

Aneuhaploids in bread wheat

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SUMMARY

Euploid and aneuploid plants of *Triticum aestivum*, variety Chinese Spring were pollinated with pollen of *Hordeum bulbosum*. Euhaploids and aneuhaploids of Chinese Spring were obtained from the crosses. Meiotic chromosome pairing was analysed in 25 different aneuhaploids and the results were compared with those obtained from euhaploids. The evidence provided by the meiotic studies was used to identify chromosomes whose activities affected the genetic control of chromosome pairing.

Meiosis was abnormal in a 23-chromosome aneuhaploid and in the 22-chromosome sectors of a chimaeral plant. Both plants were thought to have resulted from the incomplete elimination of the genome of *H. bulbosum* from hybrid embryos. It is suggested that the meiotic abnormalities in the two aneuhaploids were caused by the residual barley chromosomes.

1. INTRODUCTION

Haploidy in wheat was first recognized by Gaines & Aase (1926), who described a haploid plant of *Triticum compactum* ($2n = 42$). Kimber & Riley (1963), in a review of haploid angiosperms, listed reports of haploids of diploid, tetraploid and hexaploid wheat species. At the same time they proposed a classification of haploids in which the major division was between euhaploids and aneuhaploids. Euhaploids are haploids with the precise chromosome complement of the gametes of normal individuals of the species. Aneuhaploids have a chromosome complement which differs from that of the euhaploid either in chromosome number or content.

Riley & Chapman (1958), in a study of haploids of *T. aestivum* ($2n = 42$), showed that there was a high level of pairing of homoeologous chromosomes in a 20-chromosome aneuhaploid (later shown to be deficient for chromosome 5B), but little chromosome pairing in a 21-chromosome euhaploid. This demonstration of the effect of a single chromosome on meiotic pairing led to the recognition of the genetic control of chromosome pairing and diploidization of wheat (Riley, 1960).

Haploids of wheat occur spontaneously at a very low frequency. Although in some experimental situations haploids have been obtained at high frequencies (Kihara & Tsunewaki, 1963; Riley, 1965), no method has previously been devised which can be used to extract a range of euhaploids and aneuhaploids. Barclay (1975) first applied to wheat the technique used by Kasha & Kao (1970) to produce barley haploids. He pollinated ears of *T. aestivum* var. Chinese Spring

with pollen of *Hordeum bulbosum* ($2n = 14$ or $4n = 28$). The hybrid embryos obtained from these pollinations grew into haploid wheat plants. Barclay showed that the chromosomes of *H. bulbosum* were eliminated in the early mitotic divisions of the hybrid embryos.

The aim of the work reported here was to produce aneuploids by the use of the technique described by Barclay. In particular it was hoped to extract 20-chromosome haploids nullisomic in turn for each of the 21 wheat chromosomes, and to study the effects of the deficiency of each chromosome on the pairing of chromosomes at meiosis.

2. MATERIALS

The wheat used in these experiments was *T. aestivum* emend. Thell. ssp. *vulgare* McKey ($2n = 6x = 42$) variety Chinese Spring. The monosomic, ditelocentric and nullisomic-tetrasomic lines of Chinese Spring were those originally isolated by Dr E. R. Sears. The monotelocentric lines were extracted from crosses between corresponding monosomic and ditelocentric lines and supplied by A. J. Worland of this Institute. *Hordeum bulbosum* L. ($2n = 2x = 14$), and a tetraploid form ($4n = 28$), were originally supplied by Dr W. Lange of the Foundation for Plant Breeding, Wageningen, the Netherlands.

3. METHODS

The wheat and barley plants were grown in a glasshouse. During the period of 8 weeks in which the pollinations were carried out, the temperature of the glasshouse fluctuated with extremes at $+5$ and $+30$ °C. Most of the pollinations were made with pollen from the tetraploid *H. bulbosum* but some were made using pollen from diploid *H. bulbosum* and others with a mixture of pollen from diploid and tetraploid plants. The techniques used for pollination and embryo culture were according to Barclay (1975). Plants which developed from the cultured embryos were grown at 20 °C and in continuous light in a controlled environment cabinet.

Somatic chromosome counts were made from squashes of root-tips taken from potted plants. The roots were pre-treated in 1-bromonaphthalene solution, fixed in glacial acetic acid and stained by the Feulgen procedure. Meiotic observations were made on squash preparations of anthers which had previously been fixed in 1:3 acetic alcohol and stained by the Feulgen procedure.

4. RESULTS

A total of 687 ears of Chinese Spring and its aneuploids were pollinated with *H. bulbosum*. The embryos of the hybrid grains were cultured and 221 haploid plants were established. Haploids were obtained from Chinese Spring euploid and from 25 different aneuploid genotypes. There were also three haploids with chromosome complements which did not match the normal gametes of their parent plants. Nine of the possible 21 nullisomic haploids were obtained.

Many pollinations failed to produce seeds. In some ears, 40% of the florets which were pollinated set seed, but in most just a few seeds were set. There were differences between the aneuploids in the mean yield of haploids per ear pollinated (Table 1). Because the pollinations were carried out in an uncontrolled environment it is not possible to assert that these differences represent different genotypic responses to pollination with *H. bulbosum*.

Table 1. *The numbers of haploids resulting from the pollination of Chinese Spring and its aneuploids with H. bulbosum*

Chinese spring genotype	No. ears pollinated	No. haploids	Mean haploids per ear
Euploid	11	11	1.00
Monosomics	336	136	0.40
Ditelocentrics	222	7	0.03
Monotelocentrics	35	5	0.14
Nullisomic-tetrasomics	83	62	0.75

(i) *Frequency of aneuhaploids*

A 41-chromosome monosomic wheat plant produces gametes with either 20 or 21 chromosomes. It has been shown by Sears (1954) that in the 21 monosomic lines of Chinese Spring about three-quarters of the functional female gametes have 20 chromosomes and one quarter 21 chromosomes. From this it was expected that the majority of haploids obtained by pollinating Chinese Spring monosomics with *H. bulbosum* would have 20 chromosomes. Table 2 shows the frequencies of 20 and 21 chromosome haploids which resulted from such pollinations. The proportion of 20 chromosome haploids was much lower than had been expected.

Ditelocentric and nullisomic-tetrasomic plants each produce a majority of gametes in which the chromosome complement is a simple reduction of the parental sporophyte and the haploids obtained from them should also have this chromosome constitution. The chromosome complement was as expected in the 26 nullisomic-disomic haploids examined at meiosis and in all but one of the haploids obtained from ditelocentrics. Thirty-six nullisomic-disomic haploids were not examined meiotically but at maturity the phenotypes of these plants did not differ from those of the plants whose chromosome constitution had been confirmed.

(ii) *Phenotypes of aneuhaploids*

Sears (1954) produced the 21 distinct nullisomics of Chinese Spring and compared them with the euploid. The deficiency of each chromosome altered the phenotype in a characteristic manner. Plate 1(a) shows nullisomic haploids compared with euploid. The modifications to the phenotypes are essentially similar to those described by Sears in the corresponding nullisomics and euploids. Haploids nullisomic for chromosome 5A or 5D died before reaching maturity. It is known that chromosomes 5A and 5D are important in determining the response of a

variety to vernalization (Law, Worland & Giorgi, 1976). The failure of the haploids nullisomic for chromosome 5A or 5D to survive in the 20 °C regime of the growth cabinet may have been due to their need of vernalization. The nullisomic–disomic

Table 2. *The frequency of euploids and aneuploids from the pollinations of Chinese Spring monosomics*

Monosomic	No. of plants of chromosome no.	
	20	21
1A	—	18
1B	—	5
1D	2	4
2A	—	2
2B	—	1
2D	—	10
3A	—	3
3B	2	9*
3D	—	5
4A	2	7
4B	—	1
4D	—	11
5A	1	5
5B	1	2
5D	2	5
6A	2	3
6B	—	7
6D	—	4
7A	1	3
7B	3	8
7D	—	7
Totals	16	120

* Including one with 23 chromosomes.

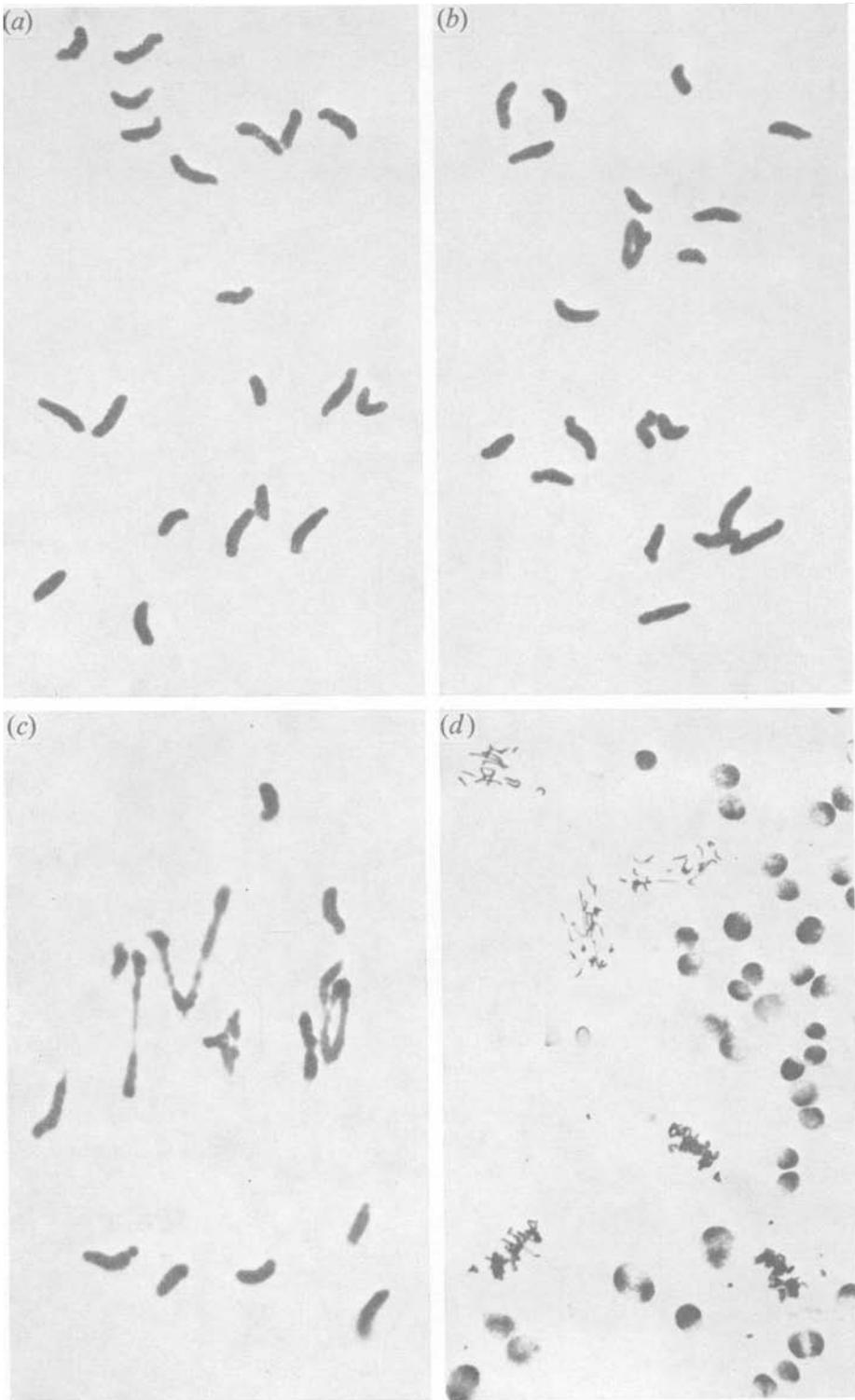
haploids obtained from pollinations of nullisomic–tetrasomic lines had phenotypes similar to those of euploids. The plants in Plate 1(b) show the effect on the phenotype caused by the deficiency of chromosome 3B and the compensating effect of the increased dosage of either of the homoeologous chromosomes 3A and 3D.

(iii) *Meiosis*

Tables 3 and 4 show the results of the meiotic analyses of euploids and aneuploids. The chromosome behaviour of eight euploid plants was analysed at first metaphase of meiosis. The low level of chromosome pairing corresponded with earlier reports (Riley & Chapman, 1957; Person, 1955). In addition 40 other 21-chromosome plants, presumed to be euploid, were examined meiotically. One had an isochromosome but no chromosome structural differences were present in the remainder and the level of chromosome pairing was like that in other euploids. Aneuploids from nullisomic–tetrasomic lines were expected to have 21 chromosomes and to be deficient for one chromosome of the wheat complement



Haploid plants of *T. aestivum* (left to right). (a) Euhaploid, nullisomic haploid 1D, 3B, 4A, 5A, 5B, 5D, 6A, 7B. (b) Euhaploid, nullisomic haploid 3B, nullisomic 3B-disomic 3A haploid nullisomic 3B-disomic 3D haploid.



First metaphase of meiosis in pollen mother cells of aneuploids of *T. aestivum*. (a) Nullisomic haploid 3B, 20 univalents. (b) Nullisomic 3B-disomic 3D haploid, 1 bivalent and 19 univalents. (c) Nullisomic 5B-disomic 5D haploid, 2 trivalents, 4 bivalents and 7 univalents. (d) 22-chromosome haploid showing doubled and undoubled cells.

Table 3. Mean chromosome pairing at first metaphase of meiosis in euphaploids and eneuphaploids (30 cells each; ranges in parentheses).

Aneuphaploids in bread wheat

Genotype	No. plants analysed	I	II			III	Others
			Rod	Ring	Total		
Nullisomic	2	19-73 (19-20)	0-13 (0-1)	—	0-13 (0-1)	—	—
	2	19-93 (18-20)	0-03 (0-1)	—	0-03 (0-1)	—	—
	2	19-24 (16-20)	0-33 (0-2)	—	0-33 (0-2)	0-03 (0-1)	—
	1	8-43 (4-15)	2-83 (0-5)	0-67 (0-4)	3-50 (0-6)	1-43 (0-4)	0-07 ^{IV} (0-1)
	2	19-54 (16-20)	0-23 (0-2)	—	0-23 (0-2)	—	—
	1	19-33 (18-20)	0-33 (0-1)	—	0-33 (0-1)	—	—
	3	19-64 (16-20)	0-18 (0-2)	—	0-18 (0-2)	—	—
Euhaploid	130	20-52 (17-21)	0-24 (0-2)	—	0-24 (0-2)	—	—
Telocentric	2	20-23 (17-21)	0-38 (0-2)	—	0-38 (0-2)	—	—
	2	20-30 (17-21)	0-35 (0-2)	—	0-35 (0-2)	—	—
	1	20-00 (17-21)	0-50 (0-2)	—	0-50 (0-2)	—	—
	1	20-87 (19-21)	0-07 (0-1)	—	0-07 (0-1)	—	—
	4	20-33 (17-21)	0-33 (0-2)	—	0-33 (0-2)	—	—
	1	20-73 (19-21)	0-13 (0-1)	—	0-13 (0-1)	—	—
	1	16-83 (12-20)	1-47 (0-4)	0-07 (0-1)	1-54 (0-5)	0-03 (0-1)	—
Telocentric 3B ^L (20 chromosomes)	1	18-40 (4-22)	0-70 (0-6)	0-03 (0-1)	0-73 (0-7)	0-20 ^{IV} , 0-07 ^V (0-1) (0-1)	
Telocentric 5B ^L (22 chromosomes)	1	18-60 (5-21)	0-90 (0-3)	0-70 (0-2)	0-27 (0-2)	0-10 ^{IV} (0-1)	
23-chromosome from monosomic 3B	1						

and disomic for another. At meiosis in these nullisomic-disomic haploids there was a high frequency of pollen mother cells in which there was one ring bivalent (Table 4, Plate 2*b*). No ring bivalents were seen in the meiotic pairing of euploids and it can be presumed that those in the nullisomic-disomic haploids resulted from the pairing of the two homologues.

Table 4. *Mean chromosome pairing at first metaphase of meiosis in 21-chromosome nullisomic-disomic haploids (30 cells each; ranges in parentheses)*

Genotype	No. plants analysed	No. plants analysed	I	II			III
				Rod	Ring	Total	
Nulli-diso							
1A-1D	1	1	19.27 (17-21)	0.27 (0-2)	0.60 (0-1)	0.87 (0-2)	—
1B-1A	3	2	18.65 (16-21)	0.20 (0-2)	0.95 (0-1)	1.51 (0-3)	0.01 (0-1)
1B-1D	1	1	18.13 (15-21)	0.57 (0-2)	0.87 (0-1)	1.43 (0-3)	—
2D-2A	2	2	18.53 (17-21)	0.28 (0-2)	0.95 (0-1)	1.23 (0-3)	—
2D-2B	9	2	18.80 (15-21)	0.22 (0-2)	0.88 (0-1)	1.20 (0-3)	—
3B-3A	12	2	19.00 (19)	0.06 (0-1)	0.93 (0-1)	1.00 (1)	—
3B-3D	21	2	19.00 (17-21)	0.10 (0-1)	0.90 (0-1)	1.00 (0-2)	—
3D-3A	2	1	17.40 (15-19)	0.83 (0-2)	0.97 (0-1)	1.80 (1-3)	—
5B-5D	4	2	7.40 (3-13)	3.33 (1.6)	2.46 (1-5)	5.80 (3-7)	0.66 (0-4)
5D-5B (20 °C)	7	2	19.10 (17-21)	0.40 (0-2)	0.55 (0-1)	0.95 (0-2)	—
5D-5B (12 °C)		1	20.73 (19-21)	0.13 (0-1)	—	0.13 (0-1)	—

A comparison of the aneuploids with the euploids shows that only the deficiency of chromosome 5B increased the level of pairing. Both the nullisomic 5B haploid and those nullisomic 5B-disomic 5D (Plate 2*c*) had high pairing 5B similar to that in nullisomic haploids of the wheat variety Holdfast (Riley & Chapman, 1958). Because of the low pairing in euploids it is difficult to recognise a chromosome activity which decreases pairing. However, the low frequency of bivalent formation in the nullisomic 3B haploid (Plate 2*a*) was probably caused by the absence of the chromosome which carries out an activity increasing synapsis (Sears, 1954).

In a nullisomic-disomic haploid the meiotic pairing can be affected either by the loss of a chromosome or by the increased dosage of one of its homeologues. The final level of pairing presumably reflects the combination of these two effects. Some evidence of these combined effects on meiotic pairing can be inferred from the analyses of the nullisomic-disomic haploids. Chromosome 3A or 3D, in extra

dosage, did not compensate for the reduced pairing resulting from the deficiency of chromosome 3B. Chromosome 5D did not compensate for the deficiency of 5B. From a study of meiosis of hybrids between aneuploid lines of *T. aestivum* and *Aegilops sharonensis* ($2n = 14$), Mello-Sampayo (1973) concluded that chromosomes 3A and 3D both reduced chromosome pairing and that chromosome 3D had an effect larger than that of 3A. The higher pairing in the nullisomic 3D disomic 3A haploid (Table 4) shows that the extra dosage of chromosome 3A has not completely compensated for the loss of the activity of chromosome 3D. The meiotic pairing of chromosomes of the aneuploid nullisomic 5D-tetrasomic 5B is normal at 20 °C but fails at 12 °C (Riley, 1966). The same response to temperature was seen in aneuhaploids of this genotype. At 12 °C the two homologous chromosomes were unpaired in most pollen mother cells (Table 4).

(iv) *Haploids with non-gametic chromosome numbers*

Three aneuhaploid plants were obtained with chromosome numbers which differed from the gametic chromosome numbers of their parents.

(1) A plant which had 20 chromosomes including a telocentric, resulted from the pollination of a plant monotelocentric for the long arm of chromosome 3B. If, as is probable, the telocentric was that for 3B, then another chromosome had been lost. It is not possible therefore to offer an explanation for the higher pairing seen in this plant.

(2) A 23-chromosome plant was obtained from the pollination of a plant monosomic for chromosome 3B. In 20 of the 30 cells examined at meiosis there were 21 univalents and one bivalent, which was often a ring. Pairing in the other cells was higher including trivalents or quadrivalents such that in one cell only four chromosomes were unpaired. A similar distribution of pollen mother cells with either high or low pairing was reported by Kempanna & Riley (1962) in wheat-rye hybrids which were simultaneously deficient for chromosome 3B and 5B. A few 46-chromosome pollen mother cells were seen at meiosis in the 23-chromosome haploid, but only two could be analysed. One cell had 23 bivalents, the other 19 bivalents and 8 univalents. The pattern of meiotic pairing permits speculation as to the constitution of the haploid. The frequency of a single ring bivalent suggests that the plant was disomic for one of the chromosomes of the haploid complement of wheat. Because the 23-chromosome haploid arose from the pollination of a plant monosomic for chromosome 3B it could have been deficient for chromosome 3B. It is difficult to explain the origin of all 23 chromosomes without considering the possibility that at least one of them was a chromosome from *H. bulbosum*. However, no support for this could be found from a study of the phenotype of the plant, which was similar to that of a euploid.

(3) A plant resulting from the pollination of a ditelocentric 5B^L plant was found by a root-tip somatic count to have 21 chromosomes, one of which was a telocentric. At meiosis, pairing was lower than in the euploid. This reduction in pairing could have been due to the absence of the short arm of chromosome 5B which is known to have an activity which increases synapsis (Riley & Chapman,

1967). A second tiller from the plant had 22 chromosomes at meiosis. Further fixations showed that the plant was chimaeral so that tillers had either 21 or 22 chromosomes. Meiosis in the 22-chromosome tillers was abnormal. Most pollen mother cells had 22 chromosomes but there were some with 44 and others with numbers between 22 and 44 (Plate 2*d*). Many cells had 22 univalents; in other 22-chromosome cells there were bivalents and multivalents. Multivalents were seen in 44-chromosome cells. It was not possible to determine the constitution of the 22-chromosome sectors of the haploid. Because of the low frequency of ring bivalents in the 22-chromosome cells it is unlikely that the haploid was disomic for one of its chromosomes. The extra chromosome may therefore have been one from *H. bulbosum*.

5. DISCUSSION

These experiments have shown that aneuploids can be obtained by pollinating the aneuploids of Chinese Spring with *H. bulbosum*. The frequency of haploids resulting from the pollinations was lower than that obtained by Barclay (1975). This may have been because Barclay's experiments were conducted under a regime of constant light and temperature from pollination to the embryo culturing stages. Kao & Kasha (1971), using a similar technique to produce haploids of barley, have shown that environment is one of the key factors which influence the rate of production of haploids. The failure to produce aneuploids from some of the monosomic lines reduced the value of the experiment. It is difficult to account for the high proportion of euploids produced by the pollinations of the monosomics. It may be that after chromosome elimination nullisomic embryos are formed in the expected gametic proportions but do not develop fully. Alternatively, the chromosomes of *H. bulbosum* may not be eliminated from the embryo in the absence of a particular wheat chromosome or chromosome arm.

The failure of the chromosome elimination process could especially account for the results obtained from crosses involving monosomic 1A. The nullisomic 1A is not one of the weakest of the series, so there was no reason to expect that the nullisomic haploid would not develop. Yet despite a high yield of haploids from the pollinations, no nullisomic haploid was found. By the use of trisomic plants of *Hordeum vulgare* in crosses with *H. bulbosum*, Ho & Kasha (1975) obtained evidence that particular *H. vulgare* chromosomes are involved in the control of chromosome elimination. In crosses of *H. vulgare* with tetraploid *H. bulbosum* (Konzak, Randolph & Jensen, 1951), there was no chromosome elimination and the hybrid embryos grew into hybrid plants. No hybrid plant between wheat and *H. bulbosum* was obtained by Barclay (1975) and none was found in the present studies. It is not known, therefore, in hybrid embryos deficient for a particular chromosome, whether chromosome elimination takes place and the nullisomic haploid embryos die or whether there is no elimination and the hybrid embryos fail to grow into hybrid plants. It should be possible to resolve this problem by a cytological examination of the appropriate hybrid embryos at the critical period for chromosome elimination.

The presence of additional chromosomes, namely B chromosomes of *Aegilops speltoides* in hybrids of *T. aestivum* × *Ae. speltoides*, caused abnormalities in the spindle formation of the pre-meiotic mitosis (Vardi & Dover, 1972). The occurrence at meiosis of pollen mother cells with doubled chromosome numbers was attributed to the spindle abnormalities. If the 22 and 23 chromosome haploids arose through incomplete elimination, the residual chromosomes of *H. bulbosum* may have caused spindle abnormalities in the pre-meiotic mitosis, which resulted in the formation of pollen mother cells with doubled chromosome numbers.

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