Gametocidal Genes Induce Chromosome Breakage in the Interphase Prior to the First Mitotic Cell Division of the Male Gametophyte in Wheat

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ABSTRACT

Male gametogenesis was cytologically analyzed in wheat lines homozygous or hemizygous for gametocidal (Gc) factors with different modes of action. The first and second meiotic divisions in all lines were cytologically normal. The postmeiotic mitoses were normal in the homozygous lines; however, chromosome fragments and bridges were observed in the mitoses of the hemizygous lines. The morphology of the chromosome fragments suggests that the Gc genes induce chromosome breaks in the G_1 phase prior to DNA synthesis of the first postmeiotic mitosis. The age of an anther was correlated with the frequency of aberrant second mitosis. Younger anthers contained a higher number of pollen undergoing normal second mitosis. This observation suggests that the arresting of the cell cycle occurs as the result of chromosome breaks during the first mitosis. Because chromosome bridges were more frequent than fragments in the second mitosis, breakage-fusion-bridge cycles possibly occurred during gametogenesis, which led to further chromosomal rearrangements. The Gc factors located on chromosomes 2S of *Aegilops speltoides* and $4S^{sh}$ of *Ae. sharonensis* induce severe chromosome breakage in pollen lacking them. However, the Gc factor on telosome $2C^cL$ of *Ae. cylindrica* only induced chromosome breaks at a low frequency. The observed partial fertility of Gc lines is presumably due to cell cycle arrest and the competition among gametes with and without chromosome breakage.

GAMETOCIDAL (Gc) factors in wheat (*Triticum aestivum* L. em Thell, 2n = 6x = 42, AABBDD) are strong segregation distorters that affect plant fertility through differential functioning of the gametes. Plants hetero- or hemizygous for a Gc factor produce two types of gametes, with or without the Gc factor. In most cases, only gametes with the Gc factor are functional, and the Gc factor is selectively transmitted to the progeny (Endo and Tsunewaki 1975; Endo 1990; Maan 1975).

Gc factors are introduced into wheat through interspecific hybridization and backcrossing with related Aegilops species during the transfer of cytoplasms (for review see Endo 1990). Alien chromosomes with Gc factors are selectively or sometimes exclusively retained. In some cases, alien chromosome segments with Gc factors are translocated to homoeologous chromosomes of wheat (Tsujimoto and Tsunewaki 1984; T. R. Endo, personal communication). Gc chromosomes were identified in different Aegilops species belonging to the sections: *Cylindropyrum* [3C of *Ae. caudata* L. (2n = 14, CC; Endo and Katayama 1978) and $2C^c$ of *Ae. cylindrica* Host. (2n = 28, $C^cC^cD^cD^c$; Endo 1979, 1996)]; *Polyeides* [$4M^g$ of *Ae. geniculata* Roth (2n = 28, $U^gU^gM^gM^g$; Friebe *et al.* 1998) and $3C^t$ of *Ae. triuncialis* L. (2n = 28, $U^tU^tC^tC^t$; Endo and Tsunewaki 1975)]; and *Sitopsis* [$2S^l$ and $4S^l$ of *Ae. longissima* Schweinf. & Muschl. (2n = 14, S^lS^l; Maan 1975, 1980; Endo 1985; Friebe *et al.* 1993), $2S^{sh}$ and $4S^{sh}$ of *Ae. sharonensis* Eig (2n = 14, $S^{sh}S^{sh}$; Maan 1975; Endo 1982; Miller *et al.* 1982), and 2S and 6S of *Ae. speltoides* Tausch (2n = 14, SS; Tsujimoto and Tsunewaki 1983, 1988; Kota and Dvorak 1988)]. With respect to chromosome 6S of *Ae. speltoides*, the mechanism of action is different because breakage occurs in gametes with the gametocidal chromosome (Kota and Dvorak 1988). The interactions among different gametocidal genes were analyzed in double-monosomic chromosome addition or substitution lines for the different Gc chromosomes. Endo (1990) and Tsujimoto (1995) summarized the available data and concluded that Gc factors on the same homoeologous group have similar functions ($2S = 2S^l = 2S^{sh}$, $4S^l = 4S^{sh}$). The Gc factors on chromosomes 3C of *Ae. caudata* and $3C^t$ *Ae. triuncialis* have similar functions but are different from either of the group-2 or group-4 Gc factors. The Gc factor on chromosome $2C^c$ of *Ae. cylindrica* has a different mode of action from all other Gc factors (Endo 1996).

Of additional interest is the interaction of the Gc factors with the genetic background of different wheats. For example, the Gc chromosome $2C^c$ of *Ae. cylindrica* is exclusively transmitted to the progeny in the cultivar Jones Fife, but not in Chinese Spring (CS) wheat (Endo 1988). A dominant suppressor (*Igc1*) of the Gc factor located on chromosome $3C^t$ is found in the cultivar Norin 26 (Tsujimoto and Tsunewaki 1985a). In the

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presence of *Igc1*, the Gc chromosome $3C^t$ is not preferentially transmitted but induces chromosome mutations.

Structural chromosomal aberrations are observed in the progeny of plants that were monosomic for a Gc chromosome. Chromosome mutations are detected in almost all Gc systems. Based on this observation, Endo (1990) proposed that the primary function of a Gc factor is to induce chromosome breaks in gametes lacking the Gc factor. The gametes are nonfunctional if the breakage is severe. If the damage caused by chromosome breakage is not sufficient to kill the gamete, it may still function and be transmitted to the offspring. Finch *et al.* (1984) detected chromosome fragments at anaphase of the first postmeiotic mitosis in meiospores of a monosomic $4S^{sh}$ chromosome addition line in wheat.

In the present paper, we report detailed cytological observations on male gametogenesis in three different Gc systems in CS wheat that differ in their ability to induce chromosome breakage. The Gc factors on chromosomes 2S and $4S^{sh}$ have strong Gc effects, and gametes lacking them are not involved in fertilization (Maan 1975; Miller *et al.* 1982; Tsujimoto and Tsunewaki 1984). The effect of the Gc factor on chromosome $2C^c$ is more moderate, and the Gc chromosome is not preferentially transmitted to the offspring. We observed chromosome breakage at anaphase of the first pollen mitosis in all plants hemizygous for a Gc factor. The morphology of the chromosome fragments suggests that Gc acts before the S phase of the first pollen mitosis. The different modes of actions of the three Gc factors are discussed.

MATERIALS AND METHODS

Plant materials: *T. aestivum* cv. Chinese Spring and the CS-derived lines homozygous or hemizygous for three different Gc factors were used (Table 1). In this paper, hemizygous instead of heterozygous is used to describe the status of lines that have a single dose of a Gc factor, assuming that CS does not have any Gc allele. All lines were kindly provided by T. R. Endo (Kyoto University, Japan) and are maintained by the Wheat Genetics Resource Center, Kansas State University.

Cytological observations: Plants were grown in a greenhouse at 18–20° with an 18-hr light period. Anthers at appropriate stages of meiosis and pollen mitosis were selected after staining with 1% acetocarmine from first tillers and fixed in a mixture of three parts absolute ethanol and one part glacial acetic acid. After fixing overnight, the anthers were treated with 1 N HCl at 60° for 10 min and stained in Schiff's Reagent (Fisher Scientific, Pittsburgh, PA) for several hours. Slides were prepared using the acetocarmine squash method. Photomicrographs were taken with a Zeiss photomicroscope III using Kodak Imagelink HQ microfilm 1461 (Eastman Kodak, Rochester, NY).

RESULTS

Meiosis: Meiosis and chromosome pairing at metaphase I in pollen mother cells of all lines listed in Table 1 were cytologically normal except in line MA2C^{c}L. In this line, a trivalent was observed in seven out of 52 PMCs involving the 2C^{c}L telosome and group-2 chromosomes of wheat (B. Friebe, S. Nasuda, T. R. Endo and B. S. Gill, unpublished results). None of the tetrads or immature male gametophytes possessed micronuclei, indicating that no chromosome breakage occurred at this stage.

Pollen mitoses in normal and Gc-factor homozygous lines: Pollen mitosis was cytologically normal in CS and the Gc-homozygous lines T2B-2S/T2B-2S, T4B-$4S^{sh}$/T4B-$4S^{sh}$, and DA2C^{c}L (Tables 2 and 3). Trinucleate pollen was produced after two rounds of postmeiotic mitosis (Figure 1). The first pollen mitosis occurs when the spike emerges from the flag leaf. At metaphase of the first pollen mitosis, the sister chromatids and position of centromeres are visible (Figure 1e). No chromosome bridges or fragments were observed at anaphase (Figure 1f) and telophase (Figure 1g). The vegetative nucleus decondensed soon after telophase of the first pollen mitosis (Figure 1h). Approximately one week later, the generative nucleus passed through the second pollen

TABLE 1

Plant materials used in this study

Line (Chromosome constitution)	State of Gc factor	Origin
CS		
T2B-2S/T2B-2S	Homozygous for *Gc1a*	*Ae. speltoides*
T2B-2S/2B	Hemizygous for *Gc1a*	*Ae. speltoides*
T4B-$4S^{sh}$/T4B-$4S^{sh}$	Homozygous for *Gc2*	*Ae. sharonensis*
T4B-$4S^{sh}$/4B	Hemizygous for *Gc2*	*Ae. sharonensis*
DA2C^{c}L	Homozygous for an unnamed Gc factor	*Ae. cylindrica*
MA2C^{c}L	Hemizygous for an unnamed Gc factor	*Ae. cylindrica*

CS, *Triticum aestivum* cv. Chinese Spring; T2B-2S, translocation involving chromosome 2B of wheat and 2S of *Aegilops speltoides*; T4B-$4S^{sh}$, translocation involving chromosome 4B of wheat and $4S^{sh}$ of *Ae. sharonensis*; DA2C^{c}L, disomic addition of a long arm telosome derived from chromosome $2C^c$ of *Ae. cylindrica*; MA2C^{c}L, monosomic addition of 2C^{c}L.

TABLE 2

Frequency of aberrant anaphase or telophase of first pollen mitosis in CS wheat and lines homozygous or hemizygous for Gc factors

Line	No. of pollen grains observed	Normal (%)	Abnormal (%)[a]
CS	115	115 (100.0)	0 (0.0)
Gc-homozygous			
T2B-2S/T2B-2S	50	50 (100.0)	0 (0.0)
T4B-4S^{sh}/T4B-4S^{sh}	50	50 (100.0)	0 (0.0)
DA2C^{c}L	57	57 (100.0)	0 (0.0)
Gc-hemizygous			
T2B-2S/2B	69	38 (55.1)	31 (44.9)[b]
T4B-4S^{sh}/4B	75	36 (48.0)	39 (52.0)[b]
MA2C^{c}L	88	80 (90.0)	8 (9.1)

[a] Abnormal pollen has bridge(s), chromosome fragment(s), or both.

[b] χ^2 values to 1:1 segregation are 0.710 ($P = 0.399$, d.f. = 1) for T2B-2S/2B and 0.120 ($P = 0.729$, d.f. = 1) for T4B-4S^{sh}/4B.

mitosis and produced a pair of crescent-shaped sperm nuclei (Figures 1i to 1l). Ana/telophase cells of the second pollen mitosis show neither bridges nor chromosome fragments (Figure 1k).

In anthers of normal CS and lines homozygous for a Gc factor, more than half of the pollen population was at anaphase or telophase (Figure 2). Furthermore, in these lines a large portion of pre-anaphase pollen (categorized as "Inter-metaphase" in Figure 2) was at metaphase. These observations indicate that pollen in anthers of normal CS and in homozygous lines were highly synchronized. No abnormal second pollen mitosis was observed in these lines.

First pollen mitosis in Gc-factor hemizygous lines: Chromosome fragmentation and/or chromosome bridges are observed in pollen at anaphase and telophase stages of the first pollen mitosis in lines hemizygous for a Gc factor (Figures 3a–3d, 3i–3l, 3q and 3r). In the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B, the fragmentation was so massive that all pollen suffering breakage had numerous acentric fragments between the spindle poles. The acentric fragments differed in size and shape. Some fragments were as large as whole chromosome arms (Figure 3b). Chromosome fragments with sizes identical to sister chromatids were observed, suggesting that chromosome breakage occurs prior to chromosome replication (Figures 3b and 3l).

No obvious differences in the frequency or pattern of aberrant first pollen mitosis at anaphase and telophase stages were observed between the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B. The frequency of pollen with aberrant anaphases or telophases was 44.9% in T2B-2S/2B and 52.0% in line T4B-4S^{sh}/4B (Table 2).

In line MA2C^{c}L, the frequency of pollen with aberrant anaphases or telophases was much lower (9.1%) (Table 2). The number of chromosome fragments and/or brid-

TABLE 3

Pollen maturity in anthers shortly before dehiscence in CS wheat and lines homozygous or hemizygous for Gc factors

Line	Total no. pollen	Mature pollen (%)	Immature (%) 3 nuclei	2 nuclei	1 nucleus	0 nucleus	χ^2 test[a]
CS	690	660 (95.7)	12 (1.7)	16 (2.3)	2 (0.3)	0 (0.0)	A
Gc-homozygous							
T2B-2S/T2B-2S	242	208 (86.6)	10 (6.3)	8 (2.3)	11 (3.1)	5 (1.6)	A
T4B-4S^{sh}/T4B-4S^{sh}	719	652 (90.7)	13 (1.8)	24 (3.3)	27 (3.8)	3 (0.4)	A
DA2C^{c}L	504	476 (94.4)	14 (2.8)	12 (2.4)	0 (0.0)	2 (0.4)	A
Gc-hemizygous							
T2B-2S/2B	359	219 (61.0)	61 (17.1)	59 (16.4)	13 (3.6)	7 (1.9)	B
T4B-4S^{sh}/4B	802	487 (60.7)	101 (12.6)	144 (18.0)	58 (7.2)	12 (0.4)	B
MA2C^{c}L	726	701 (96.5)	10 (1.4)	12 (1.7)	3 (0.4)	0 (0.0)	A

[a] Lines that differ significantly from each other in proportion at 5% are given different letters. χ^2 value for the heterogeneity among seven lines is 79.53 (d.f. = 24, $P < 0.005$).

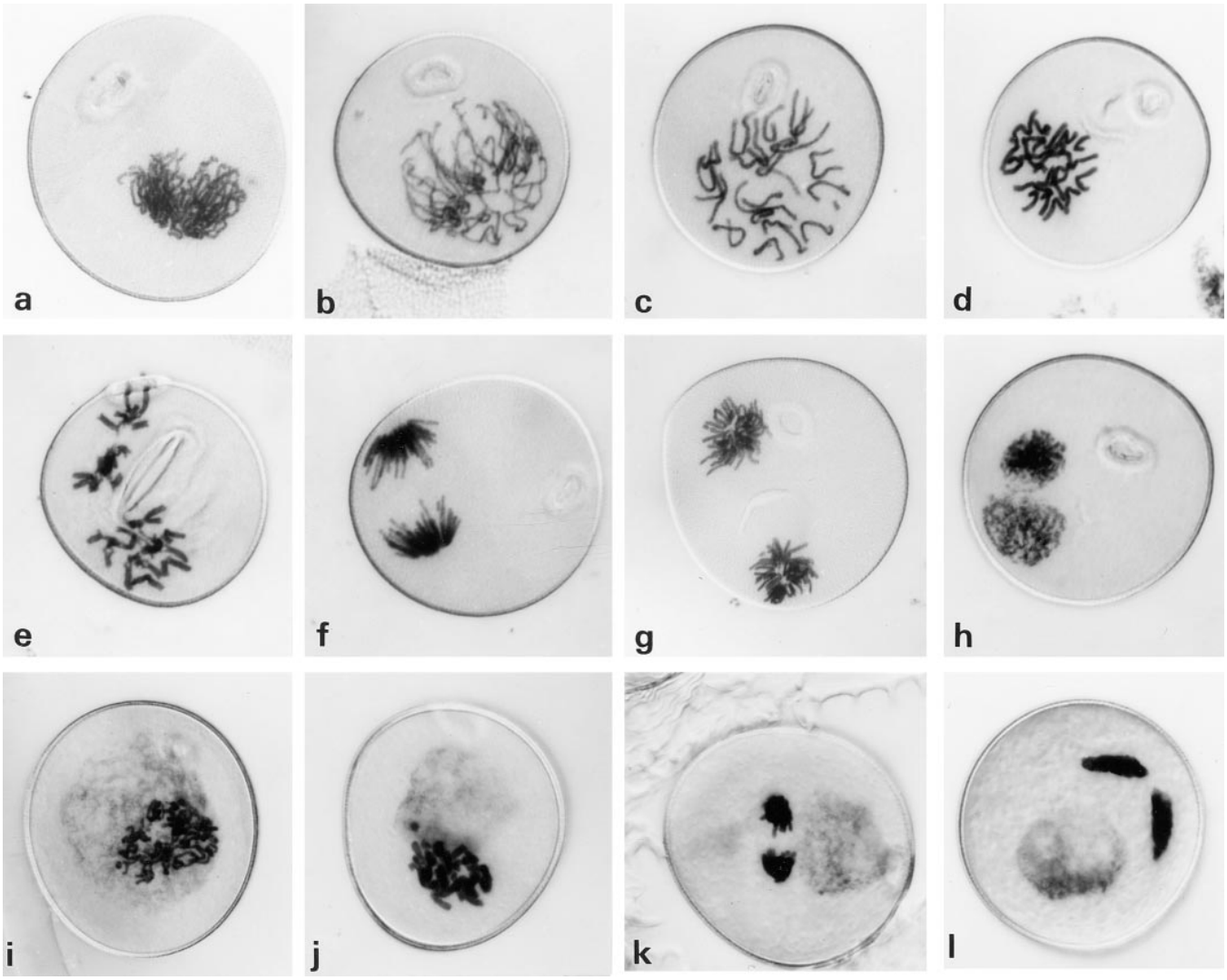

Figure 1.—Normal first (a to g) and second pollen mitoses (i to k) in wheat. Pollen grains go through prophase (a to d), metaphase (e), anaphase (f) and telophase (g) of first pollen mitosis and result in the pollen having one generative and one vegetative nucleus (h). About one week later, the generative nucleus undergoes prophase (i), metaphase (j), ana/telophase (k) of second pollen mitosis. Mature pollen (l) has one vegetative nucleus and two crescent-shaped sperm nuclei. Note that no chromosome fragments or bridges are seen in the ana/telophases of the first and second pollen mitosis (f, g, and k).

ges observed for each mitotic division also was lower (Figures 3q and 3r). These observations indicate that the Gc action of the 2C^{c}L telosome is significantly weaker than that of *Gc1a* and *Gc2* in the induction and amount of chromosome breakage.

Second pollen mitosis in Gc-factor hemizygous lines: Three anthers of different ages were examined in lines hemizygous for a Gc factor. The age of the anther was defined by the frequency of mature pollen grains. Fewer pollen grains at anaphase or telophase were observed in anthers of the heterozygous lines (21.1% in T2B-2S/2B, 20.8% in T4B-4S^{sh}/4B, and 27.4% in MA2C^{c}L on average), indicating a less synchronized development of the microsporophytes. Aberrant anaphase and telophase of second pollen mitosis also were observed (Figure 2). Compared to the first pollen mitosis, ana/telophase bridges were more frequent than chromosome fragmentation. The frequency of aberrant anaphases or telophases was correlated with the age of the anther in the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B (Figure 4). Thus, normal anaphases and telophases are more frequent in younger than in older anthers. This observation of nonsynchronized second pollen mitosis suggests that in the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B, pollen without chromosome breakage undergoes the second pollen mitosis before pollen that suffers from chromosome breakage. No correlation was observed between the age of the anther and the frequency of aberrant ana/telophases in line MA2C^{c}L presumably because of the lower frequency of chromosome breakage in this line. Although the Gc factor on the telosome 2C^{c}L causes less chromosome breakage com-

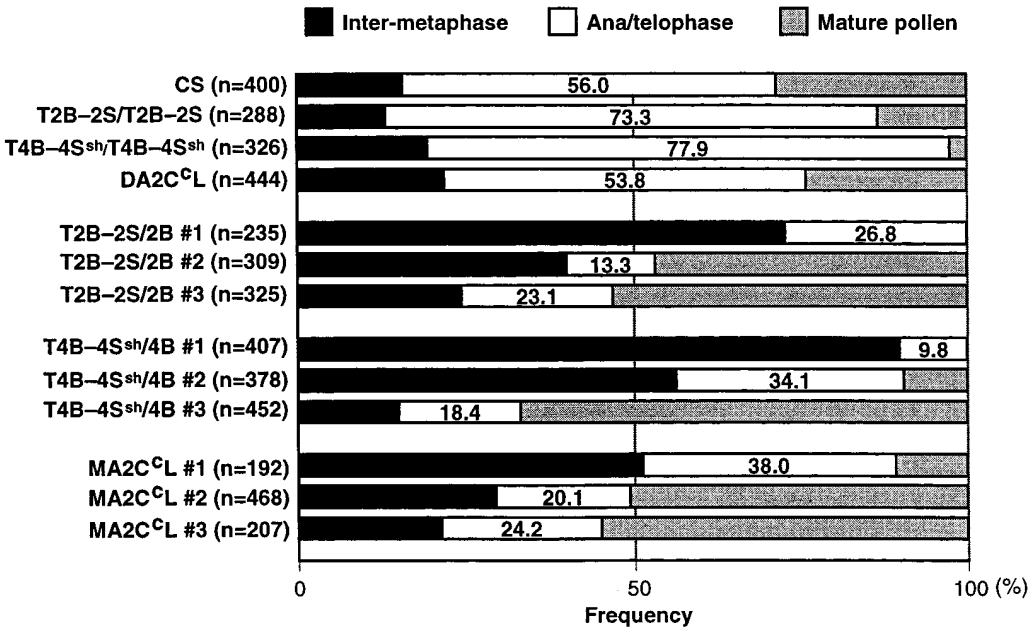

Figure 2.—Distribution of microspores in different stages of the second pollen mitosis. For CS, and Gc-homozygous lines T2B-2S/T2B-2S and T4B-4S^{sh}/T4B-4S^{sh} and DA2C^{c}L, one anther each was examined. For lines hemizygous for the Gc factor, three anthers at different ages were examined. Number of pollen scored is given in the parentheses.

pared to the Gc factors located on the translocation chromosomes T2B-2S and T4B-4S^{sh}, the lower amount of induced breaks might result in a less synchronized second pollen mitosis.

Maturity of pollen in the anthers prior to dehiscence: The morphology and number of nuclei in the microsporophytes immediately prior to anther dehiscence was determined. Mature pollen grains had characteristic crescent-shaped sperm nuclei and a decondensed vegetative nucleus (Figure 1l). Immature pollen was divided into four classes according to the number of nuclei (Table 3). Lines homozygous for a Gc factor had as high a frequency of mature pollen as normal CS. Line MA2C^{c}L also had a high frequency of mature pollen grains, indicating normal male fertility. However, in the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B, the frequency of mature pollen grains was 61.0% and 60.7%, respectively, indicating lower male fertility induced by chromosome breakage. In these lines, gametophytes still in the second pollen mitosis were observed.

Fertility of female gametes: Seed set of the first and second florets (excluding the lowermost and uppermost spikelets) of open-pollinated spikes were scored (Table 4). Seed set of open-pollinated spikes represents the fertility of the female gametes. Pollen semisterility does not seem to have an effect on the fertility of the female gametes, because anthers opened normally and shed considerable pollen. The hemizygous lines T2B-2S/2B and T4B-4S^{sh}/4B had low rates of seed set, indicating reduced fertility of the female gametes. The other lines had a seed set equal to normal CS. A strong negative correlation between seed set and the frequency of aberrant first pollen mitosis ($r = -0.988$, d.f. = 5), and a positive correlation between seed set and the frequency of mature pollen grains in the anthers just before dehiscence ($r = 0.948$, d.f. = 5) were observed. Thus, Gc factors probably affect fertility in both male and female gametes in a similar manner.

DISCUSSION

Gc genes are segregation distorters that have been found in diverse eukaryotic organisms. When segregation distortion occurs, one of the alleles at heterozygous loci transmits to the progeny at higher frequencies than the expected Mendelian ratio (Sandler *et al.* 1959). Different segregation distorters were identified in a wide range of plants, animals, and fungi (for reviews in different systems, see Lyttle 1991a). In animals, the *t* haplotypes of mouse (for review Silver 1993) and the Segregation distorter (*SD*) system of *Drosophila melanogaster* (for review Lyttle 1991b, 1993) are best studied. In plants, Rick (1966) reported gamete eliminator (*Ge*) in tomato, which causes abortion of gametes because of allelic interaction. *Ge* induces abortion of the gametes carrying the opposite allele, although the homozygotes show no adverse effect on the formation of the gametes. A similar genetic model exists for the pollen killer in wheat (Loegering and Sears 1963) and in rice (Sano *et al.* 1979). The preferential transmission of alien chromosomes can be explained by assuming that a similar sterility factor(s) is located on the alien chromosome (Cameron and Moav 1957; Maguire 1963; Endo and Tsunewaki 1975). The underlying molecular mechanisms leading to the abortion of the gametes are largely unknown.

Gc factors induce chromosome breakage in the first postmeiotic interphase: The observed Gc factors induce chromosome breakage in the first postmeiotic interphase. Because both meiotic divisions were normal in

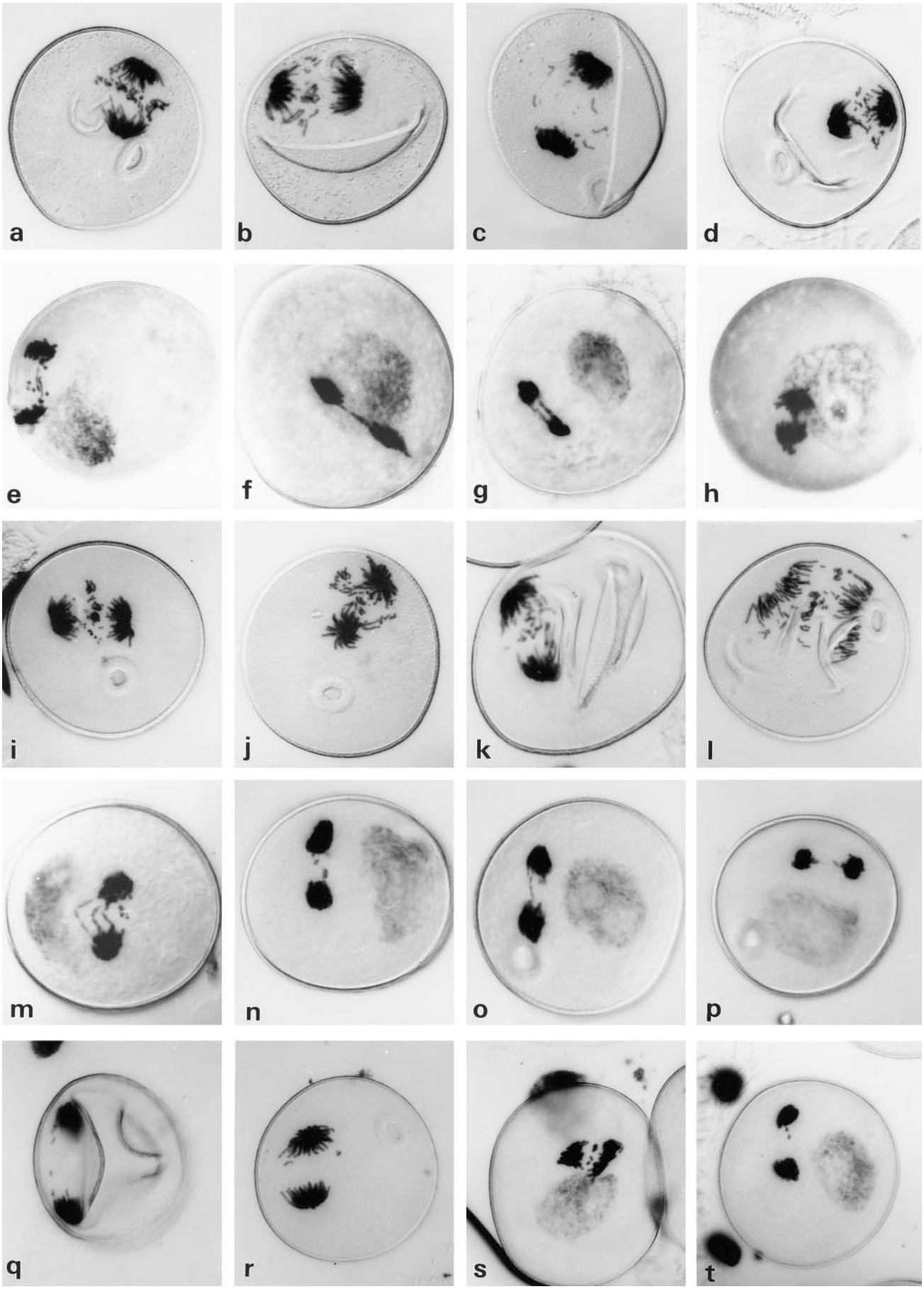

Figure 3.—Aberrant ana/telophase of the first (a to d, i to l, q and r) and second (e to h, m to p, s and t) pollen mitoses in the Gc-hemizygous lines T2B-2S/2B (a to h), T4B-4S^{sh}/4B (i to p), and in the monosomic chromosome addition line MA2C^{c}L (q to t).

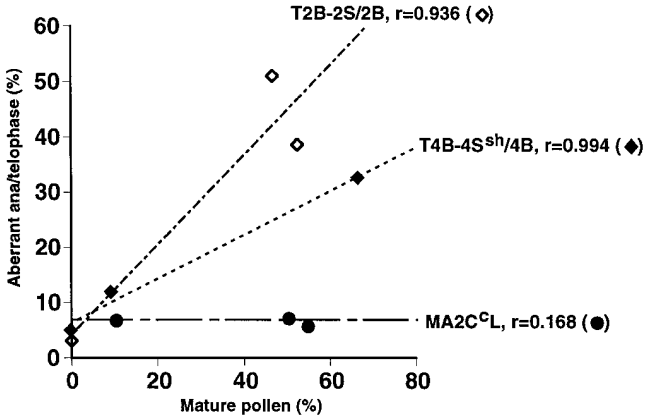

Figure 4.—Relationships between the age of anthers in terms of percent mature pollen and the frequency of aberrant ana/telophases in the second pollen mitosis. Open squares, closed boxes, and closed circles indicate T2B-2S/2B, T4B-4S^{sh}/4B, and MA2C^{c}L, respectively.

all Gc lines, the activity of the Gc genes is expressed in single-nucleus pollen. The occurrence of broken chromosome fragments with similar-sized sister chromatids suggests that the chromosome breaks are induced before the S phase of the first pollen mitosis. Tsujimoto and Tsunewaki (1985b) reported that most mutations induced by Gc factors occur before the first zygotic division. Endo (1990) suggested that Gc factors are expressed in gametogenesis, but did not specify the stage of their expression. We successfully identified the stage of the Gc expression at G_1 between the end of meiosis and the S phase of the first pollen mitosis.

Because the Gc factors in the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B are translocated to wheat chromosomes, these factors are expected to segregate in a 1:1 ratio. The frequency of pollen with aberrant first pollen mitosis in these lines is about 50% and fits the theoretical segregation ratio (Table 2). The Gc factors are transmitted almost exclusively to the offspring (Endo 1982; Tsujimoto and Tsunewaki 1983). Therefore, it is reasonable to assume that chromosome breaks always occur in gametes without the Gc factor, and the semi-sterility observed in these lines is caused by chromosome breakage induced before the S phase of the first pollen mitosis.

TABLE 4

Seed set in open-pollinated spikes in CS wheat and lines homozygous or hemizygous for Gc factors

Line	No. florets observed	Seed set rate (%)
CS	400	98.8
Gc-homozygous		
T2B-2S/T2B-2S	400	97.0
T4B-4S^{sh}/T4B-4S^{sh}	400	98.3
DA2C^{c}L	406	91.3
Gc-hemizygous		
T2B-2S/2B	400	49.0
T4B-4S^{sh}/4B	400	50.3
MA2C^{c}L	192	89.1

In the line MA2C^{c}L, three-fourths of the pollen grains are expected to lack the 2C^{c}L telosome and show aberrant first pollen mitosis, because the Gc telosome 2C^{c}L is monosomic. The observed frequency of aberrant first pollen mitosis was much lower than the expected 75% (Table 2) (Endo 1988). Thus, the Gc factor on chromosome 2C^{c} does not induce gametic sterility and allows transmission of induced chromosome mutations to the offspring in the CS background. Endo and Gill (1996) isolated a series of deletion lines using the Gc chromosome 2C^{c}. They reported that about half of the progeny of the monosomic 2C^{c} addition line had one or more structural chromosome changes. The difference between the observed low frequency of aberrant first pollen mitosis and the high recovery of deficiencies may be explained by a small number of chromosome breaks, most of which were undetected cytologically in the present investigation.

Second pollen mitosis is arrested by the chromosome breakage: The second pollen mitosis is highly synchronized in CS and in lines homozygous for a Gc factor (Figure 2). The synchrony of cell division is disturbed in lines hemizygous for the Gc factors. The second mitosis occurred later in pollen with chromosome breakage, then in pollen without chromosome breaks (Figure 4). This indicated that induced chromosome breaks in the first pollen mitosis arrested the second pollen mitosis.

Eukaryotic cell cycles are highly regulated (Murray and Hunt 1993). The cell cycle is controlled by the activation and inactivation of a maturation promoting factor. There are two checkpoints in the cell cycle, one before and one after S phase. If the DNA is damaged, the cell cycle is arrested at these checkpoints. The cell cycle also is arrested in the middle of mitosis by the failure of a single chromosome to attach properly to the mitotic spindle (Gorbsky 1997). In the present study, chromosome breakage was observed at ana/telophase of the first pollen mitosis. Thus, pollen prior to the second postmeiotic division possessed damaged DNA that might have triggered a delay or arrest of the cell cycle. The high frequency of immature three- or two-nuclei pollen in anthers of the heterozygous lines T2B-2S/2B and T4B-4S^{sh}/4B just before dehiscence (Table 3) may be the result of cell cycle arrest induced by chromosome breakage. Although aberrant second pollen mitosis was not often observed in line MA2C^{c}L, the synchronization of the second pollen mitosis was disturbed (Figure 2), indicating the presence of chromosome breaks during the first pollen mitosis that were not observed cytologically.

Breakage-fusion-bridge (BFB) cycles may be initiated by the chromosome breakage induced by Gc factors:

Although chromosome fragments are prevalent in the first pollen mitosis, chromosome bridges occur more often than chromosome fragments in the second pollen mitosis. This result suggests that BFB cycles are initiated by chromosome breakage in the first pollen mitosis. The number of chromosome bridges in cells at the second pollen mitosis was less than the number of fragments observed in the first pollen mitosis, indicating that some broken chromosome ends are healed before entering the second pollen mitosis. Werner *et al.* (1992) observed that first generation deficiencies in wheat induced by the action of the Gc gene on chromosome $2C^c$ possessed telomere-specific repeats as visualized by *in situ* hybridization analysis. Tsujimoto (1993) also showed that telomeric repeats were added to the broken ends within one generation after the chromosome breakage occurred. These results suggest that in wheat, broken chromosome ends are healed by the addition of telomeric repeats.

BFB cycles may be of the chromatid or chromosome type (McClintock 1941, 1952). We observed both single- and double-chromatid bridges in the second pollen mitosis, indicating that both types of BFB occurred. Besides initial structural rearrangement, the chromatid type of BFB leads to duplication and deficiencies. The chromosome type of BFB also may result in translocations. Lukaszewski (1995) examined both chromatid and chromosome types of BFB cycles induced by reverse tandem duplications in wheat. The chromatid-type BFB continued in early endosperm divisions but ceased in embryogenesis, whereas the chromosome type of BFB continued through embryogenesis but eventually ceased before meiosis.

Recently, Tsujimoto *et al.* (1997) cloned a broken end of a wheat chromosome deficiency induced by the Gc factor on $2C^c$ of *Ae. cylindrica*. The breakpoint mapped in the middle of the nucleolus organizer region (rRNA gene). They found a repeat of the telomere sequence at the end of the deleted chromosome. A 31-bp reverse tandem duplication of rDNA sequence was detected between the rRNA gene and the telomeric repeat. There was an 8-bp sequence of unknown origin between the duplications whose presumed function was to join the sister chromatids. These data provide molecular evidence for the occurrence of the chromatid type of BFB cycles during the origin of deficiencies and are consistent with our cytological observations of chromosome bridges in the second pollen mitosis.

Mode of action of Gc factors: Three different Gc factors in the same CS background were analyzed. All Gc factors investigated induced chromosome breakage following meiosis in G_1 phase prior to DNA synthesis of the first mitotic cell division. In plants with Gc factors on chromosomes 2S and $4S^{sh}$, many chromosome fragments were produced in about half of the pollen. On the contrary, the Gc factor on the telosome $2C^cL$ induces chromosome breakage less frequently. The number of chromosome fragments and/or bridges is much lower compared with lines T2B-2S/2B and T4B-$4S^{sh}$/4B. The difference of Gc action also is reflected in the pollen fertility and in preferential transmission of the Gc factor (Tsujimoto and Tsunewaki 1984; Endo 1988; present article). In the cases of Gc factors on the 2S and $4S^{sh}$ chromosomes, semi-sterility and preferential transmission were observed, whereas the line MA$2C^cL$ had normal fertility and the chromosome $2C^c$ showed typical monosomic segregation.

So far, two sets of the deletion lines of wheat were established by using Gc factors. Endo and Gill (1996) used the Gc factor on chromosome $2C^c$ to establish deficiencies for all wheat chromosomes, and Tsujimoto *et al.* (1996) used the Gc factor on chromosome 2S to produce deficiencies for the long arm of chromosome 5A. The construction of cytologically-based physical maps reveals striking differences between the two sets of deficiencies. Hohmann *et al.* (1995) characterized 63 deficiencies for group-7 chromosomes produced by Endo and Gill (1996) and found that only seven lines had additional interstitial deletions. On the contrary, Ogihara *et al.* (1994) analyzed the deficiencies produced by Tsujimoto *et al.* (1996) and reported a much higher frequency of chromosomal rearrangements. Of

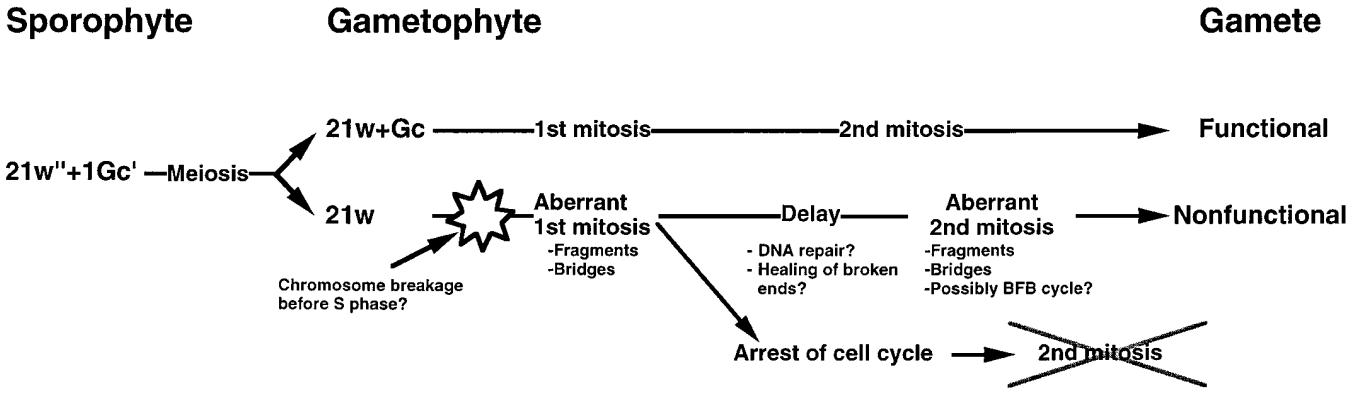

Figure 5.—A scheme showing the mode of action of Gc genes in CS background leading to semi-sterility.

19 lines analyzed, 15 had translocations and/or interstitial deletions. This difference is in agreement with our observation that the Gc factor on 2S chromosome induces more breakages than the Gc factor on telosome $2C^cL$.

Chromosome breakage, cell cycle arrest, and preferential transmission of Gc chromosome: Based on the observations of three different Gc factors, we propose a modified version of the model of Gc action introduced by Endo (1990). Our scheme for Gc action and its relation to the preferential transmission of Gc factors is shown in Figure 5. Meiosis of the Gc lines is completely normal. The primary function of the Gc factors is induction of chromosome breaks in the first postmeiotic interphase in the gametophyte. The presence of chromosome fragments with similar sizes of sister chromatids indicates that chromosome breakage is initiated before chromosome replication. While microsporophyes without chromosome breakage normally proceed to the second mitosis, those with chromosome breakage had a delayed second mitosis. Repair of damaged DNA and healing of broken chromosome ends may be required before entry into the second mitosis. In extreme cases with many chromosome rearrangements, the cell cycle is completely arrested and further development of pollen is stopped at the two nuclei stage. Chromosome bridges are more frequently observed than chromosome fragments in aberrant second pollen mitosis. Possibly, BFB cycles occur during the gametogenesis and produce further rearrangements of chromosomes. Although the frequency of pollen with chromosome breakage was about 50% in the first mitosis in the heterozygous lines T2B-2S/2B or T4B-$4S^{sh}$/4B, more than 60% of the pollen was mature in the anthers just prior to dehiscence. This result suggests that cells with breaks can develop into mature gametophytes.

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