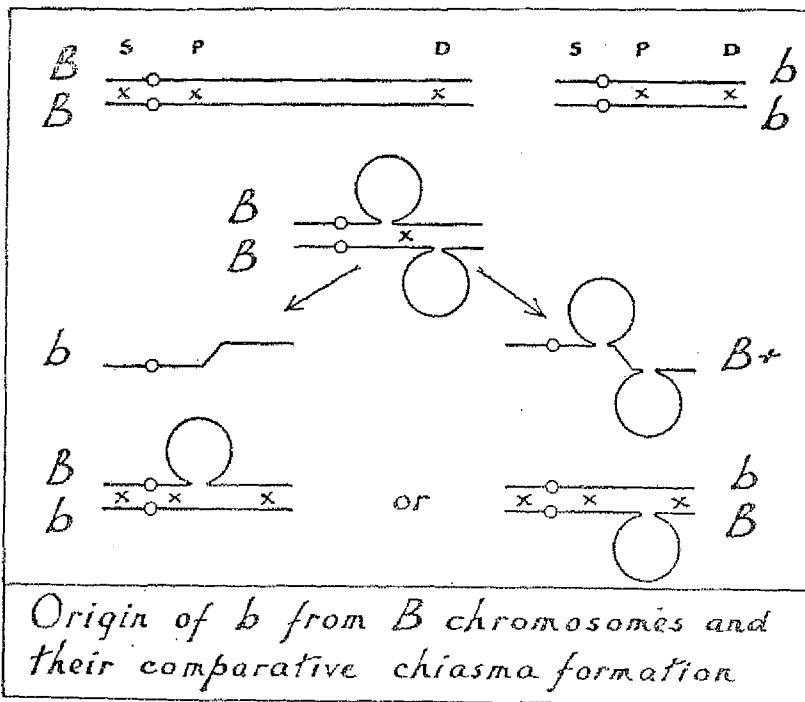
Text-fig. 7.

could take place through crossing-over between duplicated parts of two identical B chromosomes. If it did so there would also be produced a B+ chromosome with yet another duplication such as we have not yet identified (Text-fig. 7).

The chiasma frequency of B chromosomes in 2B plants diverges sharply from that of the A chromosome series (Darlington, 1934). B chromosomes are two-thirds of the length of the shortest A chromosome (no. 10) and should have a chiasma frequency greater than unity to be in proportion. The frequency observed is 0·5.

A second divergence from the A type of pairing is shown by the variance which in all classes of B plants (with one exception, 3 B (ii)) is far higher: there is a more frequent failure of pairing, than would arise with a similar chiasma frequency in A chromosomes (Table 3). The obvious explanation of this excessive variance as well as of the lower aggregate frequency would be a partial failure of pachytene pairing. McClintock (1933) states, however, that B chromosomes pair fairly regularly. It follows that they pair differently from the A chromosomes, and indeed less effectively from the point of view of crossing-over potential. Such a difference has been described by McClintock as due to non-homologous pairing; it is favoured by the heterochromatic character of the B's. This kind of pairing we assume to take place by torsion instead

Table 3. *Distribution of chiasmata to AA and BB chromosomes,*
showing absence of positive or negative correlation within samples
*from single flowers of single plants*

	Chiasmata of BB	0	1	2	3	4
2 BB	Cells (11)	6	4	1	—	—
	Mean chiasmata of AA	23·16	23·75	28·0	—	—
3 BB (i)	Cells (9)	1	1	3 .	4	—
	Mean chiasmata of AA	21·0	21·0	22·3	22·8	—
3 BB (ii)	Cells (12)	1	7	5	—	—
	Mean chiasmata of AA	—	25·7	25·6	—	—
4 BB (i)	Cells (10)	1	4	5	—	—
	Mean chiasmata of AA	23	24·5	23·2	—	—
4 BB (ii)	Cells (15)	1	6	5	2	1
	Mean chiasmata of AA	24·0	25·8	26·0	25·5	22
5 BB, bb	Cells (20)	1	5	4	4	6
	Mean chiasmata of AA	23·0	26·0	26·0	27·0	25·3

of attraction. If the paired threads do not correspond they will never cross-over. And even the correctly paired parts, if very short, will not always cross-over. Occasional torsion pairing will therefore reduce the aggregate frequency of crossing-over and increase its variance.

The importance of torsion pairing in relation to crossing-over may be estimated in another indirect way. A chromosomes in our stocks show inversion crossing-over in 3 or 4% of cells (Text-fig. 8 A, B), and in one cell we have seen two bridges. A B chromosome bridge with fragment was found only once (Text-fig. 8 C). To judge from the frequency of deletions we have seen in B's, inversions should also be frequent. From the frequency of zip-pairing, which McClintock has found to override homology, inversion crossing-over should however be largely suppressed, for the correct pairing of an inversion loop is not always effected even in ordinary chromosomes. It is therefore worth recording that our single instance of inversion crossing-over was in a bivalent B in a plant with

only these two B's (Text-fig. 8 C). We found none in all the plants with multiple B's which McClintock has shown to have such frequent non-homologous torsion pairing at pachytene. We conclude that our single bridge results from homologous pairing of a genuine inversion and that the inverted non-homologous pairing does not lead to crossing-over.

A third divergence of B chromosomes from orthodox rules is shown by the absence of competition for chiasmata between A and B chromosomes. such as Mather (1939) has found between A chromosomes alone (Table 3). Again we see that in the B chromosomes we are evidently
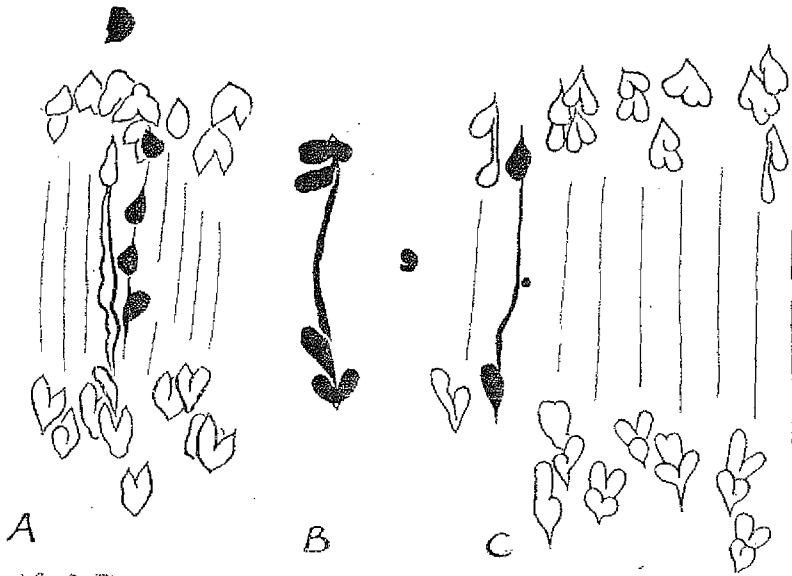
Text-fig. 8. First anaphases. A, A chromosome bridge and acentric fragment and lagging B quadrivalent. B, A chromosome bridge and acentric fragment. C, B bivalent bridge and fragment. ×2000.

dealing with a violently disparate system, one which owes its properties to its heterochromatic character.

The series of observations on 2 B, 3 B, 4 B, 5 B and 6 B cells, on the other hand, shows a concordance of a kind which no previous material has offered the opportunity of discovering, and a concordance, too, which does not seem to depend on the heterochromatin in their composition.

It will be seen that the high associations shown in Text-figs. 5 and 6 are scarcely compatible with the low chiasma frequency of B's in 2 B plants, and this is in fact true (Table 4 and Text-fig. 9). The higher

number of B's have a higher chiasma frequency. But this simple distinction is not enough. The even-numbered systems show a regular

Table 4. *Chiasma frequencies of B and b chromosomes present in different numbers (cf. Text-fig.* 9)

Plant	No. of cells	No. of B's	Chiasma frequency of BB		Chiasma frequency of AA per bivalent
			Per cell	$\frac{1}{2}$ Xta per chromosome	
2⁴/37	10	2	0·50	*0·50*	2·38
1/36	9	3	·1·90	*1·27*	2·00
2⁸/37	12	3	1·42	*0·95*	2·57
2¹¹/37	10	4	1·40	*0·70*	2·37
2¹⁹/37	15	4	1·73	*0·87*	2·55
13⁶/38	20	5 (2 b)	2·45	*0·98*	2·58
3/36	10	5 (1 b)	2·60	*1·04*	2·42
	10	6 (1 b)	2·60	*0·87*	2·45
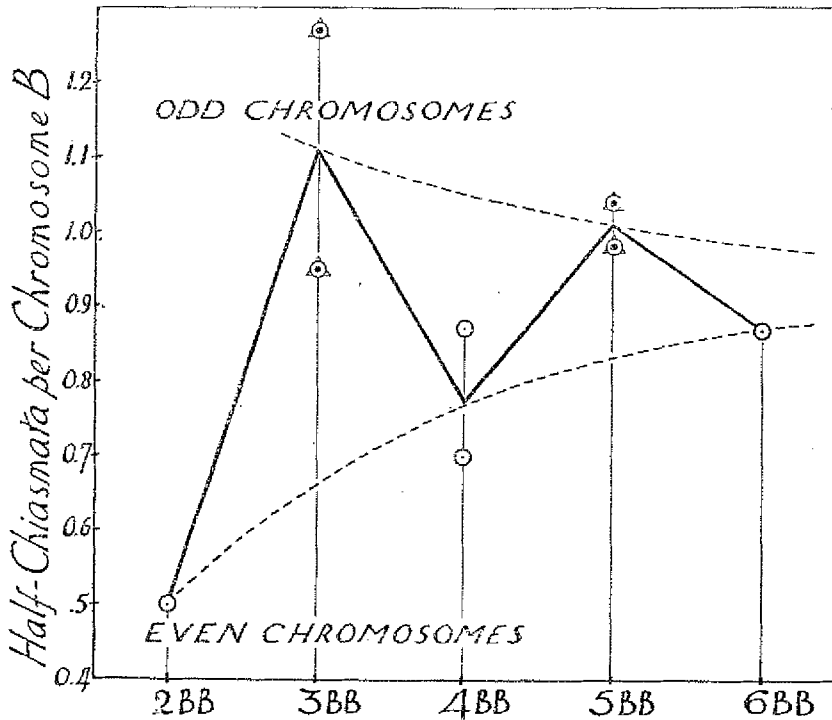
Text-fig. 9. Chiasma frequencies of B chromosomes in plants of different valencies (from Table 4).

increase, while the odd-numbered systems show an increase according to the proportion of odd chromosomes.

These two sources of increase—totality and oddness—can be understood in terms of their one common consequence, increase in the number of points of contact in pairing. In regard to oddness, these points are shown by changes of partner which are known to be more numerous in trisomics than in tetrasomics. In regard to totality, where the chromosomes are small, the points of contact are bound to be more numerous in tetrasomics than in the disomics, provided that the whole nucleus is not correspondingly multiplied (Darlington, 1940b).

When active chromosomes are concerned a similar comparison can be made only with a balanced multiplication of the whole complement. The increase in the size of the nucleus then introduces a third factor, the reduction factor (Upcott, 1939), owing to the general slowing down of pairing. The inert B chromosomes therefore provide a test of a principle that can be tested in no other way and the result seems to depend on general mechanical principles and not on the special properties of heterochromatin.
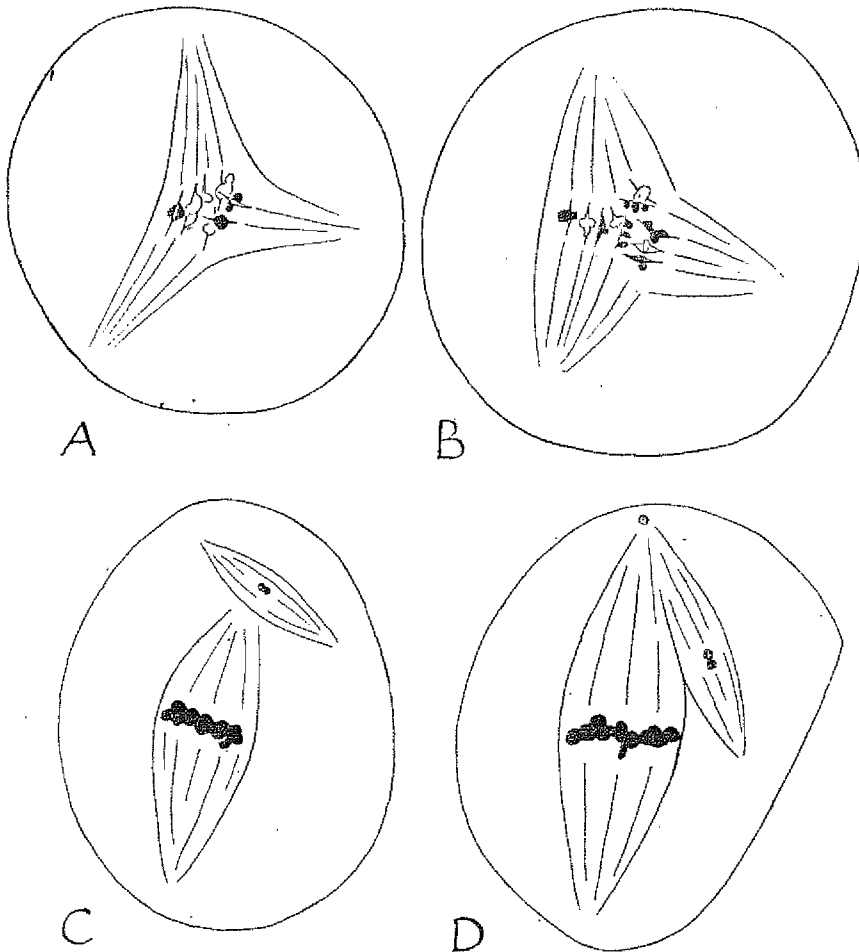
5. B CHROMOSOMES AND THE SPINDLE

Multivalent B chromosomes assume the linear, convergent and parallel forms of co-orientation proper to multiple figures at first metaphase (Text-figs. 4–6). The first of these is not found in A chromosomes for the simple reason that the large A's never have chiasmata near their centromeres so that their trivalents take up too much room on the spindle to allow of a linear co-orientation.

Univalent B's usually lie well removed from the equator, and they then take up a form not apparently found in A univalents or indeed in any unpaired chromosomes at meiosis (Darlington, 1934). The centromere is stretched in the axis of the spindle just as we saw in paired chromosomes with P-chiasmata and, in the same way too, the short arm is concealed so that the chromosome appears telocentric. Fig. 3 (i) illustrates a single exception to this rule.

The stretching of the spindle at anaphase pushes an unpaired B far beyond the paired chromosomes, so that at the second division it often forms a separate spindle. The structure of this spindle becomes clear when the material is preserved in 70% alcohol for some months. Successive stages then reveal its property of cohesion. The separate B spindle is too small to coalesce symmetrically with the main spindle, but the poles fuse (Text-fig. 10 C, D). Such arrangements no doubt reduce the frequency of loss of the B chromosome from the gamete nuclei.

This observation helps to explain the series of stages sometimes

found in the development of the first division spindle. Here a fusion of separate chromosome spindles probably takes place with some rapidity. Sometimes, however, it gives rise to a tripolar spindle which is more

Text-fig. 10. A and B, tripolar spindles at first metaphase. C and D, early independence and later cohesion of a B chromosome spindle at second metaphase. × 1000.

persistent (Text-fig. 10 A). And, rarely, the spindle seems to reach equilibrium in this position, so that an irregular anaphase will follow.

These examples of irregular cohesion show us the opposite side of the picture to that given by failure of cohesion as a particular genetic abnormality in grasses (Darlington & Thomas, 1937).

6. THE SELECTION EQUILIBRIUM

The effects of failure of pairing and loss of B chromosomes at meiosis are first seen in the ensuing pollen grain mitosis (Text-fig. 11). There is in the first place a wide range in the number of B's, a range which may best be measured by the coefficient of variation (V/M). This value is 0·33 for the plant with four B's. It is evidently exaggerated by double reduction, or differential segregation at both meiotic divisions. This is shown by one haploid pollen grain which has more B chromosomes than
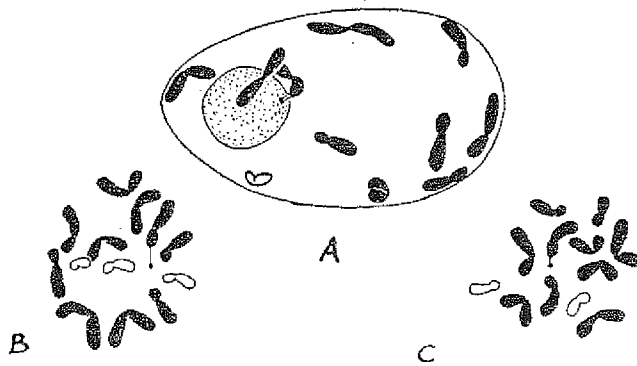
Text-fig. 11. Pollen grain mitoses with one, two or three B chromosomes from a 4 B plant. × 2000.

Table 5. *Loss and variance of B chromosomes between meiosis and first pollen grain mitosis in 4 B plant*

PG	10+0	÷B	÷2B	+3B	÷4B	÷5B	T	B's	M
No.	1	13	30	6	—	1	51	96	1·9

Total B's expected without loss: 102.
Chance of loss: 6%.
Variance (V): 0·63.
Coefficient of variation (V/M): 0·33.

its zygotic parent. Such double reduction occurs in fragments of *Tradescantia* (Darlington, 1929) and must be due to the passage of whole univalents without division into particular daughter nuclei of the tetrads, a not improbable event.

In the second place, the frequency of B's in the pollen grains has a lower mean than half the parental zygotic number and the reduction in mean may be taken as a measure of the chance of loss of each B, in this case a chance of loss at meiosis. This value is 6% in the plant with four B's (Table 5). It of course represents a fraction of the unpaired B chromosomes, a fraction greater than 6%.

These estimates are useful when we come to the next stage, the progeny of B plants. Reciprocal crosses show a higher loss on the female than on the male side (Table 6). Where the number of B chromosomes is odd rather than even, we expect, and we find, a higher coefficient of variation. We also find that the coefficient of variation is higher with B's coming from the male side than from the female side. And finally the coefficient of variation is lower with high-B than with low-B plants, a result attributable to the increase in chiasma formation, and the consequent greater regularity of pairing, that we have found with more B's.

One interesting combination of the results of $O \times B$ and $B \times O$ has been pointed out to us by Dr Mather: $B \times B$ should have the sums of the means and variances of the first two crosses and the same V/M value. Agreement between the expected and observed distributions may be

Table 6. *Loss and variance of B chromosomes in transmission to progeny*

(0–2 B parents calculated from Longley (1927); 3–6 B parents from present results)

Parents...	0	B	2 B	3 B	4 B	5 B	6 B	Plants	B's	Loss %	M	V	V/M
B × O	109	58	3	—	—	—	—	170	64	*24·7*	0·38	0·27	0·72
O × B	76	22	15	—	—	—	—	113	52	*8·0*	·0·46	0·52	1·13
B × B													
Obs.	105	91	55	14	—	—	—	265	243	*8·3*	0·92	0·81	0·88
Exp.	*114·3*	*93·9*	*43·3*	*12·9*	*0·6*	—	—	*265*	*221·7*	*16·3*	*0·84*	*0·79*	*0·94*
2 B × 2 B	6	19	13	30	5	—	—	73	155	*0*	2·12	1·47	0·69
? 3 B × 6 B	—	—	1	5	5	—	—	11	37	—	3·36	0·45	0·13
4 B × 5 B	—	—	2	2	2	2	1	9	34	*16·0*	3·78	1·95	0·51
5 B self	—	—	1	1	5	2	2	11	47	*14·0*	4·27	1·42	0·33

tested by the calculation of χ^2 if we group 3 B and 4 B classes: $\chi^2 = 4·016$, D.F. $= 3$, $P = 0·30–0·20$, showing good agreement (Table 6).

So far we have taken the transmission of B's as resulting directly and without selection from their behaviour at meiosis, and our analysis does not allow us to contradict this simple assumption. Selection, as we shall see, might very well have modified the results, but there is no evidence of it in the experiments.

Now, however, with the knowledge of what happens within families of uniform parentage, we can turn to the populations. Randolph's determinations (1928 a) were made on two classes, genetic cultures and commercial varieties (Table 7). Four of the cultures are F_2 and F_3 families and these show the lowest coefficients of variation. The fifth, perhaps less inbred, shows the highest value. The varieties, which must be less inbred than the cultures but perhaps more selected, show intermediate values. But what is remarkable is that the values of both cultures and varieties are so little higher than in the single families with corresponding means.

They have for the most part the same unimodal distribution with a scarcely increased V/M value. Such a situation can be maintained only by selection in favour of an optimum number of B chromosomes in each stock.

There is a second and entirely independent source of evidence of selection pressure acting on the occurrence of B chromosomes. At every meiosis, as we have seen, there is a certain chance of loss varying as between male and female and as between high and low, and odd and even B plants. Furthermore, the B's suffer a continual chance of loss of parts as we have seen in the origin of the deficient b's. Nevertheless, the B chromosomes continue in the population and have continued for a long period. This is shown (as in *Secale*) equally by their present lack

Table 7. *Coefficients of variation in the number of B chromosomes in cultivated populations (after Randolph, 1928a)*

	No. of B's ...	1	2	3	4	5	6	7	8	9	n	M	V	V/M
	Culture													
21	*su Tu tu*	4	5	—	1	—	—	—	—	—	10	0.80	0.84	1.05
15	(F_2) *B, $ts_2 \times Ts_2ts_2$*	1	4	5	1	—	—	—	—	—	11	1.55	0.67	0.43
16	(F_2) *B g Tu tu*	2	5	6	7	—	—	—	—	—	20	1.90	1.04	0.55
11	(F_3) *a C R Pr Su lg*	1	1	5	2	4	1	—	—	—	14	2.71	1.92	0.71
1	(F_3) *A C R Pr Su*	—	—	3	2	4	1	—	—	—	10	3.30	1.12	0.35
	Variety													
	H. G. Nugget	6	2	—	—	—	—	—	—	—	8	0.25	0.21	0.84
	G. Bantam	10	6	3	—	—	—	—	—	—	19	0.63	0.58	0.92
	N. Y. S. Flint	2	2	4	2	—	—	—	—	—	10	1.60	1.04	0.65
	Bl. Mexican	2	6	8	12	5	4	4	1	1	43	3.2	3.30	1.03

of apparent relationship with the normal chromosomes and by their wide distribution in unrelated stocks. There must therefore be a selection pressure acting on whole plants to increase the number of B's in the population exactly equal to the selection pressure acting on the chromosomes in the cells to reduce their number. We thus have two selective forces acting at different levels of integration and producing in different stocks different equilibria. Indeed the measurement of the known mechanical loss is a measurement of the unknown populational gain.

There is thus not merely an optimum number of B's in each population but that optimum number is greater than the mean number observed.

A measurable selection in favour of B's means that these supposedly inactive chromosomes have an activity and a measurable activity. That activity would seem, from their heterochromatic properties, to depend on their special nucleic acid metabolism. This view is supported by Longley's observations (1938) that in thirty-three primitive maize stocks

in the U.S.A., thirteen have B chromosomes and these are more frequent where the A chromosomes have fewer heterochromatic knobs. And this combination in turn is more frequent in regions more remote from Mexico. B chromosomes occur in *Euchlaena mexicana* but their presence is to be attributed to contamination with maize and not to their independent origin in this species (Longley, 1937). Thus it seems that, as the cultivation of maize has progressed, the B chromosomes have taken over and enlarged the function of the knobs; and in doing this they have provided a more elastic means of regulating the nucleic acid metabolism of the nucleus as a whole.

Whatever the activity of the B chromosomes may be, it is evidently of a different kind from that of the A chromosomes, and should therefore be subject to different laws of evolutionary change. That this is so we see in two ways. The appearance of the deleted b chromosome three times in our experiments shows that original and spontaneous changes are more frequent than in the A chromosomes. And, further, since the B chromosomes as wholes are not indispensable, the loss of their parts cannot be immediately and seriously deleterious. Nevertheless, we see that the B chromosomes are preserved at about the same size in all cultures and at all times, since they were first found by Kuwada in 1911, and the gain in size which we might expect as the reciprocal of loss does not seem to occur. There are therefore conditions limiting the effective variability of the B chromosomes, conditions which are, mechanically, more stringent than those that apply to the larger active chromosomes. We would suggest that three such conditions can already be specified:

(i) That reduction in size leads to too frequent loss at meiosis for the small b's to survive as alternatives to large B's, although the B's may themselves have begun as even smaller bodies.

(ii) That increase in size leads to too frequent loss at mitosis for similar survival. The large B already has a sub-efficient centromere, as shown by its irregularity at mitosis. Its centromere will not *carry* a greater load.

(iii) That internal rearrangements not leading to change in size are largely indifferent, since the parts of the B are scarcely differentiated in function. We must therefore expect considerable rearrangements, such as are indicated by McClintock's determination of a terminal centromere in her stocks and our determination of an intercalary one in ours, without any great change of size. Their kind and frequency will repay study in crosses of different B stocks such as seem in the past to have been somewhat miraculously avoided.

It is worth while knowing something of these principles, for the B chromosomes of maize are just one example of an evolutionary progression of which we are beginning to see the steps. In many species we see new fragment chromosomes appearing from time to time. Most of these probably come to nothing. In *Fritillaria, Tradescantia* and *Ranunculus* we see them reduplicated to such an extent that they must be taken to have acquired a use in the species, the race or the clone. In *Secale* they are so widespread as to have maintained this use for a long time. In *Zea Mays* they have established special equilibria in special varieties. These different equilibria are a symptom of the rapid adjustment of this rapidly evolving species, or genus as we may say, since *Zea* as a genus owes its existence to human selection of *Euchlaena*.[1] Whether in fact the urgent selection practised by man in favour of bigger and better corn, demanding as it has done the reconstruction of cell activity on a new level, is responsible for the spread of B chromosomes in certain stocks, even when transplanted back to *Euchlaena*, we cannot yet say. But it is a question to which maize breeders can supply the answer.

Turning to animals, we see in the Heteroptera an even more advanced condition of the subinert supernumerary. In some species, like *Nepa cinerea*, it has acquired constancy of reproduction, while in others, like *Cimex* and *Metapodius*, it varies in number. It is indispensable for the population although not yet for the individual. In this group parts of the sex chromosomes have been used as a basis for the supernumerary system, and it fits them well mechanically on account of their special trick of segregation. But the physiological implications are the same as in plants, where ordinary chromosomes are used as the basis of supernumeraries: they provide a new supply of heterochromatin which is readily adjustable to a changing economy.

Our conclusion is therefore clear. Some species are conveniently organized with their hereditary materials in one type of chromosome. Others arrive at a division of labour. The physiology of this division and its mechanical and evolutionary stability are problems that can be handled by the comparison of other species that are undergoing a reconstruction of their cell life.

7. SUMMARY

1. B chromosomes of *Zea Mays* are short heterochromatic chromosomes hitherto supposed to be inert but existing in equilibrium in many genetic cultures and varieties.

[1] A view we must continue to hold, in spite of Mangelsdorf & Reeves (1939).

2. Their frequency of crossing-over is less than proportionate to their length by the A chromosome standard, and its distribution is different. B chromosomes regularly form chiasmata closer to the centromere than is possible in A chromosomes. This is due to the position of the centromere which is nearer one end than in the A type.

3. Their unlimited reduplication makes it possible to show that their crossing-over frequency is increased by odd numbers. This effect is due to more frequent changes of partner at pachytene, on the anchorage principle.

4. B's are liable to irregularity at mitosis (owing to deficient centromeres) and loss at meiosis (owing to non-pairing). They are also specially liable to deletion (owing to heterochromatin content) and then give smaller b chromosomes.

5. A double selection pressure thus acts to reduce their size and frequency in the population and must be compensated by an equal and opposite selection in their favour.

6. The equilibrium that is reached implies that mechanical loss in the population of cells is equal to the physiological gain in the population of organisms.

7. Thus the cell observations measure the total opposite selection pressure and reveal a population, such as we might expect in *Zea Mays*, under high *selectional stress*.

8. Longley's observations may be taken to indicate that under artificial selection B chromosomes are replacing the heterochromatic regions of A chromosomes in the nucleic acid metabolism of the cell.

9. Similar subinert supernumeraries in other organisms fall into an evolutionary progression, indicating division of labour in a hitherto disregarded function of the chromosomes: the maintenance of their own mitotic economy.

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EXPLANATION OF PLATE 9

Figs. 1–4. First metaphases in pollen mother cells of *Zea Mays* with B chromosomes.
      × 3000.

Figs. 5 and 6. Second metaphases. × 1000.

Fig. 1. Trivalent B (third in Text-fig. 5).

Fig. 2. Univalent B and b chromosomes.

Fig. 3. Bb bivalent with S chiasma (third in Text-fig. 6) and univalent b chromosome.

Fig. 4. bb bivalent with D chiasma (Text-fig. 6) and B univalents off the plate.

Fig. 5. B chromosome lost in the cytoplasm.

Fig. 6. B chromosome with spindle cohering to the main spindle (Text-fig. 10 D).