SOMATIC INSTABILITY OF TELOCENTRIC CHROMOSOMES IN WHEAT AND THE NATURE OF THE CENTROMERE[1]

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THE centromere, a necessary part of all chromosomes, is the region responsible for congression and poleward movement. It is called "kinetochore" by those who wish to emphasize that it is the center of kinetic chromosome activity. This name is also more suitable because the centromere often is far from central in its location. In the following, the name centromere is retained because of its relation to the term "telocentric," which denotes a one-armed chromosome with a terminal centromere.

Although in some materials with favorably large chromosomes, some investigators have seen a complex structure (LIMA-DE-FARIA 1949; MATTSON 1963), and this had been suggested even earlier (NEBEL 1939), the centromere of most mitotic chromosomes is seen only as a constriction, while in meiotic chromosomes it appears as a simple, homogeneous, translucent body (RHOADES 1940). The lack of staining with the usual chromatin stains shows that there is less DNA than in the rest of the chromosome at this time, which could be related to the fact that it is active at this stage by analogy with the "puffs" of giant chromosomes (BEERMAN 1963) or compared to the converse situation of the Barr bodies (THOMPSON 1965).

Because of their universal occurrence, and their usual optical emptiness, it has been considered that " . . . the centromeres of different chromosomes are all alike in their form and behavior" (DARLINGTON 1939). This behavior is highly organized and sensitive to environmental and chemical influences and is likely to have as a basis a complex structure, even though this structure is usually microscopically invisible. LIMA-DE-FARIA (1958) in pachytene chromosomes of maize could distinguish two large and one small pair of chromomeres within the centromere region.

Ordinarily centromeres divide quite regularly at mitosis and meiosis, but when a chromosome is unpaired, misdivision may occur at meiosis and give rise to telocentric chromosomes. If the centromere has a complex structure, the transverse split of misdivision might occur in different portions, resulting in telocentrics that differ in the completeness of their centromeric regions (Figure 1). It is known that acentric fragments are lost; lacking a kinetic center, they fail to reach the poles and are excluded from the telophase nuclei. Since it has been observed that telocentrics are also lost somatically or may change to isochromo-

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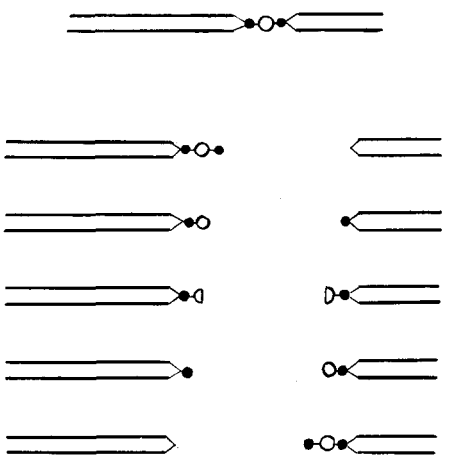


FIGURE 1.—Unpaired chromosome with centromere region composed of three subunits and the results of misdivision at various positions.

somes, it seems not unreasonable to hypothesize that their somatic behavior depends on the completeness or incompleteness of their centromeres.

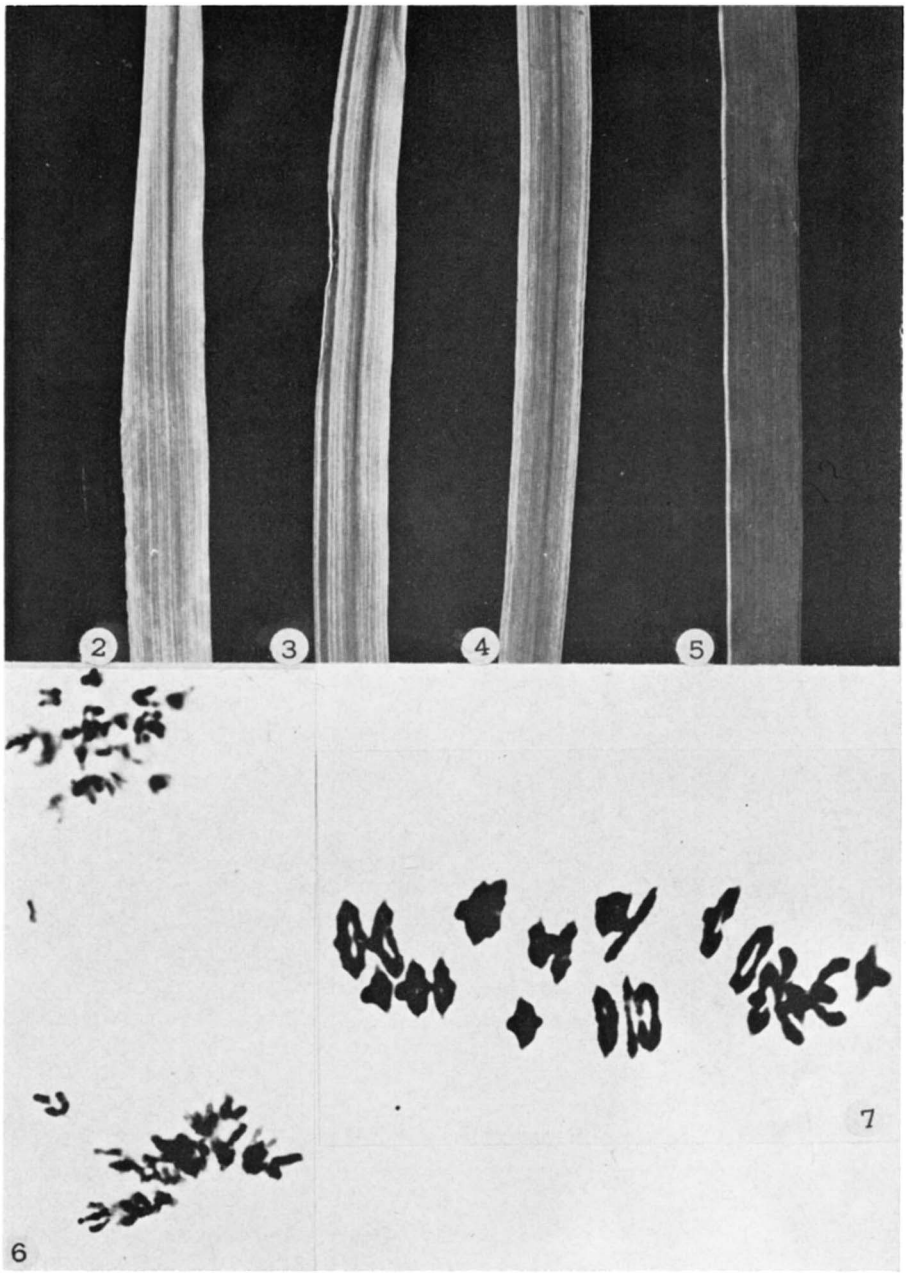
The purpose of the experiments reported here was to determine whether misdivision can occur in different sections of the centromere region as detected by different types of telocentrics obtained.

Polyploid wheat is a particularly suitable material for a cytogenetic study of this problem. Tampering with centromere region may result in chromosomal loss, which would be lethal in a diploid organism but is tolerated in hexaploid wheat, and the genetic consequences of such loss are already known (SEARS 1954). In the variety Chinese Spring, used in these experiments, misdivision of monosomes is frequent at meiosis and provides a ready source of telocentric chromosomes (SEARS 1952).

MATERIAL

To study the results of misdivision, chromosome 3B (III) was chosen. The short arm of this chromosome was marked with Neatby's virescent, *v*, a hemizygous-ineffective gene. A plant or a sector with one chromosome 3B carrying *v* is green, with two 3B's virescent, and with three 3B's white. Loss or gain of the chromosome bearing this gene can be recognized through the occurrence of green or white sectors on a virescent plant (Figures 2 to 5). The sectors can equally well be used to determine the somatic stability of a telocentric chromosome for the short arm of chromosome 3B which carries the marker gene.

Deficiency of the long arm of chromosome 3B, which carries a gene for synapsis, results in much irregularity of chromosome transmission, and even if one long arm is present some reduction of synapsis may occur. It was therefore desirable in this study to use lines not deficient for the long arm of chromosome 3B. This was accomplished by combining the six *v*-carrying, short-arm telocentrics each with the same pair of telocentrics for the long arm (obtained from E. R. SEARS). The resulting "double ditelosomic" lines, had 22 pairs of chromosomes, consisting of 20 normal pairs plus chromosome 3B present as two telocentric pairs (Figure 7). In this material, pairing at meiosis and meiotic transmission was quite regular.



FIGURES 2–7.—(2.) Virescent leaf. (3.) Virescent leaf with green-white twin sector. (4.) Virescent leaf with two narrow green sectors to the left of the somewhat dark middle of the leaf. (5.) Green leaf with white sector. (6.) Misdivision of monosome 3B. Telophase I. One telocentric moving to the upper pole, the other three arms moving to the lower pole. (7.) Meiotic metaphase I in a double ditelosomic line. Note the 20 normal pairs of chromosomes and two telocentric pairs near the middle of the plate.

RESULTS

Offspring of self pollinated monosomic 3B carrying Neatby's virescent consisted of about 75% green plants (mono- and nullisomic 3B) and about 25% virescents (disomic 3B. Table 1a). The virescent plants were analysed cytologically to determine whether they carried two entire chromosomes or whether a new telocentric for the virescent-bearing arm had been formed.

The frequency of plants with a heteromorphic bivalent, each including newly derived telocentrics, was 4.9% (Table 1b). However, there was a large class of plants (14.5%) that started out as virescent seedlings but when analysed at meiosis were found to have only a monosome. At this time virescent plants had become much more green so that they could no longer be distinguished from green plants with certainty. From a few plants in which root tips were checked, it was concluded that most of these plants originally had a telocentric for 3B, which however was so unstable that it was lost somatically in the development of the plant. Another possibility is that, owing to monosomic shift, a few of these plants had an unrelated monosome; that is, that they were disomic-3B and monosomic for some other chromosome.

A cytological check of meiosis showed misdivision of the monosome at first division in 25.7% of 435 microsporocytes from 19 different plants of monosomic 3B (Figure 6). Misdivision at second division increases the number of telocentrics. Thus, if misdivision of about the same frequency occurs on the female side, where there is no selection against deficient gametes, the large class of plants (14.5%) that started out as virescent seedlings but only contained a monosome at meiosis could be accounted for by very unstable telocentrics, lost early in ontogeny.

In the spring of 1965 a disomic control and two double ditelosomic lines were grown for a comparison of somatic sectoring. Preliminary experiments had indicated that these might differ with regard to the frequency of sectoring. Forty-two plants of each line were grown in a coded, semirandomized arrangement. Sectors were recorded and at the termination of the experiment their frequency was adjusted to take into account the number of leaves made by each plant. Records for main stem and tiller leaves were kept separately, but final combination of

TABLE 1

a. *Offspring of monosomic 3B* (v)

Total No.	Green		Virescent		White	
3148	2318	(73.6%)	818	(25.9%)	12	(0.5%)

b. *Cytological analysis of virescent and white offspring of mono 3B* (v)

	Virescent						White	
Total No.	Disomic		Heterom. biv.		Monosomic*		Normal + iso	
553	439	(79.4%)	27	(4.9%)	80	(14.5%)	7	(1.2%)

* Virescent as seedlings, but green at flowering. Telocentric assumed present in the seedling (verified in five root tips out of nine examined) but lost during the development of the plant.

TABLE 2

Somatic sectors in virescent plants: A disomic control and two lines double ditelosomic for chromosome 3B

Replication No.	Control			Double-ditelo 7			Double-ditelo 1		
	No. Leaves	No. Sectors	Percent Sectors	No. Leaves	No. Sectors	Percent Sectors	No. Leaves	No. Sectors	Percent Sectors
1	31	2	6.4	30	4	13.3	22	3	13.6
2	47	2	4.2	25	5	20.0	21	4	19.0
3	48	2	4.2	37	6	16.2	29	7	24.1
4	67	4	6.0	68	4	5.9	23	5	21.7
5	28	0	0.0	23	1	4.3	22	2	9.1
6	36	2	5.5	45	4	8.9	22	3	13.6
7	39	1	2.6	20	0	0.0	33	3	9.1
8	28	1	3.6	27	4	14.8	29	3	10.3
9	55	1	1.8	52	4	7.7	70	11	15.7
10	47	2	4.2	58	2	3.4	57	10	17.5
11	42	1	2.4	47	4	8.5	45	3	6.7
12	76	3	3.9	61	6	9.8	73	18	24.6
13	66	1	1.5	63	4	6.3	37	10	27.0
14	62	1	1.6	51	1	2.0	57	5	8.8
Total	672	23		607	49		540	87	
Mean			3.4			8.1			16.1

Analysis of variance, percent sectors

Source	df	Sum of Squares	Mean Squares	
Replications	13	453.95	34.92	not significant
Treatment (2)		1076.02	538.01	significant
Disomic *vs.* 7+1	1	721.01	721.01	significant
7 *vs.* 1	1	355.01	355.01	significant
Exp. Error	26	598.46	23.02	
Total	41	2128.43		

3 plants per replicate

these data did not alter the results. Significantly more sectors were found in the double ditelosomic lines than in the controls, and the two lines also differed significantly from each other (Table 2).

A second experiment was run in a similar manner but enlarged to six double ditelosomic lines and 90 plants of each. The plants were grown in a semirandomized coded arrangement in the greenhouse in the fall of 1965. Because the expressivity of *v* was low at the high temperatures which prevailed in the early fall, the first two or three leaves of these plants were too nearly normal green for scoring the green (*v*-) sectors. The data are presented in Table 3.

Analysis of variance and Duncan's new multiple range test on the data, adjusted for the number of leaves, showed that the control had significantly fewer sectors than any of the double ditelosomic lines. Line No. 1 was again significantly different, being less stable than the others. All the other five lines showed nonsignificant differences between each other in their somatic stability.

DISCUSSION

In the data presented, every somatic sector is recorded as a separate loss or gain.

TABLE 3

Somatic sectors in virescent plants:
A disomic control and six lines double ditelosomic for chromosome 3B

	Control		1		4		5		7		8		11	
Replication No.	No. leaves	Percent sectors	No. leaves	Percent sectors	No. leaves	Percent sectors	No. leaves	Percent sectors	No. leaves	Percent sectors	No. leaves	Percent sectors	No. leaves	Percent sectors
1	42	9.5	38	23.7	39	10.3	37	10.8	29	6.9	41	12.2	45	11.1
2	45	6.7	39	10.3	23	13.0	42	16.7	37	16.2	40	32.5	36	5.6
3	54	1.9	40	20.0	32	15.6	45	11.1	45	13.3	41	14.6	47	8.5
4	54	5.6	46	10.9	42	11.9	36	8.3	33	18.1	45	20.0	52	7.7
5	49	0.0	51	15.7	37	29.7	42	38.1	37	18.9	47	12.8	39	20.5
6	51	3.9	45	27.9	39	20.5	52	23.1	33	12.1	46	10.9	43	18.6
7	52	9.6	50	36.0	42	23.8	41	9.8	40	17.5	44	22.7	46	8.7
8	59	8.8	43	23.3	40	10.0	47	25.5	50	6.0	45	17.8	54	14.8
9	51	11.8	36	13.9	35	11.4	51	15.7	30	23.3	46	13.0	48	6.3
10	43	2.3	45	42.2	33	12.1	34	17.6	37	5.4	32	6.3	49	8.2
11	57	8.8	35	28.6	39	10.3	44	11.4	40	7.5	42	30.9	45	11.1
12	57	1.8	55	27.3	28	14.8	49	12.2	45	8.9	44	4.5	47	12.8
13	65	1.5	47	21.3	42	11.9	44	9.1	42	19.0	52	13.5	49	12.2
14	50	6.0	37	16.2	42	9.5	55	12.7	46	4.3	45	11.1	52	11.5
15	57	10.5	51	11.8	46	34.8	57	10.5	45	8.9	49	18.4	52	21.2
16	58	3.4	42	45.2	44	22.7	50	12.0	49	12.2	39	20.5	51	7.8
17	53	11.3	45	37.8	43	11.1	45	15.6	43	11.6	44	9.1	43	18.6
18	54	1.9	50	12.0	52	0.0	51	13.7	47	6.4	45	6.7	53	26.4
Total	951		795		698		822		728		787		852	
Mean % Sectors		5.6		23.6		15.2		15.2		11.7		15.4		13.0

Analysis of variance, percent sectors

Source	df	Sum of Squares	Mean Square	
Replications	17	900.68	52.98	not significant
Treatments (6)		3044.78	507.46	significant
Control *vs.* others	1	1534.87	1534.87	significant
No. 1 *vs.* others	1	1330.42	1330.42	significant
Other treatments	4	179.49	.....	not significant
Exp. Error	102	4645.46	45.54	
Total	125	9604.75		

Duncan's new multiple range test showed nonsignificant differences between lines 4, 5, 7, 8 and 11.
5 plants per replicate

Most of the sectors are due to simple losses since they consist of green stripes on the virescent background. Twin sectors green-white could be equally well due to the formation of an isochromosome as to nondisjunction since both raise the dosage of the short arm to three in one cell and leave only one in the other.

It is quite possible that a maldistribution in the meristematic region may affect several consecutive leaves and thus be recorded as several events. The large number of plants used in this study should equalize any error arising from this, unless the earlier mitoses in some lines are more prone to loss. In fact, the assumption underlying the calculation of the data in percent of leaves is that the more leaves a plant made, the more cell divisions took place, and the more chances for loss occurred. Even after such an adjustment is made, the frequency of loss in plants of the same line is still very variable.

The second assumption is that once a telocentric has been established it cannot repair its centromere region, and the more defective this region is, the greater is the chance that the telocentric will be lost somatically. The fact that line No. 1 remained significantly less stable than the others through several generations is evidence that repair can not take place.

Whether a misdivision of a monosome results in telocentrics or isochromosomes may be due to the stage of reproduction of the centromeric chromomeres and connecting threads. If the critical centromeric chromomere were undivided, the resulting misdivision might be more likely to give rise to isochromosomes. Misdivision might be thought of as occurring at the point where duplication or division has not yet been completed. Some evidence for this idea can be found in the fact that monosomes that get onto a metaphase plate early apparently always misdivide (BROWN 1958; Figures 5 to 6, and OKAMOTO, personal communication). A monosome dividing after the bivalents separate more frequently divides normally (see RESULTS in this paper), i.e., it gives rise to two normal half chromosomes.

From the data presented here, it appears that at least three classes of telocentrics arise as a result of misdivision of monosome 3B. A large number (75%) of these telocentrics are very unstable and can not be recovered at meiosis. These may be deficient for a large part of the centromere region (see Figure 1). The second class, which can be maintained but which is lost very frequently somatically, presumably has more but not a complete centromere region. This class, represented in this study by only one line (No. 1), originated from an isochromosome which misdivided later to give a telocentric. If isochromosomes arise by misdivision near the center section of the centromeric region (Figure 1), they would presumably have a duplicated 50% of this region. If the centromere region were a reverse repeat, as suggested by LIMA-DE-FARIA (1949), this would be equivalent to a normal nondeficient centromere region. It would be of interest in this connection to see whether it is possible to obtain isochromosomes with varying amounts of centromere region.

The more stable telocentrics (lines 4, 5, 7, 8, and 11) should have an almost complete centromere region (Figure 1). The fact that they are still less stable than the normal two-armed chromosome, leads one to suspect that a small defi-

ciency remains. If this were not true an intrinsic instability due to the terminal position of the centromere would have to be assumed.

The data on somatic stability of telocentrics reported here suggest that the centromere region has a complex structure. Several questions are still unanswered, however. It might be of interest in this connection to compare several telocentrics derived from isochromosomes. Likewise, if an acrocentric with almost no second arm were available, it might give a further clue to the effect of a complete centromere on somatic stability. Telocentrics of other chromosomes than 3B, if they can be appropriately marked, should also be studied, especially with respect to possible differences from 3B.

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SUMMARY

Monosome 3B, marked with Neatby's virescence gene on the short arm, misdivides at meiosis to give up to 20% short arm telocentric chromosomes in the selfed offspring. The largest number of telocentrics, although present in the seedling as indicated by its virescent color, are lost somatically before meiosis. A second class of telocentric is very unstable somatically, while a third class is more stable but is still lost significantly more frequently than normal chromosomes. The relative instabilities of the telocentric chromosomes are attributed to the degree of completeness of the centromere region received when they were originally formed.

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