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Changes in the Structure of B^4 Chromosomes in Maize[1]

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Summary. A class of mosaic endospersm involving the marker Su_1 was observed among the progeny of individuals hyperploid for the chromosome B^4 and genetically analyzed. The exceptional individuals showing mosaic endosperm were found when the hyperploid material was used as pollen source. While in some cases mosaicism was limited to the endosperm tissue, with no apparent consequences in the embryo, in others the mosaicism was transmitted to the progeny, which showed changes in the structure of the B^4 chromosome, with the formation of unstable chromosomes whose genetic behaviour was similar to that of ring chromosomes. This interpretation was cytologically confirmed. In other cases the B^4 chromosome analyzed in mosaic endosperm individuals underwent altered transmission frequencies or loss, suggesting that its original structure had been modified by breakage-fusion-bridge cycles. The changes in this chromosome revealed by the mosaic phenotype are discussed in relation to the original structure of the B chromosome and the B^4 hyperploid condition.

Introduction

Mosaic phenotypes may be of chromosomal nature in maize, reflecting a pattern of instability of a particular chromosome, when they indicate, in a suitable genetic background, the repeated loss of one or more dominant traits. Phenotypes of this kind are produced in a variety of ways, whenever a breakage-fusion-bridge cycle is started (McClintock, 1938, 1941, 1948, 1950). However, the ability to transmit mosaicism through the generations, although with typical irregularity, is presumably restricted to particularly unstable chromosome structures such as reverse tandem duplications in chromosome 9 (McClintock, 1941, Schwartz, unpublished, cited by Doerschug, 1967 and Doerschug, 1967). Although ring chromosomes normally self-perpetuate in a stable manner in *Drosophila*, they are often unstable in maize and other plants, and in other organisms including man (McClintock, 1938; Schwartz, 1953, 1958; Emmerling, 1955, 1959; Miles, 1971; Lejeune, 1967, 1968; Levan, 1956; and others). They can produce transmissible mosaics, especially if carried as supernumerary chromosomes. An exception to the instability of ring chromosomes in maize was studied by Schwartz (1958). Unstable dicentric structures have been studied by many authors (reviewed by Saccardo, 1971), who observed the inheritability of some.

The unstable chromosomes so far studied were obtained from radiation-treated materials or were isolated from their progeny after further rearrangements occurred spontaneously. Because of their unstable nature and behaviour, such chromosomes are difficult to handle genetically and, unless selected, they would rapidly be eliminated. Nevertheless they represent excellent material for investigating not only the behaviour of unstable chromosomes, but also the genetic effects of repeated chromosome breakage, the production of highly rearranged chromosome structures and the possible inception of new controlling elements (McClintock, 1950 and Doerschug, 1967).

Different kinds of chromosome instability have also been observed in the presence of B chromosomes and knobs (Bianchi et al., 1961; Carlson 1969, 1970, 1973; Longley, 1956; Randolph, 1941; Rhoades, Dempsey and Ghidoni, 1967; Rhoades and Dempsey, 1972) leading to the phenomena of non-disjunction, breakage and chromosome elimination. These types of instability are undoubtedly related to the nature of hetero-chromatic portions whose role in the nucleus has not been thoroughly investigated. The present study is concerned with the mosaic endosperm class which repeatedly appears in the progeny of a translocation between chromosome 4 and a B chromosome. This translocation, which is known as *TB-4a*, was obtained by Roman (1947) after X-ray treatment of pollen grains collected from plants carrying supernumerary chromosomes of the B type. The chromosomes responsible for producing mosaics are the B^4 chromosomes which were the subject of earlier observations on transmission and stability (Ghidoni, 1968, 1969, 1970). Similarly, Robertson (1967) studied the transmission of the B^9 chromosome, while its instability was investigated by Carlson (1969, 1970 and in this volume).

It is the purpose of the present study to show that selecting for kernels with a mosaic phenotype often yields progenies with an altered frequency of transmission of the B^4 segments and a high frequency of unstable derivatives of these chromosomes, reflecting, in most cases, changes in the arrangement of the original structure.

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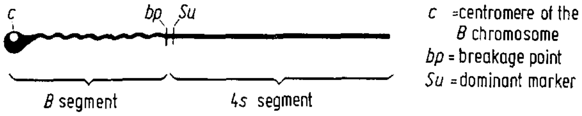
[1] The author dedicates the present paper to Prof. Marcus M. Rhoades with esteem and gratitude.

Material and Methods

The chromosome under study is a supernumerary B^4 marked by the dominant Su_1 which was extracted from the translocation *TB*-4*a* and placed in a homozygous recessive su_1 background (Su_1 = starchy, plump endosperm; su_1 = sugary, wrinkly endosperm). The B^4 chromosome is a compound chromosome composed of part of the B chromosome (the centromere region and its adjacent portion) and the segment of the short arm of chromosome 4 which lies distally to the marker Su_1, as shown below:



The genotype considered in the present study was a hyperploid of the following kind:

$$4\ su,\ 4\ su,\ +\ B^4\ Su\ .$$

The location of the marker *Su* close to the breakage point allows the fate of this chromosome to be followed, since the crossing-over frequency between the B^4 and the normal chromosome 4 is negligible in the region *bp* — *Su*. Crosses were made using the hyperploid parent as the pollen source for testers homozygous su_1. The progeny consisted of sugary kernels (absence of B^4) and starchy kernels (presence of B^4). Mosaics in the endosperm for the character controlled by the *Su* gene were observed as mixtures of the two phenotypes, starchy, plump / sugary, wrinkly. Individuals have been grown from kernels showing mosaic endosperm in various years since 1967. Chromosome observations were made on squashed root tips stained by the Feulgen method, and on microsporocytes smeared in acetocarmine. Microphotographs were taken in bright field with Zeiss equipment, or in phase contrast with Leitz Ortholux.

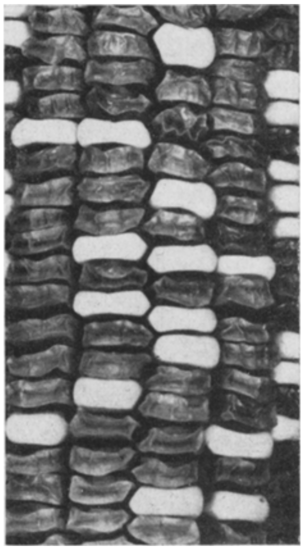


Fig. 1

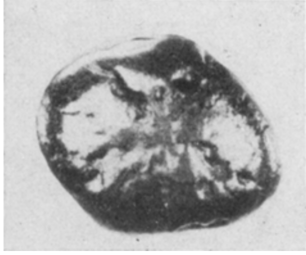


Fig. 2

Fig. 1. Phenotypes of the progeny of the cross *su* × *su*, *su*, B^4*Su*. The plump kernels (stable *Su*) indicate the transmission of the supernumerary chromosome with its original linear structure. This chromosome was not inherited by the wrinkly (*su*) kernels

Fig. 2. A kernel with the unstable phenotype *Su*/*su* is occasionally found among the stable *Su* types described in fig. 1. The repeated loss of the dominant marker *Su* was probably caused by a bridge-breakage-fusion-cycle in the endosperm. In some of these mosaic individuals this phenotype is inheritable while in others it is limited to the endosperm tissue

Results

The progeny of the cross described above consisted of two types of individuals, shown in Fig. 1, whose phenotypic differences were due to the presence or absence of the supernumerary chromosome B^4. It was expected that the presence of this chromosome in the embryo and endosperm would correspond, assuming that the B^4 chromosome does not undergo nondisjunction in the second haploid mitosis of the pollen, in the presence of a normal chromosome 4 and in the absence of the distal portion of the *B* chromosome (Roman, 1949; Ghidoni, 1968). Non-correspondence between embryo and endosperm, although normally not observed in this material, appeared first in mosaic kernels, starchy/sugary, of which an example is shown in Fig. 2. A single individual of this type was found in 1966 and planted in the summer of 1967. Upon self-pollination of the plant obtained (No. 415-1), new mosaic individuals appeared on the ear, on which the majority of the kernels were of the sugary type. Some of these mosaic types were then planted and either selfed or crossed to su_1; the data are reported in Table 1. While most plants gave

Table 1. *Progeny test of mosaic individuals collected after selfing Plant no. 415-1*

Pedigree (Progeny of 415-1*)	Description of crosses by phenotypes	Results					
		su	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
695-1	unstable *Su* ⊗	159	19	7	26	185	14.0
695-2	unstable *Su* ⊗	122	10	4	14	136	10.3
695-3	unstable *Su* ⊗	107	11	2	13	120	10.8
695-4	unstable *Su* ⊗	98	15	3	18	116	15.5
696 × 695-4	*su* × unstable *Su*	234	22	0	22	256	8.6
695-5	unstable *Su* ⊗	185	0	0	0	185	0
696 × 695-5	*su* × unstable *Su*	276	0	0	0	276	0
695-6	unstable *Su* ⊗	186	19	5	24	210	11.4

* = original unstable *Su* individual which transmitted the mosaicism; ⊗ = self cross

transmission af mosaic types, plant no. 5, although obtained from a kernel with mosaic endosperm, did not transmit this phenotype when selfed or crossed to su_1. More mosaics of independent origin were collected from the progeny of similar crosses in different years. Their frequency was roughly 1% (258 mosaics out of 25,000 stable *Su* phenotype individuals). The plants obtained from them were progeny tested, and the results are reported in Tables 2, 3, 4 and 5. The data show a high frequency of non-correspondence between embryo and endosperm. A correspondence was found only in the cases of transmission of mosaic phenotypes, which made a total of 19 independent changes in the structure of the B^4 chromosome responsible for a switch from the stable to the unstable condition (see Fig. 3). Another case of transmissible mosaicism in the same

Table 2. *Progeny test of mosaic individuals independently originated and planted in 1968*

Pedigree	Description of crosses by phenotypes	Results					
		su	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
994-1	unstable *Su* ⊗	160	0	0	0	160	0
993×994-2	*su*×unstable *Su*	107	0	17	17	124	13.7
994-3	unstable *Su* ⊗	194	0	0	0	194	0
994-4	unstable *Su* ⊗	125	0	0	0	125	0
994-5	unstable *Su* ⊗	216	0	0	0	216	0
994-6	unstable *Su* ⊗	169	0	0	0	169	0
994-7	unstable *Su* ⊗	137	0	0	0	137	0
993×994-7	*su*×unstable *Su*	249	0	0	0	249	0
993×994-8*	*su*×unstable *Su*	84	13	(1)	14	98	14.3
994-9	unstable *Su* ⊗	204	0	0	0	204	0
994-10	unstable *Su* ⊗	131	0	0	0	131	0
994-11 } *	unstable *Su* ⊗	200	8	0	8	208	3.9
993×994-11 }	*su*×unstable *Su*	235	9	0	9	244	3.7
994-12	unstable *Su* ⊗	95	0	0	0	95	0

* = Individuals which transmitted the mosaicism; ⊗ = self cross

Table 3. *Progeny test of mosaic individuals independently originated and planted in 1969*

Pedigree	Description of crosses by phenotypes	Results					
		su	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
1303-1 ×1313	unstable *Su*×*su*	179	0	85	85	264	32.2
-2(·) ×1313	unstable *Su*×*su*	128	(1)	42	43	271	15.9
-3 ×1313	unstable *Su*×*su*	99	0	32	32	131	24.4
-7* ×1313	unstable *Su*×*su*	323	13	0	13	336	3.9
-10* ×1313	unstable *Su*×*su*	140	0	0	0	140	0
-11 ×1313	unstable *Su*×*su*	277	(1)	70	71	348	21.0
-12 ×1313	unstable *Su*×*su*	197	0	45	45	242	18.6
-15 ×1313	unstable *Su*×*su*	265	0	0	0	265	0
-17 ×1313	unstable *Su*×*su*	131	0	60	60	191	31.4
-18 ×1313	unstable *Su*×*su*	186	0	54	54	240	22.6
-22(·) ×1313	unstable *Su*×*su*	195	0	45	45	240	18.8
-27(·) ×1313	unstable *Su*×*su*	324	0	76	76	400	19.1
-28 ×1313	unstable *Su*×*su*	221	0	91	91	312	29.1
Reciprocal crosses:							
1313×1303-1	*su*×unstable *Su*	639	0	81	81	720	11.2
1313×1303-2(·)	*su*×unstable *Su*	457	2	21	23	480	4.8
1313×1303-3	*su*×unstable *Su*	403	(1)	29	30	432	7.0
1313×1303-7*	*su*×unstable *Su*	479	27	0	27	506	5.3
1313×1303-10*	*su*×unstable *Su*	572	4	0	4	576	0.7
1313×1303-11	*su*×unstable *Su*	441	0	77	77	5198	14.8
1313×1303-12	*su*×unstable *Su*	591	(2)	75	77	668	11.5
1313×1303-15	*su*×unstable *Su*	476	0	0	0	476	0
1313×1303-17	*su*×unstable *Su*	314	0	54	54	368	14.6
1313×1303-18	*su*×unstable *Su*	407	(1)	55	56	463	12.1
1313×1303-22(·)	*su*×unstable *Su*	221	0	19	19	240	7.9
1313×1303-27(·)	*su*×unstable *Su*	370	0	15	15	385	3.9
1313×1303-28	*su*×unstable *Su*	284	0	40	40	244	16.4

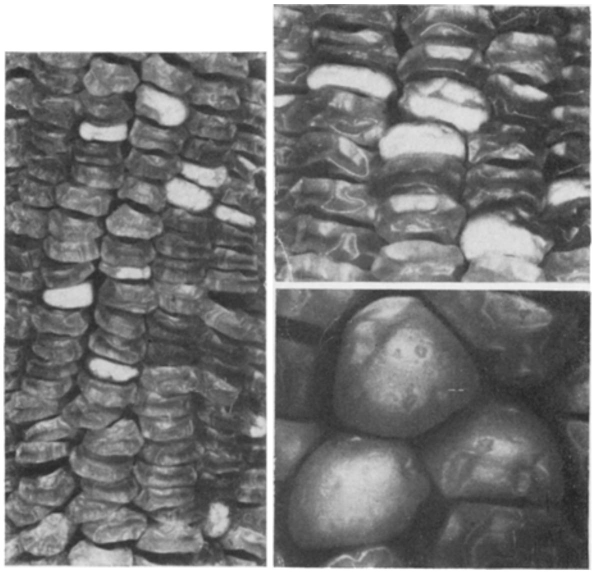
* = Individuals which transmitted the mosaicism; (·) = Individuals which transmitted the B^4 chromosome with atypical frequencies

Table 4. *Progeny test of mosaic individuals independently originated and planted in 1970*

Pedigree	Description of crosses by phenotypes	Results					
		su	unstable *Su*	stable *Su*	total *Su*	total *Su*+*Su*	% *Su*/*Su*+*su*
1540-1 ×1546	unstable *Su*×*su*	105	0	35	35	140	25.0
-2 ×1546	unstable *Su*×*su*	62	0	26	26	88	29.5
-3 ×1546	unstable *Su*×*su*	64	0	0	0	64	0
-4 ×1546	unstable *Su*×*su*	137	0	53	53	190	27.9
-5 ×1546	unstable *Su*×*su*	58	0	21	21	79	26.6
-6 ×1546	unstable *Su*×*su*	64	0	19	19	83	22.9
-7 ×1546	unstable *Su*×*su*	473	0	0	0	473	0
-8 ×1546	unstable *Su*×*su*	319	0	0	0	319	0
-9* ×1546	unstable *Su*×*su*	180	5	4	9	189	4.8
-10 ×1546	unstable *Su*×*su*	322	0	0	0	322	0
-11 ×1546	unstable *Su*×*su*	299	0	0	0	299	0
-12 ×1546	unstable *Su*×*su*	190	0	97	97	287	33.8
-13 ×1546	unstable *Su*×*su*	69	0	45	45	114	39.5
-14 ×1546	unstable *Su*×*su*	268	0	0	0	268	0
-15 ×1546	unstable *Su*×*su*	216	0	61	61	277	21.8
-16(·) ×1546	unstable *Su*×*su*	315	0	45	45	360	12.5
-17 ×1546	unstable *Su*×*su*	178	0	82	82	260	31.6
-18 ×1546	unstable *Su*×*su*	59	0	17	17	76	22.4
-19(·) ×1546	unstable *Su*×*su*	78	0	11	11	89	12.4
-20 ×1546	unstable *Su*×*su*	244	0	80	80	324	24.6
-21 ×1546	unstable *Su*×*su*	79	0	18	18	97	18.5
-22 ×1546	unstable *Su*×*su*	249	0	156	156	405	38.5
-23(·) ×1546	unstable *Su*×*su*	303	0	108	108	411	21.8
-24* ×1546	unstable *Su*×*su*	428	10	18	28	456	6.1
-25 ×1546	unstable *Su*×*su*	190	0	19	19	209	9.1
-26 ×1546	unstable *Su*×*su*	190	0	73	73	263	27.8
-27 ×1546	unstable *Su*×*su*	142	0	49	49	191	25.6
-28 ×1546	unstable *Su*×*su*	23	0	12	12	35	34.1
Reciprocal crosses							
1546×1540-1	*su*×unstable *Su*	220	0	22	22	242	9.2
1546× -2	*su*×unstable *Su*	150	1	36	37	187	19.6
1546× -3	*su*×unstable *Su*	179	0	0	0	180	0
1546× -4	*su*×unstable *Su*	277	0	20	20	297	6.7
1546× -5	*su*×unstable *Su*	16	0	1	1	17	5.9
1546× -6	*su*×unstable *Su*	281	0	22	22	303	7.3
1546× -7	*su*×unstable *Su*	316	0	0	0	316	0
1546× -8	*su*×unstable *Su*	492	0	0	0	492	0
1546× -9*	*su*×unstable *Su*	—	—	—	—	—	—
1546× -10	*su*×unstable *Su*	478	1	60	61	539	11.3
1546× -11	*su*×unstable *Su*	420	0	0	0	420	0
1546× -12	*su*×unstable *Su*	219	0	19	19	238	7.9
1546× -13	*su*×unstable *Su*	133	1	15	16	149	10.7
1546× -14	*su*×unstable *Su*	330	0	0	0	330	0
1546× -15	*su*×unstable *Su*	80	0	20	20	100	20.0
1546× -16	*su*×unstable *Su*	—	—	—	—	—	—
1546× -17	*su*×unstable *Su*	279	2	60	62	341	18.2
1546× -18	*su*×unstable *Su*	68	0	12	12	80	15.0
1546× -19(·)	*su*×unstable *Su*	215	0	13	13	228	5.7
1546× -20	*su*×unstable *Su*	453	1	92	93	546	17.1
1546× -21	*su*×unstable *Su*	107	0	12	12	119	10.1
1546× -22	*su*×unstable *Su*	430	0	54	54	484	11.2
1546× -23(·)	*su*×unstable *Su*	355	0	87	87	442	19.8
1546× -24*	*su*×unstable *Su*	497	9	0	9	506	0.2
1546× -25	*su*×unstable *Su*	516	0	0	0	516	0
1546× -26	*su*×unstable *Su*	89	0	17	17	106	16.0
1546× -27	*su*×unstable *Su*	312	1	44	45	357	7.9
1546× -28	*su*×unstable *Su*	241	1	25	26	267	9.7

* = Individuals which transmitted the mosaicism; (·) = Individuals which transmitted the B^4 chromosome with atypical frequencies

kind of material was found by F. Salamini (personal communication). Plants which showed atypical transmission of a B^4 chromosome, or complete lack of transmission of the *Su* phenotype, or transmission of a stable *Su* in a typical frequency, are all to be regarded as cases of non-correspondence between embryo and endosperm since they were all derived from kernels with mosaic endosperm; they totalled 152 out of 171 cases which produced progeny. The outcome of progeny testing of the independently originated mosaics is summarized in Table 6. The high frequency of failure (87/258) should be noted. Cultures made in different years gave comparable results in all classes examined, and are a satisfactory test of the repeatability of the phenomena observed. Class 3 and class 4 were separated by comparing the frequency of transmission of the B^4 chromosome with that of its original stable form. It is possible that some deviations from the typical frequency are obtained by chance, but the frequency of such deviations is much higher than expected. Class 5 represents the considerable fraction (56/171) in which no transmission of the B^4 was observed using the marker *Su*.



◀ Fig. 3. The mosaic phenotype is inherited in a typically irregular manner as in the case shown above; *left*: an irregular cluster of mosaic kernels; *right*: details of the mosaicism *Su/su* in clusters

Kernels with transmitted mosaics, obtained from two cultures of independent origin (695-1 and 994-2, respectively reported in tables 1 and 2), were planted and grown in 1969 and reciprocally crossed with a su_1 tester. The results are reported in Tables 7 and 8 respectively. Two main facts emerged from these data. First, the frequency of transmission of the unstable phenotype differed significantly in the two cultures, being considerably higher in nearly all the plants reported in Table 8 in both reciprocal crosses. Secondly, transmission through the pollen was higher in both families, with few exceptions. The unstable phenotype was always observed when the hyperploid parent furnished the pollen, while, in the reciprocal cross, apparently stable *Su* phenotypes were also observed, presumably because two chromosomes marked with *Su* were present in the endosperm. In this case the appearance of recessive sectors requires simultaneous loss of all *Su* alleles present.

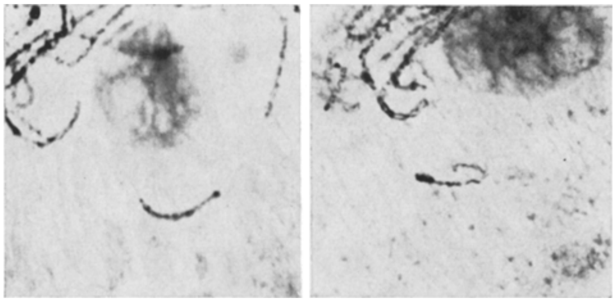


Fig. 4. Pachytene of an isolated B^4 chromosome with the original linear structure. The *B* centromere, surrounded by heterochromatin, is on the right end in the *left* picture, and on the left end in a backfolded segment in the *right* picture

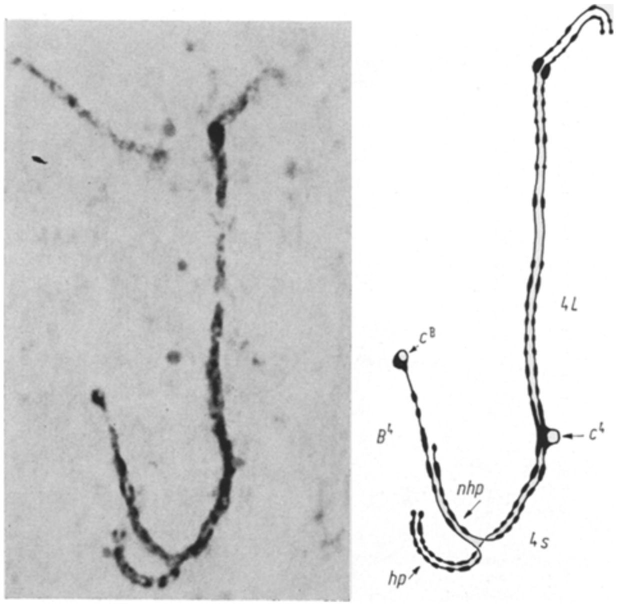


Fig. 5. *Left*: Pachytene with two normal chromosome s4 and a supernumerary B^4. *Right*: Camera lucida of the same figure. c^B = centromere of the *B* chromosome portion of B^4. c^4 = centromere of chromosome 4. *hp*: region of homologous pairing between 4 and B^4. *nhp*: region of non-homologous pairing between 4 and B^4

The confirmed transmissibility of mosaicism (with significantly different frequencies, at least in the two cases which were progeny tested), the changed frequencies of transmission of the B^4 chromosome compared with the original type, and the odd behaviour in reciprocal crosses, suggest the formation of new chromosome structures.

Root tips were taken from six plants grown from the transmissible mosaic kernels reported in Table 8. The presence of a small supernumerary chromosome was ascertained in the samples examined, with the

Table 5. *Progeny test of mosaic individuals independently originated and planted in 1971*

Pedigree	Description of crosses by phenotypes	Results					
		su	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
2087-1 × 2088	unstable *Su* × *su*	210	0	0	0	210	0
-2 × 2088	unstable *Su* × *su*	47	0	24	24	71	33.9
-3 × 2088	unstable *Su* × *su*	58	0	29	29	87	33.3
-4* × 2088	unstable *Su* × *su*	518	11	0	11	529	2.1
-5 × 2088	unstable *Su* × *su*	107	0	0	0	107	0
-6(·) × 2088	unstable *Su* × *su*	78	0	12	12	90	13.3
-7* × 2088	unstable *Su* × *su*	380	11	25	36	416	8.7
-8* × 2088	unstable *Su* × *su*	284	0	0	0	284	0
-9 × 2088	unstable *Su* × *su*	—	—	—	—	—	—
-10 × 2088	unstable *Su* × *su*	61	0	15	15	76	19.7
-11 × 2088	unstable *Su* × *su*	268	0	0	0	268	0
-12(·) × 2088	unstable *Su* × *su*	493	2	12	14	507	2.8
-13 × 2088	unstable *Su* × *su*	140	0	60	60	200	30.0
-14 × 2088	unstable *Su* × *su*	239	0	0	0	239	0
-15 × 2088	unstable *Su* × *su*	302	0	0	0	302	0
-16 × 2088	unstable *Su* × *su*	—	—	—	—	—	—
-17 × 2088	unstable *Su* × *su*	179	0	0	0	179	0
-18 × 2088	unstable *Su* × *su*	335	0	99	99	434	22.8
-19 × 2088	unstable *Su* × *su*	370	0	124	124	494	25.1
-20* × 2088	unstable *Su* × *su*	216	0	0	0	216	0
-21(·) × 2088	unstable *Su* × *su*	265	0	16	16	281	5.7
-22 × 2088	unstable *Su* × *su*	89	0	0	0	89	0
-23 × 2088	unstable *Su* × *su*	304	0	0	0	304	0
-24 × 2088	unstable *Su* × *su*	274	0	182	182	456	39.9
-25 × 2088	unstable *Su* × *su*	260	0	0	0	260	0
-26(·) × 2088	unstable *Su* × *su*	28	0	3	3	31	9.7
-27 × 2088	unstable *Su* × *su*	109	0	0	0	109	0
-28 × 2088	unstable *Su* × *su*	65	0	0	0	65	0
-29 × 2088	unstable *Su* × *su*	198	0	96	96	295	32.5
-30 × 2088	unstable *Su* × *su*	97	0	0	9	97	0
-31 × 2088	unstable *Su* × *su*	59	0	15	15	74	20.1
-32 × 2088	unstable *Su* × *su*	174	0	0	0	174	0
-33(·) × 2088	unstable *Su* × *su*	55	0	3	3	58	5.2
-34 × 2088	unstable *Su* × *su*	311	0	0	0	311	0
-35 × 2088	unstable *Su* × *su*	244	0	0	0	244	0
-36 × 2088	unstable *Su* × *su*	196	0	0	0	196	0
-37 × 2088	unstable *Su* × *su*	99	0	26	26	125	20.8
Reciprocal crosses							
2088 × 2087-1	*su* × unstable *Su*	371	0	0	0	371	0
2088 × -2	*su* × unstable *Su*	203	0	29	29	232	12.5
2088 × -3	*su* × unstable *Su*	335	0	23	23	358	6.4
2088 × -4*	*su* × unstable *Su*	289	10	0	10	299	3.3
2088 × -5	*su* × unstable *Su*	347	0	0	0	347	0
2088 × -6(·)	*su* × unstable *Su*	—	—	—	—	—	—
2088 × -7*	*su* × unstable *Su*	226	47	2	49	275	17.8
2088 × -8*	*su* × unstable *Su*	294	2	0	2	294	0.7
2088 × -9	*su* × unstable *Su*	396	0	64	64	460	13.9
2088 × -10	*su* × unstable *Su*	295	(2)	136	138	433	31.4
2088 × -11	*su* × unstable *Su*	309	0	0	0	309	0
2088 × -12(·)	*su* × unstable *Su*	437	0	121	121	558	21.4
2088 × -13	*su* × unstable *Su*	451	(1)	144	145	596	24.1
2088 × -14	*su* × unstable *Su*	—	—	—	—	—	—
2088 × -15	*su* × unstable *Su*	97	0	0	0	97	0
2088 × -16	*su* × unstable *Su*	119	0	0	0	119	0
2088 × -17	*su* × unstable *Su*	324	0	43	43	367	11.7
2088 × -18	*su* × unstable *Su*	340	(1)	40	41	381	10.5
2088 × -19	*su* × unstable *Su*	312	0	22	22	334	6.6
2088 × -20*	*su* × unstable *Su*	328	68	0	68	396	17.2
2088 × -21(·)	*su* × unstable *Su*	351	5	64	69	420	16.4
2088 × -22	*su* × unstable *Su*	320	0	0	0	320	0
2088 × -23	*su* × unstable *Su*	247	0	0	0	247	0
2088 × -24	*su* × unstable *Su*	471	1	124	125	596	20.8
2088 × -25	*su* × unstable *Su*	355	0	0	0	355	0
2088 × -26(·)	*su* × unstable *Su*	236	1	169	170	406	41.5
2088 × -27	*su* × unstable *Su*	198	0	0	0	198	0
2088 × -28	*su* × unstable *Su*	—	—	—	—	—	—

Table 5 (continued)

Pedigree	Description of crosses by phenotypes	Results					
		Su	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
2088 × 2087-29	*su* × unstable *Su*	470	0	41	41	511	8.0
2088 × -30	*su* × unstable *Su*	278	0	0	0	278	0
2088 × -31	*su* × unstable *Su*	11	0	2	2	13	15.4
2088 × -32	*su* × unstable *Su*	165	0	0	0	165	0
2088 × -33(·)	*su* × unstable *Su*	238	0	43	43	281	15.6
2088 × -34	*su* × unstable *Su*	196	0	0	0	196	0
2088 × -35	*su* × unstable *Su*	248	0	0	0	248	0
2088 × -36	*su* × unstable *Su*	260	0	0	0	260	0
2088 × -37	*su* × unstable *Su*	177	0	29	29	206	14.1

* = Individuals which transmitted the mosaicism; (·) = Individuals which transmitted the B^4 chromosome with atypical frequencies

exception of plant no. 4. It was difficult to describe the detailed structure of this chromosome. While pachytene figures were useful for observations of the normal B^4 in its stable form (rod-like structure), as it appears in Fig. 4 and 5, they were of little help in microsporocytes taken from the same plants which furnished the root tips. The small supernumerary chromosome always showed a compact, folded-back structure. Pre-meiotic prophases proved useful in showing a ring-like structure for this chromosome, of the type shown in Fig. 6.

Discussion

The frequency of newly originated mosaic kernels in the material studied, which was hyperploid for the chromosome B^4, was found to be about 1%. This frequency is high in comparison with normal genotypes where mosaic types appear with much lower frequency. The mosaic individuals studied here were isolated almost exclusively (except for one or two uncertain cases) from crosses in which the hyperploid parent was used as the male parent. It could be objected that mosaics are less frequently expressed in the endosperm, where two doses of the B^4 chromosome are contributed by the polar nuclei, and therefore they could have escaped detection. However, Tables 7 and 8 show that the apparently stable phenotypes were observed only in roughly 50% of the cases where transmission of the mosaic-forming B^4 chromosomes had occurred through the egg. Therefore the lack of detection of a significant fraction of mosaics borne in the female germ line suggests that the origin of these exceptional types may be connected with the male germ line. Part of the reason, however, for defective detection must be ascribed to the lower recovery of mosaic types, once originated, through the egg. This aspect was not examined thoroughly, although further data, not reported here, obtained on the same material, confirmed the lower female transmission. The heterogeneity within the families is due to the continuous changes that this chromosome undergoes. It is possible that the ring B^4, carried as a supernumerary chromosome, suffers selection because of its structure and hyperploid condition during megaspore formation, during embryo sac formation (three divisions vs./two in the male gametophyte) and by ovule abortion. Because of its nature, the ring B^4 seems unsuitable for a careful study of the possibility of its selective elimination in the basal megaspore.

Table 6. *Summary of progeny tests of mosaics independently originated and planted in years between 1967 and 1972 (Detailed data of the 1972 culture were not put on a table)*

Year of culture	1967	1968	1969	1970	1971	1972	Total
New Mosaics Planted	1	17	42	63	58	77	258
Class 1: Failures	0	5	15	35	22	10	87
Class 2: Transmissible Mosaics	1	2	2	2	4	8	19
Class 3: *Su atypical* transmission	0	0	7	3	5	19	34
Class 4: *Su typical* transmission	0	1	12	17	10	22	62
Class 5: *su* transmission only	0	9	6	6	17	18	56
Mosaics examined	1	12	27	28	36	67	171

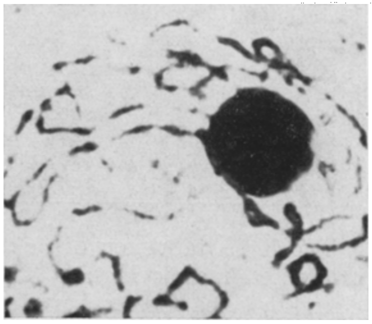


Fig. 6. A pre-meiotic prophase nucleus. On the right side, lower part, a ring B^4 chromosome is shown

Table 7. *Progeny test of the transmissible mosaic no. 695-1. Reciprocal crosses only are reported for every plant. Higher frequencies of transmission of mosaicism were observed when the plants were tested as the male parents*

Pedigree	Description of crosses by phenotypes	Results *Su*	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
1299-1 ×1313	unstable *Su*×*su*	345	1	1	2	347	0.58
-2 ×1313	unstable *Su*×*su*	309	4	11	15	324	4.6
-3 ×1313	unstable *Su*×*su*	360	0	2	2	362	0.55
-5 ×1313	unstable *Su*×*su*	231	0	4	4	235	1.7
-10×1313	unstable *Su*×*su*	163	3	2	5	168	2.98
-11×1313	unstable *Su*×*su*	265	5	4	9	274	3.3
-12×1313	unstable *Su*×*su*	294	3	2	5	299	1.67
-14×1313	unstable *Su*×*su*	251	1	3	4	255	1.57
-17×1313	unstable *Su*×*su*	187	1	27	28	215	13.0
-18×1313	unstable *Su*×*su*	120	0	0	0	120	0
-21×1313	unstable *Su*×*su*	235	5	4	9	244	3.7
-22×1313	unstable *Su*×*su*	238	4	6	10	248	4.0
-23×1313	unstable *Su*×*su*	345	1	0	1	346	0.29
-24×1313	unstable *Su*×*su*	384	0	0	0	384	0
-25×1313	unstable *Su*×*su*	157	3	6	9	166	5.4
Reciprocal crosses							
1313×1299-1	*su*×unstable *Su*	156	17	0	17	173	9.8
1313× -2	*su*×unstable *Su*	346	26	0	26	372	7.0
1313× -3	*su*×unstable *su*	391	29	0	29	420	6.9
1313× -5	*su*×unstable *Su*	178	26	0	26	204	12.7
1313× -10	*su*×unstable *Su*	344	16	0	16	360	4.5
1313× -11	*su*×unstable *Su*	378	0	0	0	378	0
1313× -12	*su*×unstable *Su*	426	6	0	6	432	1.39
1313× -14	*su*×unstable *Su*	262	14	0	14	276	5.1
1313× -17	*su*×unstable *Su*	393	27	0	27	420	6.4
1313× -18	*su*×unstable *Su*	280	20	0	20	500	6.2
1313× -21	*su*×unstable *Su*	330	30	0	30	360	8.3
1313× -22	*su*×unstable *Su*	513	47	0	47	560	8.4
1313× -23	*su*×unstable *Su*	453	3	0	3	456	0.6
1313× -24	*su*×unstable *Su*	395	13	0	13	408	3.2
1313× -25	*su*×unstable *Su*	352	16	0	16	368	4.3

Table 8. *Progeny test of the transmissible mosaic no. 994-2. Reciprocal crosses are reported for every plant. Higher frequencies of transmission of mosaicism were observed when the plants were tested as the male parents. This type of mosaicism shows higher transmission frequencies if compared with the one reported in table 7*

Pedigree	Description of crosses by phenotypes	Results *su*	unstable *Su*	stable *Su*	total *Su*	total *Su*+*su*	% *Su*/*Su*+*su*
1300-1×1312	unstable *Su*×*su*	462	20	24	44	506	8.7
1300-2×1312	unstable *Su*×*su*	425	11	12	23	448	5.1
1300-3×1312	unstable *Su*×*su*	485	16	9	25	510	4.9
1300-4×1312	unstable *Su*×*su*	467	0	0	0	467	0
1300-5×1312	unstable *Su*×*su*	434	16	14	60	464	6.5
1300-6×1312	unstable *Su*×*su*	419	13	14	27	464	6.1
Reciprocal crosses							
1312×1300-1	*su*×unstable *Su*	320	136	0	136	456	29.8
1312×1300-2	*su*×unstable *Su*	188	42	0	42	330	12.7
1312×1300-3	*su*×unstable *Su*	266	58	0	58	324	17.9
1312×1300-4	*su*×unstable *Su*	456	0	0	0	456	0
1312×1300-5	*su*×unstable *Su*	260	83	0	83	343	24.2
1312×1300-6	*su*×unstable *Su*	388	67	0	67	455	14.7

The high frequency of failures among the newly originated mosaic kernels (87/258) may be not incidental, since mosaic kernels obtained from transmissible mosaic classes did not show a high frequency of failures. Although more extensive data are needed, an explanation could be proposed: some of the mosaics observed as new inceptions may be due to a cause not necessarily involving the B^4 alone; they could be the result of more general disturbances affecting important functions during kernel development. It may be useful to compare these failures with those found by Coe (1958) when he examined variegated kernels for the allele C^I (22 failures/85 examined mosaic kernels).

Class 3 of Table 6 consists of cases where two different phenomena were observed respectively in the endosperm (the mosaicism) and in the embryo (an altered transmission frequency of the stable B^4). This class may be explained as follows: a breakage in the B^4 chromosome had occurred during or after meiosis, in a segment distal to the marker *Su*, starting a breakage-fusion-bridge cycle (observed as a mosaic involving the marker *Su*) which continued through the endosperm, and ended in the fertilized egg; the embryo developed with a modified B^4 chromosome which could be either deficient or have deficient-duplicated regions as a consequence of the breakage-fusion-bridge cycle, according to the view of McClintock (1941). This interpretation would explain the altered frequencies of transmission of the stable B^4 chromosomes of this class, which were observed in reciprocal crosses. The analysis of Class 4 can only suggest that the mosaicism, whatever its cause, was limited to the endosperm tissue while the embryo suffered no appreciable consequences.

Class 5 is concerned with the recovery of *su* individuals only as the progeny of newly originated mosaic kernels (56/171). Although heterofertilization cannot be excluded, at least in some of these cases, it seems that a more plausible explanation could be offered. Non-correspondence between embryo and endosperm is often due to a non-disjunction occurring in the second pollen mitosis. This was apparently not the case here, since no distal heterochromatin of the B chromosome was present in this material, a condition which is believed to be necessary for the non-disjunction of the B centromere (according to Roman, 1949; Longley, 1956; Ghidoni, 1968; Carlson, 1969; Ward, 1972).

Whether the mosaicism present in the endosperm of the kernels constituting this class was caused by the same phenomenon which led to the loss of the marker *Su* in one of the two sperms is unknown. However, once the mosaicism is shown to be transmissible, the complete lack of transmission through the egg and the pollen (plant no. 1300-4, Table 8), or through the egg only (plant no. 1299-18 and plant no 1299-11, Table 7), seems occasional and probably related to the pattern of instability of the unstable chromosome.

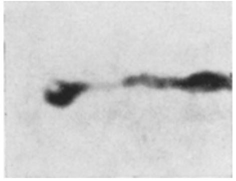


Fig. 7. Detailed structure of the *B* centromeric region magnified 2,800 × ca. The centromere appears on the left side as a slightly stained structure surrounded by heterochromatin

The formation of self-perpetuating ring chromosomes starting with a linear structure implies that two breakages must have occurred in the same cell, one on each side of the centromere. The B chromosome, observed through the ordinary microscope,

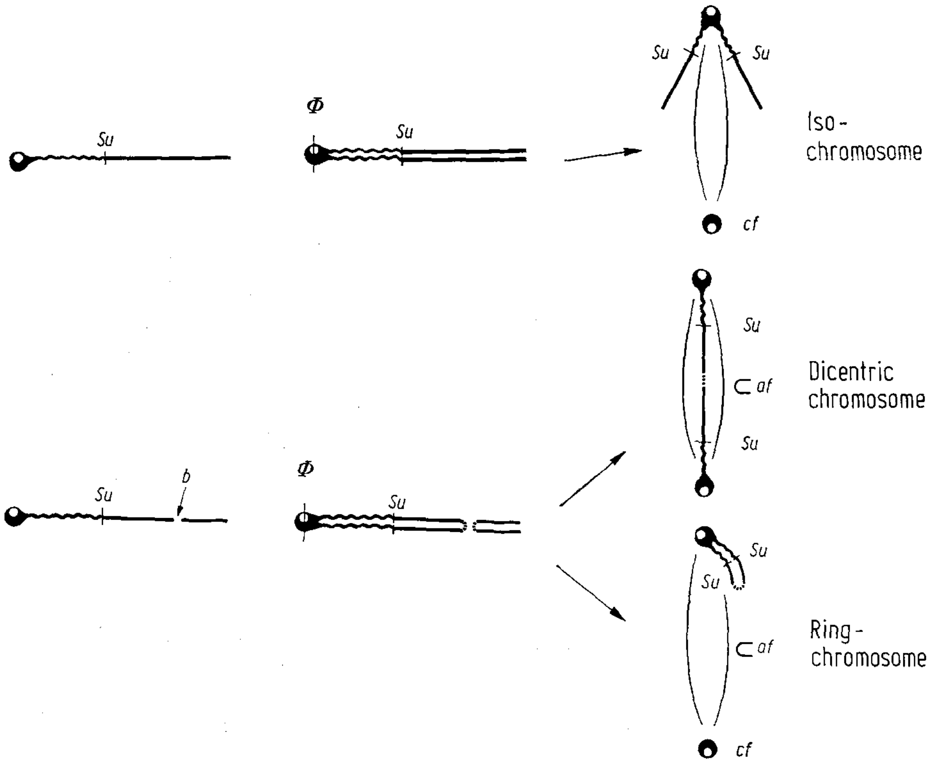


Fig. 8. A possible explanation of the appearance of mosaics *Su/su* and of sectors by the formation of dicentric and ring chromosomes (which are unstable) and of isochromosomes, following breakage of the chromosome (distally to *Su*) and centromere misdivision. *Su* = dominant marker; *b* = a breakage of the chromosome; *Φ* = centromere misdivision at anaphase; *af* = acentric fragment; *cf* = centric fragment. From *left* to *right*: behavior of B^4 during a cell division from duplication of the chromosome to anaphase

appears at the pachytene stage with the centromere located near one end but not acrocentrically (Fig. 7). This interpretation gains support from the genetic data presented here, for a second arm, although very short, should be present to allow the formation of circular structures provided with a centromere. It follows that this very short arm would be a site of frequent chromosome breakage. Alternatively, this region could be involved in an unknown manner in processes leading to the loss of *B* chromosome integrity. In both cases the breakage phenomenon may be connected with the altered duplication of this chromosome followed by difficult anaphase movement. Taking this view, one could also find an explanation for the occasional finding of smaller derivatives of supernumerary B chromosomes (Randolph, 1941). Longley (1956) proposed another hypothesis for the origin of these diminutive *B*-type chromosomes.

Although different causes could be suggested for the non-disjunction phenomenon and the cases of instability originating in the *B* and B^4 chromosomes, the findings with the chromosome B^4 and those obtained by Carlson with the B^9 chromosome can be interpreted as the effects of a common cause.

It is possible that the heterochromatin adjacent to the *B* centromere is the critical site which, by its location and nature (late or defectively replicating?), interferes with the centromere kinetics, leading in some conditions to the non-disjunction phenomenon, and, in others, to occasional breakage near the centromere or right through it. Cases of centromere misdivision were observed especially in univalent chromosomes by Nishiyama (1931, 1933), Darlington (1940), Rhoades (1940), Sanchez-Monge (1950), Upcott (1937), Koller (1938), Sears (1952), Brock (1954) and others and discussed by Darlington (1939, 1940, 1965). In the latter case, isochromosome formation (Carlson, 1970), or ring formation (Ghidoni and Carlson, this volume) if another breakage in the long arm occurs (Fig. 8), would be among the consequences.

Finally it may be proposed that the B centromere function is often altered by a breakage in the long arm (occurring then as the first breakage). This conclusion is based on the high frequency of rings recovered (requiring two breakages) as opposed to atypical B^4 chromosomes recovered (requiring single breakages), or 19 opposed to 34. Mosaic phenotypes limited to the endosperm (Class 4 and Class 5, Table 6) are not concerned in this ratio, since it is impossible to state whether one or two original breakages were involved in these mosaic endosperms.

Acknowledgements

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