The Deletion Stocks of Common Wheat

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Chromosomal breaks occurred in the progeny of a common wheat (*Triticum aestivum* L. em Thell; $2n = 6x = 42$, genome formula AABBDD) cultivar Chinese Spring with a monosomic addition of an alien chromosome from *Aegilops cylindrica* Host ($2n = 4x = 28$, CCDD) or *A. triuncialis* L. ($2n = 4x = 28$, UUCC) or a chromosomal segment from *A. speltoides* Tausch ($2n = 2x = 14$, SS). We identified 436 deletions by C-banding. The deletion chromosomes were transmitted stably to the offspring. We selected deletion homozygotes in the progeny of the deletion heterozygotes and established homozygous lines for about 80% of the deletions. We failed to establish homozygous lines for most of the deletions in the short arm of chromosome 2A and for all deletions in the short arm of chromosome 4B, because plants homozygous for these deletions were sterile. We could not obtain any homozygotes for larger deletions in the long arms of chromosomes 4A, 5A, 5B, and 5D. The deletion stocks showed variations in morphological, physiological, and biochemical traits, depending on the size of their chromosomal deficiency, and are powerful tools for physical mapping of wheat chromosomes.

The aneuploid stocks developed in a common wheat cultivar Chinese Spring are a powerful tool for genetic and breeding studies of wheat (Sears 1954, 1966; Sears and Sears 1978). These stocks are immensely useful for localization of genes on chromosomes and chromosomes arms (McIntosh 1988). Endo (1988) reported a unique genetic system for the systematic production of even more powerful novel aneuploid stocks, namely, deletion stocks with various sized terminal deletions in individual chromosome arms, useful for subarm localization of genes. When a certain chromosome from *Aegilops cylindrica* is present in Chinese Spring in the monosomic condition, chromosomal breaks occur in the gametes that lack the *A. cylindrica* chromosome and generate various chromosome aberrations, including deletions. The broken chromosome ends, if not fused to other broken ends, are stabilized by the rapid gain of telomere structure (Werner et al. 1992b). Such deletions in plants without the *A. cylindrica* chromosome are transmitted regularly to the offspring.

Isolation of deletion lines was conducted in the progeny of the *A. cylindrica* addition line by eliminating aberrations other than simple terminal deficiencies. Also, an alien chromosome from *A. triuncialis* L. and a chromosome fragment from *A. speltoides* were found recently to induce chromosome mutations in Chinese Spring in the same manner as the *A. cylindrica* chromosome (Endo, unpublished data). A preliminary attempt was made to isolate deletions in the progeny of wheats with monosomes of these chromosomes. In this article, we report the details of the production and the general features of deletion stocks of Chinese Spring.

Materials and Methods

Wheat Stocks

We used two alien monosomic addition lines and one alien translocation line of Chinese Spring to induce deletions. One alien addition line had an *A. cylindrica* chromosome (Endo 1988). This alien chromosome is homoeologous to the group 2 chromosomes (Gill KS, unpublished data). The other alien addition had an *A. triuncialis* chromosome, which has a satellite and is different from the *A. triuncialis* chromosome reported previously by Endo (1978). The alien translocation line had a modified wheat chromosome 2B with a small chromosomal segment from *A. speltoides* translocated to the end of the long arm (described as T2BS·2BL-2SL hereafter). This translocation chromosome car-

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Journal of Heredity 1996;87:295–307; 0022-1503/96/$5.00

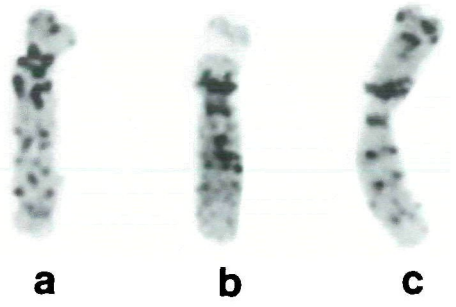


Figure 1. C-banding of (a) *A. cylindrica*, (b) *A. triuncialis*, and (c) T2BS·2BL-2SL chromosomes.

ries *the Gc1b* (gametocidal) gene (Tsujimoto and Tsunewaki 1988). Figure 1 shows the C-banding patterns of the *Aegilops* and translocation chromosomes. We isolated most of the deletions from the *A. cylindrica* addition line. A small number of deletions were isolated from the *A. cylindrica* addition line with the cytoplasm of *A. kotschyi* (Mukai and Endo 1992) and from the *A. triuncialis* addition and T2BS·2BL-2SL lines. We used the ditelosomic (Sears and Sears 1978) and nullisomic-tetrasomic (Sears 1966) lines of Chinese Spring to isolate some of the deletion chromosomes in the hemizygous condition.

Crosses and Cytology

We first backcrossed the alien addition and translocation lines to Chinese Spring, analyzed the chromosome constitutions of the progeny by C-banding (the modified C-banding; Gill et al. 1991), and grew plants that had a deletion or deletions and no alien chromosome (Figure 2). We either self-pollinated or backcrossed those plants once to Chinese Spring, followed by selfing. We cytologically screened the selfed progeny for homozygous plants with deletion chromosomes and the least degree of aberrations in the other chromosomes, that is, translocations and aneuploidy. When two or more deletions occurred in a single plant in the homozygous condition, we made no further effort to separate them.

For some deletions, when homozygotes were not obtained after the screening of the selfed progeny, we crossed the appropriate ditelosomic or nullisomic-tetrasomic lines to the deletion heterozygotes to obtain the deletion hemizygotes in the F_1 progeny. The F_2 progeny then were screened for deletion homozygotes.

We photographed 5–10 chromosomes for each of the deletions and measured the lengths of the short (excluding the satellites for chromosomes 1B and 6B) and long arms. We calculated the fraction

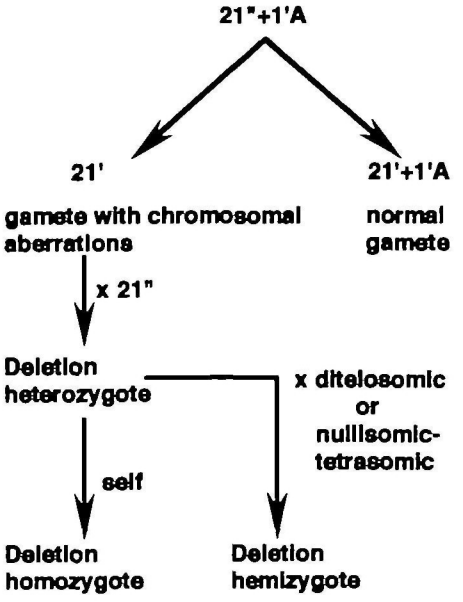


Figure 2. A scheme showing the production of the deletion stocks in common wheat. "A" stands for the *Aegilops* chromosome or chromosome segment causing chromosomal aberrations.

length (FL) of the breakpoints from the centromere for the short-arm deletions according to the formula

$$\text{FL} = \text{deletion chromosome}\ \frac{\text{deletion S arm}}{\text{L arm}} \div \frac{\text{S arm}}{\text{L arm}}\ \text{normal homologue}$$

The FL of long-arm deletions was calculated by the formula

$$\text{FL} = \text{normal homologue}\ \frac{\text{S arm}}{\text{L arm}} \div \frac{\text{S arm}}{\text{deletion L arm}}\ \text{deletion chromosome.}$$

The arm ratios were used to compensate for the difference in contraction of chromosomes between cells. The arm ratios of the normal wheat chromosomes were taken from Gill et al. (1991). The same formulae were used to calculate the FL values of the deletions in the short and long arms in the satellite chromosomes 1B and 6B, except the length of the satellite was not included. For estimating the FL value of deletions in the satellite regions of 1B and 6B, the formula

$$\text{FL} = \frac{\text{deletion satellite}}{\text{S arm}} \div \frac{\text{satellite}}{\text{S arm}}\ \text{normal homologue}$$

was used. Because of their indirect measurements, the FL values do not necessarily indicate the precise breakpoints of the deletions and, therefore, were used to order only those deletions whose breakpoints were neither in nor close to C-bands. We analyzed several of the deletions by in situ hybridization using the 18S·26S rDNA probe, as described by Mukai et al. (1991).

Results

We studied a total of 475 plants of the first progeny, backcrossed or selfed, of the *A.cylindrica* monosomic addition line. About half of the progeny had one or more chromosomal structural changes and most of the plants with the chromosomal aberrations did not carry the *A. cylindrica* chromosome, as reported previously (Endo 1988). Of the 52 backcrossed progeny of the *A. triuncialis* addition line, 44 plants did not have the alien chromosome, and 32 out of the 44 plants had some chromosomal aberrations. Of the 31 backcrossed progeny of the T2BS·2BL-2SL line, 12 plants that did not bear the translocation chromosome had some chromosomal aberrations. For the production of deletion stocks, we selected 156, 12, and 4 plants with one or more deletions and no *Aegilops* chromosome or alien translocation chromosome from the progeny of the

Table 1. Distribution of deletion breakpoints in different genomes, chromosome arms, and homoeologous groups in Chinese Spring wheat

Homoeologous group	No. of deletion breakpoints						
Genomes:	A		B		D		
Arms:	S	L	S	L	S	L	Total
1	5	6	22	18	5	8	64
2	9	6	13	11	6	12	57
3	4	8	10	12	9	3	46
4	4	13	9	14	5	15	60
5	11	23	9	18	4	12	77
6	5	8	11	15	7	11	57
7	13	25	6	16	6	9	75
Subtotal	51	89	80	104	42	70	
Total	140		184		112		436

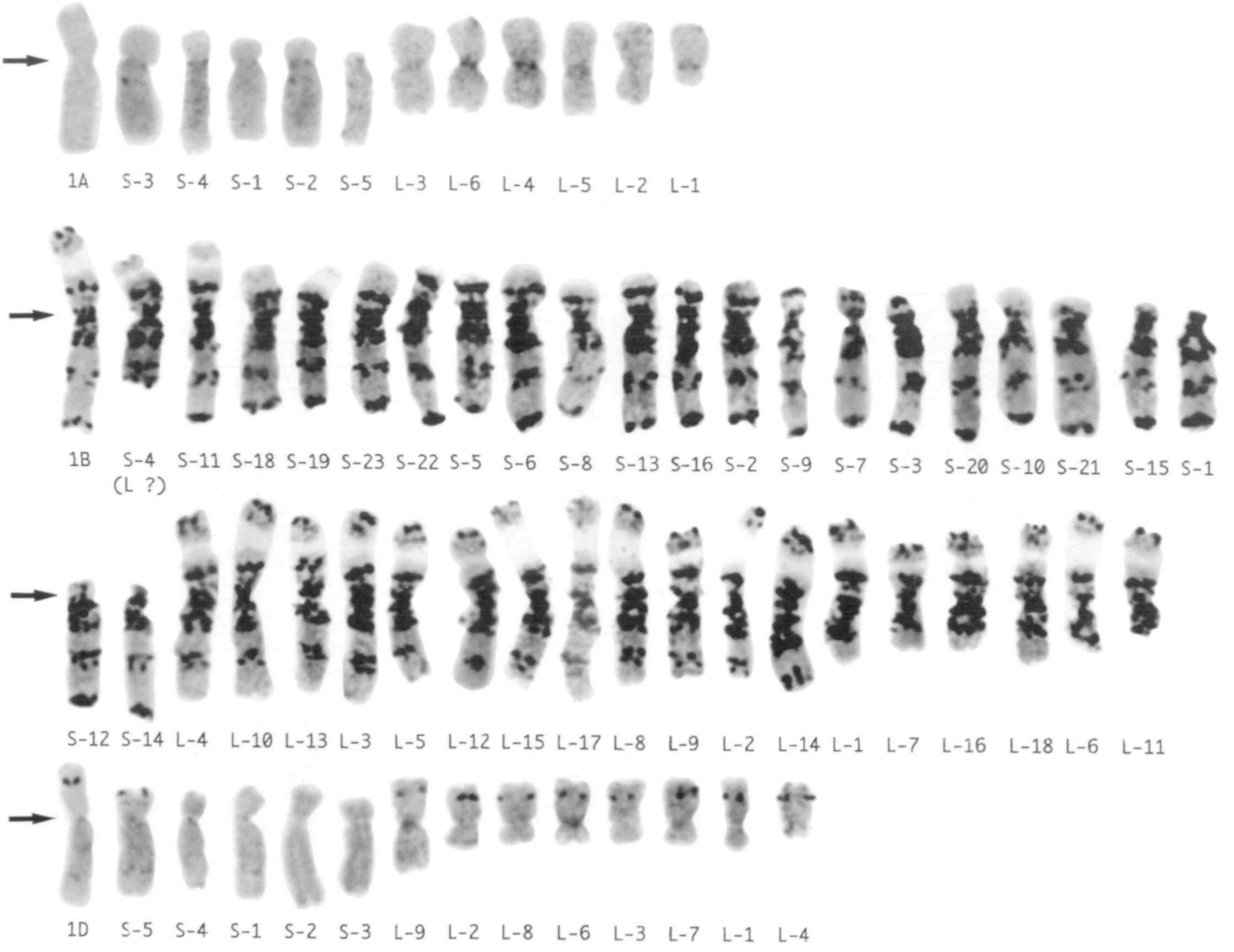


Figure 3. C-banding of the normal and deletion chromosomes of Chinese Spring wheat. Arrows indicate the centromere region. Normal chromosomes (left), followed by short- and long-arm deletion chromosomes, are shown for the 21 wheat chromosomes.

A. cylindrica addition, *A. triuncialis* addition, and T2BS·2BL-2SL lines, respectively. During the cytological screening, we found two deletions, one in the euploid and one in a ditelosomic line of Chinese Spring, and they are included in the deletion stocks reported here.

We examined by C-banding the chromosome constitutions of the first progeny of the monosomic alien addition and translocation lines, usually using single root-tip samples, and detected no chimeras of deletions among cells. However, we occasionally found chromosomes that appeared normal in the first progeny, but were aberrant in the next generation, and vice versa. In the progeny derived from different spikes of the same first-progeny plant, we found different, although rare, aberrations including different deletions of the same homologue. This fact indicated the chimeric nature of chromosomal aberrations in the first progeny, although the chromosome breaks are thought to occur in the gametes of the monosomic alien addition lines (Endo 1990a,b). At present we do not know the cause of this discrepancy. The deletion and other chromosomes were stabilized fully in subsequent generations.

So far we have identified 436 deletions and produced their homozygous or heterozygous lines (Table 1). Eight and 21 of them were isolated from the T2BS·2BL-2SL and *A. triuncialis* addition lines, respectively, and the rest were from the *A. cylindrica* addition line. For 89 (20.4%) of the deletions, we could neither achieve homozygosity nor maintain homozygous lines because of sterility. We established homozygous lines for the rest of the deletions: 289 deletions were in single-deletion lines, 45 in 39 double-deletion lines (where two different deletion chromosomes occurred in the same plant), 10 in 7 triple-deletion lines, and 3 in 3 quadruple-deletion lines. The fewer number of deletions than are expected from the number of multiple-deletion lines are due to the fact that part of the deletions involved in the multiple-deletion lines also were established in the single-deletion lines.

Figure 3 shows the C-banded deletion chromosomes. The breakpoints of deletions are shown in the C-band diagrams of Figure 4. We designated the deletions by using the chromosome arm hyphenated with consecutive numbers, for example, 1AS-1, in the order of their isolation. Some numbers are omitted in Figures 3 and 4 because some deletions were identified erroneously.

Next, we describe the general features of the deletion chromosomes and the homozygous deletion lines obtained. If not

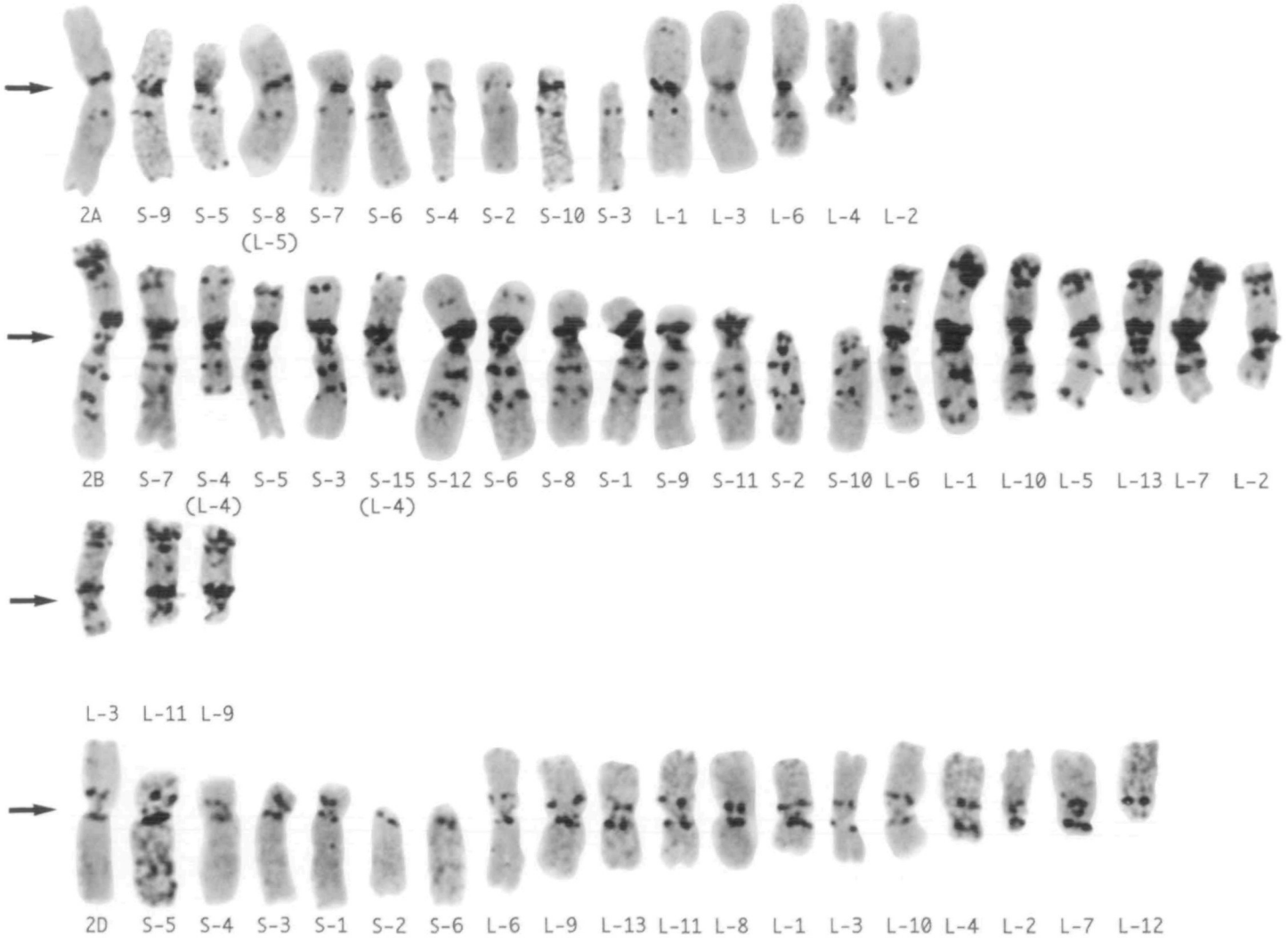


Figure 3. Continued.

noted otherwise, the seed fertility (estimated by the seed set of bagged heads), growth, and vigor of the deletion homozygotes were nearly normal or not reduced significantly.

Homoeologous Group 1

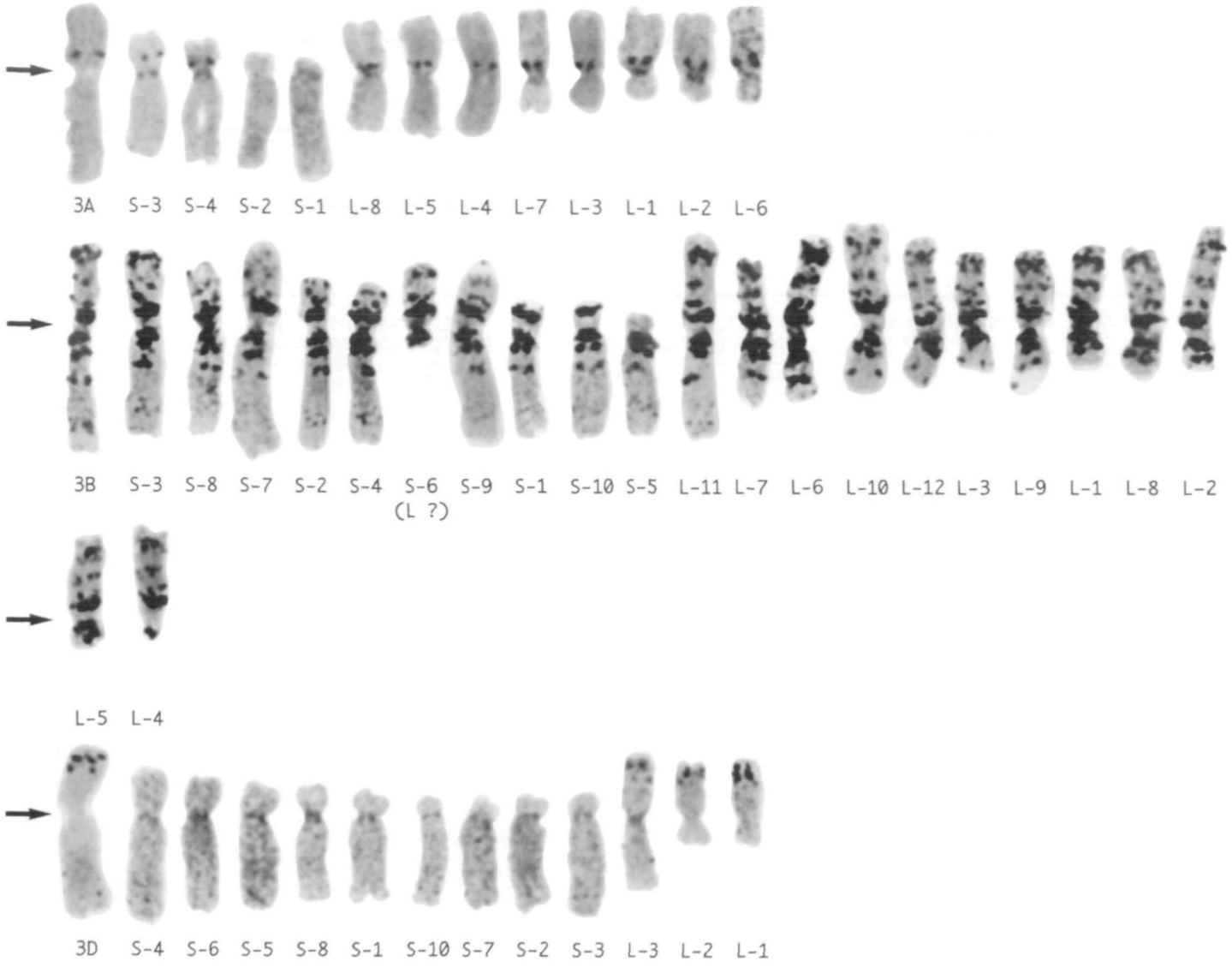
Chromosome 1A. All five deletions in the short arm and five of the six deletions in the long arm were homozygous. The long-arm deletion chromosomes were examined by in situ hybridization using the 18S·26S rDNA probe, which hybridizes to the telomeric region of the normal 1A short arm (Mukai et al. 1991); otherwise, distinguishing between the short and deleted long arms was difficult. Lack of deletions in the distal half of the long arm was due to the failure in differentiating the terminal C-band.

Chromosome 1B. We isolated 22 deletions in the short arm, six of which occurred in the satellite. Another six had breakpoints in the secondary constriction or the nucleolus organizer region (NOR). All such deletions, except 1BS-2, retained at least a part of NOR, which was detected by the in situ hybridization (Mukai et al. 1991 and present data). The NOR was not detectable by acetocarmine staining or C-banding. The smallest deletion in the satellite, 1BS-4, retained 52% of the satellite and lacked the 5S rRNA locus (Mukai et al. 1990; it was designated as 1BS-4b in the literature). Deletion 1BS-4 had an aberrant long arm (see Figure 3). Deletions 1BS-11, -12, -13, and -14 were not transmitted through pollen, because they were from the Chinese Spring line with the cytoplasm of *A. kotschyi* and lacked the fertility restorer gene, *Rfv1*. Homozygotes for these 1BS deletions cannot be obtained until the deletion chromosomes are transferred somehow into common wheat cytoplasm through pollen. Deletions 1BS-18 and -19 lacked the *Rfv1* gene, whereas 1BS-4 retained the *Rfv1* gene (for details, see Mukai and Endo 1992). Of the 18 long-arm deletions, 16 were in the homozygous state. Deletions 1BL-10 and 1BL-11 were derived from the same progeny. These short- and long-arm deletions fully covered the length of chromosome 1B.

Chromosome 1D. We produced homozygous lines for all five deletions in the short arm and six out of the eight deletions in the long arm. Deletion 1DS-2 lacked the 5S rRNA locus (Mukai et al. 1990). The homozygous 1DL-4 line, with the largest deficiency of the long arm (FL = 0.18), set 7–30 seeds per spike. Because the ditelosomic 1DS was reported to be male sterile (Sears and Sears 1978), a critical gene for male fertility must be located within the proximal 18% region of the long arm.

Homoeologous Group 2

Chromosome 2A. Of nine deletions in the short arm, only 2AS-5 (FL = 0.78) was ho-



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mozygous. Although we obtained deletions homozygous for 2AS-2, -3, -4, -6, and -7, they had irregular meloses with many univalents at metaphase I (Figure 5). They were highly sterile in both sexes. The anthers included many sterile pollen grains and often did not dehisce. The seed set of these homozygotes was sporadic, even after artificial pollination, and their offspring generated various aneuploids. The sterility probably was due to irregular meioses. The 2AS-5 homozygous line had a normal meiosis and seed set. Therefore, a gene controlling meiotic pairing must be located somewhere between the breakpoints of 2AS-5 and 2AS-7 (FL = 0.56). Sears and Sears (1978) also reported the female sterility of the ditelosomic 2AL. We produced homozygous lines for four out of the six deletions in the long arm, but their selfed seed fertility was reduced in proportion to the size of the deficiency. Two deletions, 2AS-8 and 2AL-5, occurred in one chromosome, forming a double-arm deletion chromosome (see Figure 3).

Chromosome 2B. We established homozygous lines for all 13 deletions in the short arm. They had less vigor but good seed set. All 11 deletions in the long arm were homozygous, but lines 2BL-5, -9, and -11 set less than 10 grains per spike when self-pollinated. Two double-arm deletions, 2BS-4/2BL-4 and 2BS-15/2BL-4, had the same origin. Also, the deletions 2BL-2 and 2BL-11 were derived from a single plant.

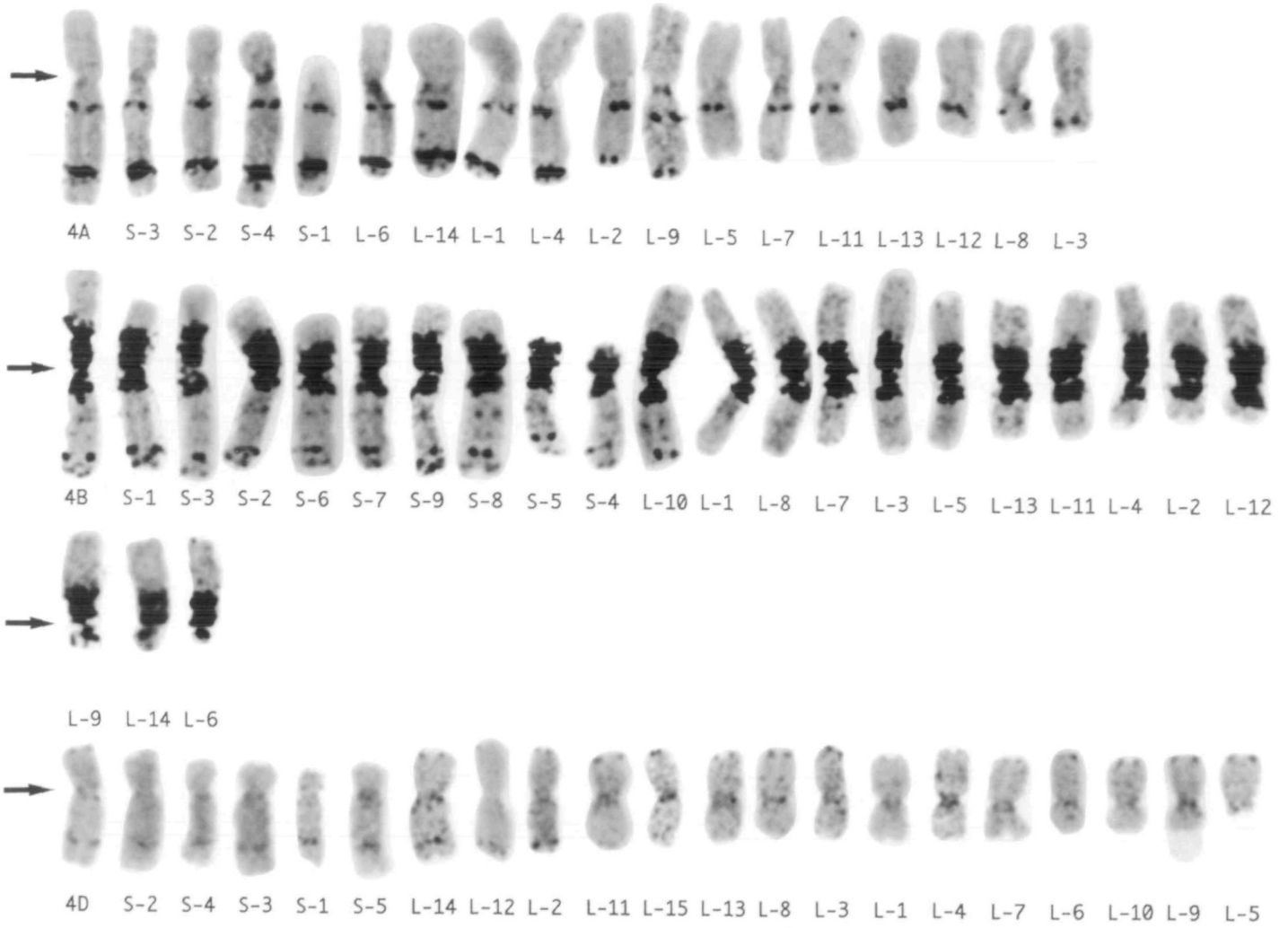
Chromosome 2D. We established homozygous lines for five out of the six deletions in the short arm and for 10 out of the 12 deletions in the long arm. The long-arm deletion homozygous lines showed a tendency for decreased selfed-seed fertility as the deletion size increased. The homozygous 2DL-2 and -7 lines set less than 10 grains per bagged spike.

Homoeologous Group 3

Chromosome 3A. All four deletions in the short arm and all eight deletions in the long arm were homozygous. The lack of the terminal band in this chromosome made it difficult to detect smaller deletions in either of the arms.

Chromosome 3B. All 10 deletions in the short arm and 11 deletions in the long arm, except 3BL-12, were homozygous. Deletion chromosome 3BS-6 lacked most of the long arm, but it was not sure the remaining part was truly of the 3B long arm (see Fig. 3).

Chromosome 3D. We produced homozygous lines for all deletions in this chromosome, nine in the short arm and three in the long arm.



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Homoeologous Group 4

Chromosome 4A. All four short-arm deletion lines were homozygous and showed reduced selfed-seed fertility. We produced homozygous lines for 11 out of the 13 deletions in the long arm, but could not obtain homozygotes for the remaining two deletions, 4AL-3 and -8, among the 25 selfed offspring of each of the deletion heterozygotes. The seed set of the long-arm deletion homozygotes decreased from normal to sporadic as the size of the deficiency increased. Among the deletions that were homozygous, 4AL-12 (FL = 0.43) had the largest deficiency. The deletion chromosome 4AL-14 was found in a Chinese Spring ditelosomic 2BL line. A gene for waxy protein, *Wx-B1,* was located between the breakpoints of cytologically indistinguishable deletions 4AL-6 and -14 (the *Wx* locus is present in both) and 4AL-1 and -4 (the *Wx* locus is absent in both) (Yamamori et al. 1994).

Chromosome 4B. We obtained deletion homozygotes for seven out of the nine short-arm deletions, except 4BS-2 and/-8, which were male sterile. This confirms previous reports that a gene controlling male fertility is located in the short arm of this chromosome (Sears and Sears 1978; the chromosome is designated as 4A in the literature) and in the distal region of the arm (Endo et al. 1991). We established homozygous lines for all 14 deletions in the long arm.

Chromosome 4D. We produced homozygous lines for all five deletions in the short arm and 13 out of the 15 deletions in the long arm.

Homoeologous Group 5

Chromosome 5A. All 11 short-arm deletions were homozygous. Their breakpoints were clustered near the distal end. Although Sears and Sears (1978) reported that ditelosomic 5AS is male sterile, we established homozygous lines, more or less male fertile, for 11 out of the 23 deletions in the long arm. As the size of the deficiency increased, especially over 50% of the long arm, the seed set decreased to several grains per spike. Deletion 5AL-21 (FL = 0.33) had the largest homozygous deficiency among the 5AL deletions. Two deletions, 5AL-15 and -22, originated from different spikes of an original backcrossed plant. Another two deletions, 5AL-20 and -23, were isolated from a speltoid and a nonspeltoid spike, respectively, of a chimeric backcrossed plant. The long-arm deletion plants, except one for 5AL-23 (FL = 0.87), had speltoid spikes. The 5AL-23 line, homozygous or heterozygous, had spikes slightly more slender than those of euploid Chinese Spring, but another deletion, 5AL-7, with a similar size deficiency (FL = 0.87) had fully speltoid spikes (Figure 6). The speltoid suppressing gene, *Q*, seems to be located close to the breakpoint of 5AL-7. This result is consistent with the previous deletion mapping of the *Q* gene, using deletions and translocations

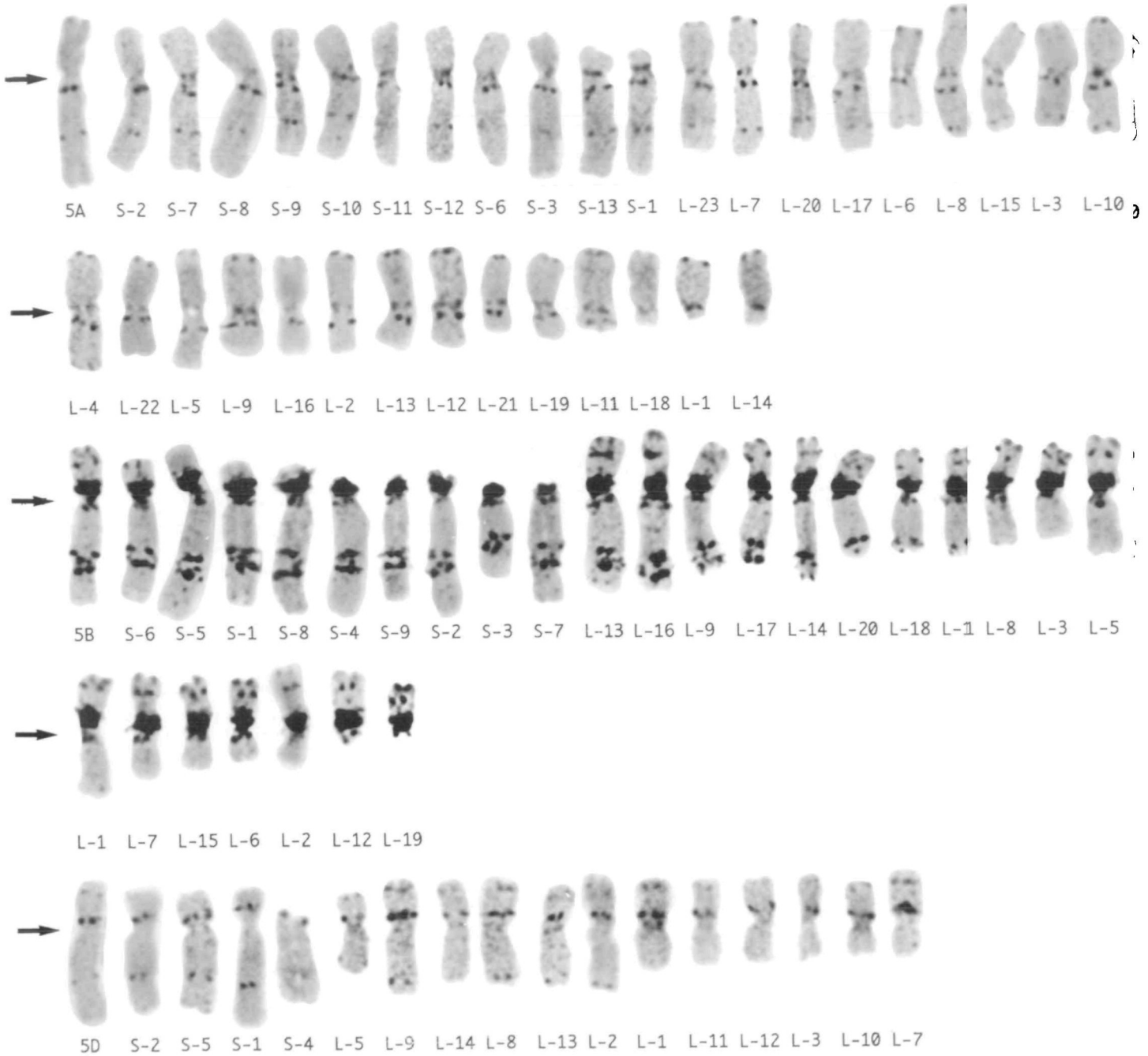

Figure 3. Continued.

induced by the so-called gametocidal chromosomes from *A. longissima* (Endo and Mukai 1988) and *A. speltoides* (Tsujimoto and Noda 1990)

Chromosome 5B. All nine deletions in the short arm were in the homozygous condition. Although ditelosomic 5BS was reported to be male sterile (Sears and Sears 1978), we could obtain male-fertile homozygous lines for 7 out of the 18 deletions in the long arm. The homozygous lines for 5BL-11, whose breakpoint was just distal to the interstitial C-band (L2.1), showed a nearly normal selfed-seed set. In contrast, homozygotes for 5BL-3 and -7, whose breakpoints were just proximal to the L2.1 C-band, were highly male sterile. The 5BL-3 homozygous plant set few seeds on a bagged spike, but the progeny set no selfed seeds. The 5BL-7 homozygous plant set few seeds by self-pollination, and the progeny also set few selfed seeds. The homozygous line for 5BL-6 (FL = 0.29), which set seeds normally by self-pollination, was trisomic or tetrasomic for 5A. Probably, the homozygote for this deletion would not be obtained when 5A is disomic. Gill et al. (1993b) found that the *Ph1* (pairing homoeologous suppresses pairing among homoeologous chromosomes) gene is present on 5BL-11 but absent on 5BL-1, which had a breakpoint just proximal to the L2.1 C-band. Figure 7 shows the meiotic chromosome configurations of the hybrids between the deletion lines 5BL-1 and -11 and *A. variabilis*. The hybrid involving 5BL-1 had many multivalents, indicating the loss of *Ph1*. But the one involving 5BL-11 had

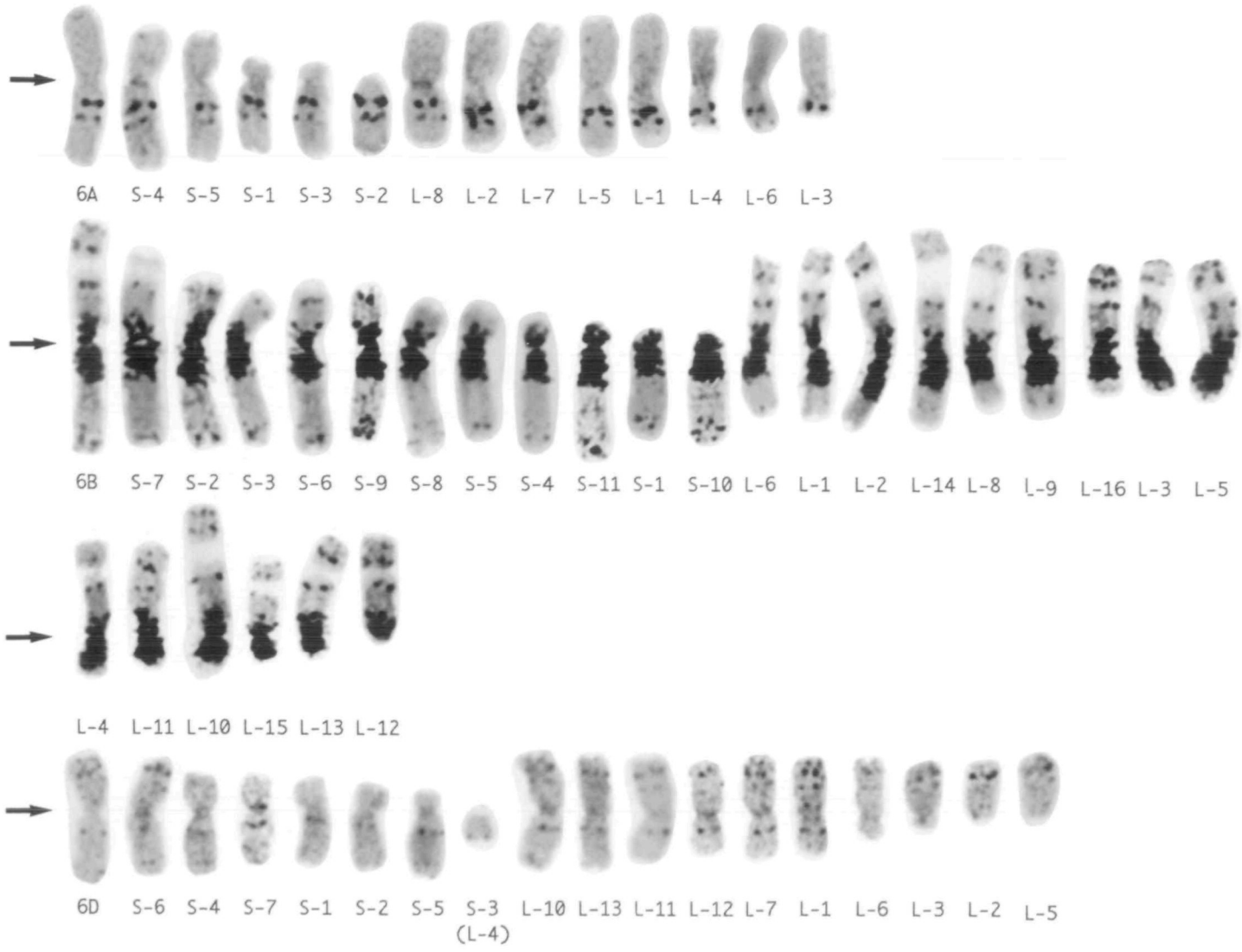

Figure 3. Continued.

only univalents, indicating the presence of *Ph1*.

Chromosome 5D. We produced homozygous lines for all four deletions in the short arm. Sears and Sears (1978) reported that the ditelosomic 5DS is male sterile, but we obtained male-fertile homozygous lines for 5 out of the 12 long-arm deletions. These long-arm deletion homozygotes set relatively many seeds, more than 20 grains per spike, by self-pollination. Deletion chromosome 5DL-8 may have had a very small terminal deletion in the short arm, because its heterozygote produced a high frequency of nullisomic gametes for 5D, as a result of poor pairing in the short arm as well as in the long arm. We generally observed reduction in meiotic pairing between normal and deletion arms, as also reported by Curtis et al. (1991).

Homoeologous Group 6

Chromosome 6A. We produced homozygous lines for three out of the five 6AS deletions, but could not get homozygotes for 6AS-2 (equivalent to the 6AL telosome) and 6AS-3 (FL = 0.27) from any of the 35 selfed progeny of the deletion heterozygotes. We established homozygous lines for all eight deletions in the long arm.

Chromosome 6B. We obtained homozygous lines for 9 out of the 11 deletions in the short arm. The four deletions whose breakpoints were in the secondary constriction had at least part of the NOR, which was revealed by the in situ hybridization analysis. All the homozygotes for the short-arm deletions, including 6BS-7 with a breakpoint in the satellite, showed severe pistillody, transformation of stamens into pistil-like organs, and, therefore, were reduced in selfed seed fertility. We obtained homozygous lines for 12 out of the 15 deletions in the long arm. The deletion homozygotes for 6BL-1, -8, -9, and -14 had awnless spikes, but those for 6BL-3, -4, -5, -6, -10, -11, -12, and -13 were awned (Figure 6). Deletion 6BL-6 has the DNA markers that are present in the other 6BL deletions (Gill et al. 1993a). Therefore, the occurrence of an interstitial deletion including the locus of an awn-inhibitor gene, *B2*, can explain the fact that the spikes of the 6BL-6 (the smallest deficiency) homozygote were awned. We found deletion 6BL-11 in an euploid Chinese Spring plant.

Chromosome 6D. We produced homozygous lines for 6 out of the 7 deletions in the short arm and 10 out of the 11 deletions in the long arm. A double-arm deletion line with 6DS-3 and 6DL-4 (see Figure 3) set a few seeds by self-pollination.

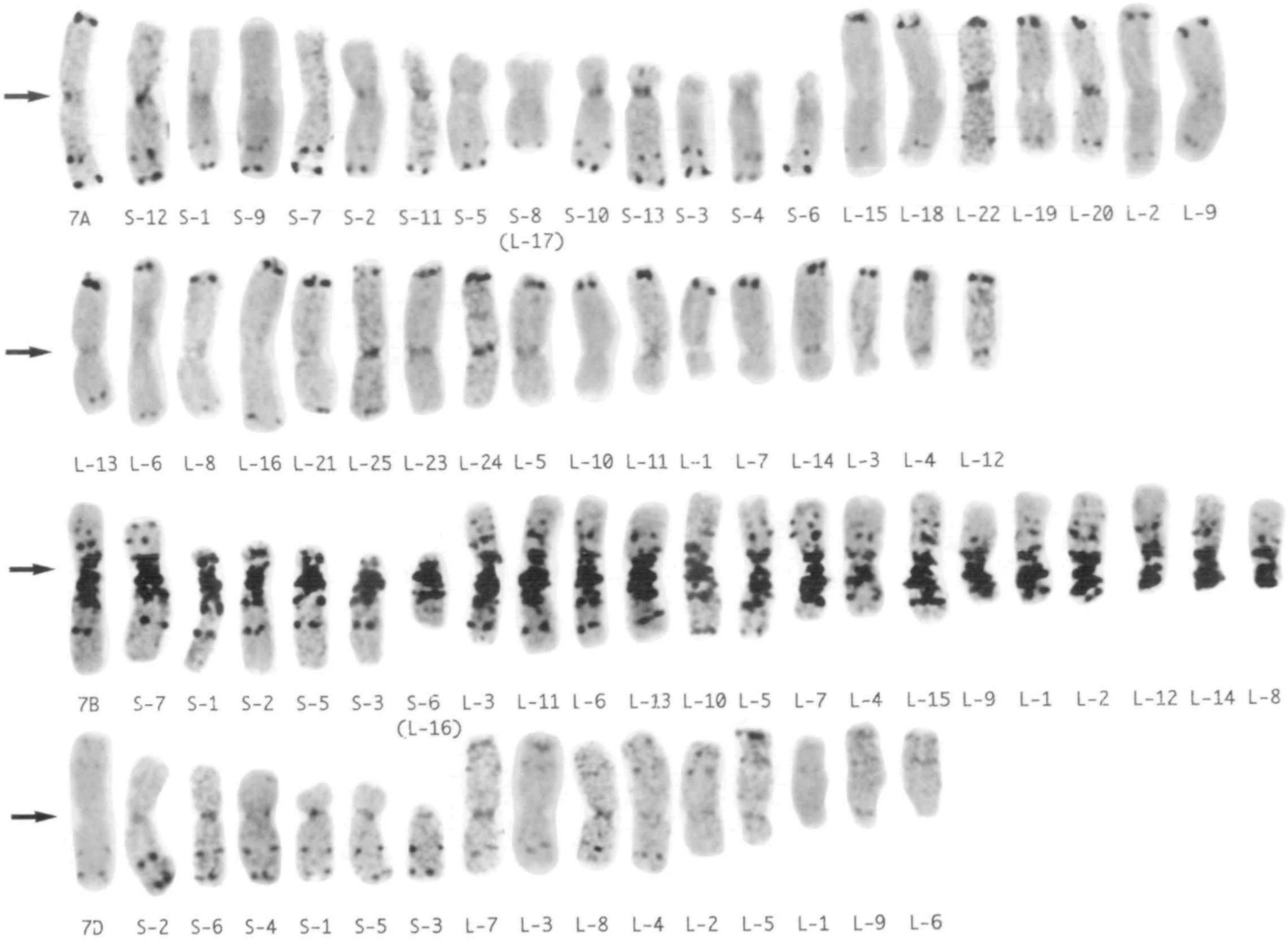

Figure 3. Continued.

Homoeologous Group 7

Chromosome 7A. We established homozygous lines for all 13 deletions in the short arm and 20 out of the 25 deletions in the long arm. They evenly covered the entire chromosome. Deletions 7AS-8 and 7AL-17 occurred in the same chromosome (see Figure 3). A waxy gene *Wx-A1* is located distal to the breakpoint of 7AS-12 and proximal to the breakpoints of 7AS-1 and -9 (Yamamori et al. 1994). These three deletions were cytologically indistinguishable from each other.

Chromosome 7B. We obtained homozygous lines for 3 out of the 6 deletions in the short arm and 11 out of the 16 deletions in the long arm. Deletion 7BS-6 and 7BL-16 were in the same chromosome (see Figure 3).

Chromosome 7D. Sears and Sears (1978) failed to recover a 7DL telosome of Chinese Spring, in spite of a large-scale screening. We isolated six deletions in the short arm and obtained their homozygous lines, except for 7DS-6. All nine deletions in the long arm were homozygous. Two deletions, 7DS-3 and 7DL-8, originated from the same progeny. A waxy protein, *Wx-D1,* was found to be absent in all the 7DS deletions (Yamamori M, personal communication).

The Chinese Spring 7DS has genetic homoeology to the 7A and 7D short arms, although it is physically longer than the 7DL (Werner et al. 1992a). Because of this, chromosome 7D was placed upside down (7DS was in the long arm position) in the figures of several previous publications (Endo 1983, 1986, 1988; Endo and Gill 1984; Gill et al. 1988, 1991; Mukai et al. 1991).

Discussion

Maintenance of Deletion Stocks

We did not find any structural changes in the deletion chromosomes after their isolation, probably because the broken chromosomal ends healed soon after breakage by the synthesis of telomeric sequences (Werner et al. 1992b). Although only a few lines were observed, a pair of homologous deletion chromosomes could pair with each other at the broken arms, as well as the intact arms, ensuring normal meiotic pairing and segregation of the disomic double-arm deletion chromosomes. Therefore, we can stably maintain the more or less fertile 338 deletion homozygous lines (289 single-, 39 double-, 7 triple-, and 3 quadruple-deletion lines) by self-pollination.

Some deletion lines have to be maintained as heterozygotes, and the progeny must be screened for homozygotes either cytologically or by other means, because the deletion homozygotes either were sterile or could not be obtained at all. All homozygous plants for the 2AS deletions whose deficiencies were larger than that

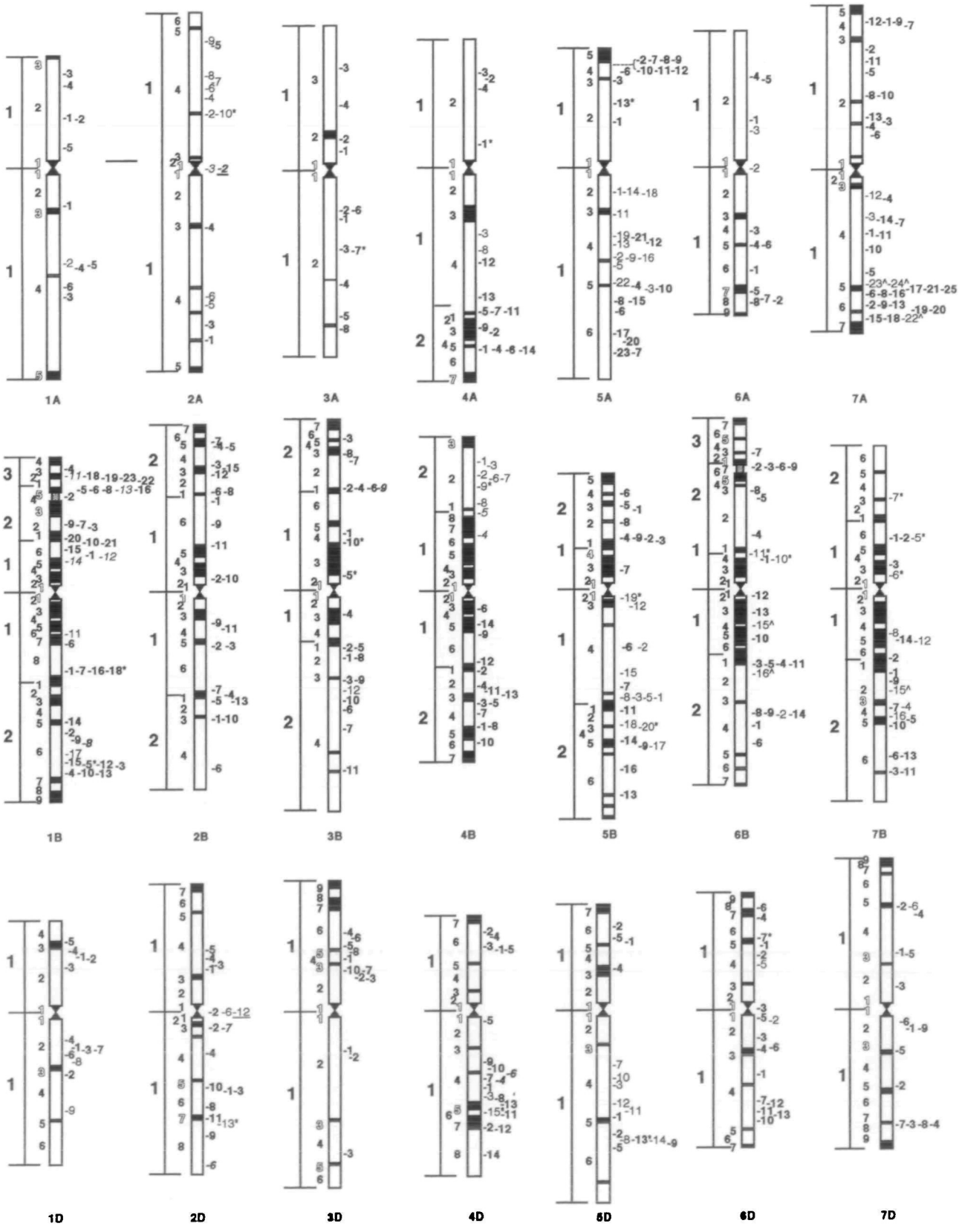

Figure 4. C-banding diagrams of Chinese Spring chromosomes showing the breakpoints of the deletions. The diagrams were taken from Gill et al. (1991). The numbers on the left of each chromosome diagram indicate the C-banded and unbanded regions; the outlined numbers indicate C-bands that did not differentiate well in this study. Bold and plain hyphenated numbers on the right of each diagram indicate the homozygous and heterozygous condition of the deletions, respectively. Underlined and plain numbers at the centromeric position indicate the deletions that lost the entire long arm and those that lost the entire short arm, respectively. The deletion lines with italicized numbers have the cytoplasm of *A. kotschyi*. The deletions marked * and ^ were induced from the *A. triuncialis* addition line and the T2BS-2BL-2SL line, respectively.

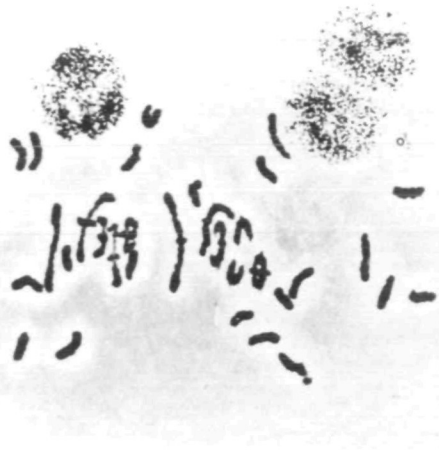

Figure 5. A meiotic chromosome configuration at metaphase I of a plant homozygous for deletion 2AS-6. Note the formation of many univalents.

of 2AS-5 (FL = 0.78) were highly male and female sterile. All 4BS deletion homozygotes were completely male sterile. To enhance the probability of recovery and for efficient screening of homozygotes, we are now producing new stocks that are disomic for the 2AS or 4BS deletion chromosomes and monosomic for the 2A or 4B short-arm telocentric chromosome. Deletion homozygotes could be obtained in the progeny of these stocks at higher recovery rates.

In another subset of deletion lines involving the long arms of chromosomes 4A, 5A, 5B, and 5D, we found no homozygotes in the selfed progeny of the heterozygotes. Because critical genes for male fertility surely are located in those arms (Sears and Sears 1978), we did not conduct further screening. However, we crossed these deletion heterozygotes with the Chinese Spring nullisomic-tetrasomics to obtain deletion-hemizygous plants, monosomic for the deletion and trisomic for its normal homoeologues (1‴ + 19″ + 1′del). We screened the selfed progeny of the deletion-hemizygous plants, but failed to recover the expected fertile deletion-homozygous plants, except for 5BL-6, compensated by the three or four doses of the homoeologues. We recovered mostly nullisomic-tetrasomic or -trisomic plants and infrequently deletion-hemizygous plants. Therefore, it would be easier for one to work with deletion hemizygotes that can be screened among the F_1 progeny of deletion heterozygotes crossed with appropriate nullisomic-tetrasomic stocks.

For the other heterozygous deletion lines, we expect to obtain deletion homozygotes by extensive selection.

Distribution of Breakpoints

Before we discuss the distribution of breakpoints, we must note that breakpoints were assayed by C-banding. Therefore, deletions were identified more easily in chromosomes with many diagnostic bands, especially with terminal marker bands, than in those lacking marker bands. The arms with the fewest deletions are mostly those lacking C-band markers, such as 1AS, 3AS, 4AS, and 6AS. If one of these arms contained a hot spot for breakage toward the distal end, as found in 5AS, then we have not detected it. In spite of these reservations, a certain trend in the distribution of the breakpoints was observed among the three genomes of wheat. Their size relationship is B < A < D (37% < 34% < 29%) based on the chromosome measurements (Gill et al. 1991), and the distribution of breakpoints correlated with the relative size of the genomes; 184 (42%), 140 (32%), and 112 (26%) for B, A, and D genomes, respectively (Table 1).

However, among homoeologous groups, among chromosomes, and among chromosome arms, the chromosome size and breakage frequency are not correlated positively. For example, the total size of group 3 chromosomes was the greatest, but they had the fewest deletions. The highest number of deletions was recovered for homoeologous group 5, which ranks third in size (Gill et al. 1991; Table 1). Chromosome 3B, the longest of the wheat chromosomes, had 22 deletions, the fewest among the B-genome chromosomes. The 7AL arm had almost twice as many deletions as the 7AS arm, in spite of their equal length and marked terminal C-bands.

Furthermore, two types of hot-spot sites of breakage were recognized: localized sites with relatively inert adjacent regions and diffused regions where relatively large areas are targets for breaks. Most of the hot spots seemed to occur at the junctures of heterochromatic and euchromatic regions. Localized hot spots were observed in the following chromosome regions (for nomenclature rules, see Gill et al. 1991; see also Figure 4): 4AL2.1 (at the boundary of band 1.4 and 2.1), 4AL2.6 (adjacent to 2.5), 5AS1.4 (adjacent to 1.5), NOR regions of 1B (S2.4) and 6B (S2.7), 1BS3.2, 3BS2.1, 5BS2.1, 5BL1.4 (just proximal to band 2.1), 7AS1.4, and 5DL1.6. The diffuse hot spots were observed in 7AL1.6,

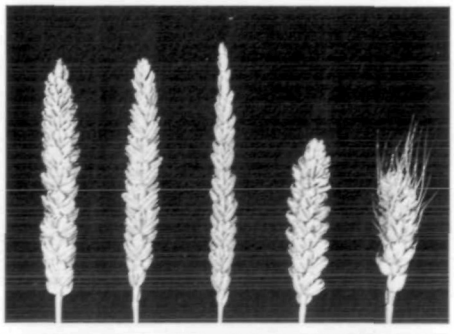

Figure 6. Spikes of euploid Chinese Spring wheat (left) and of deletion homozygotes for 5AL-23, 5AL-7, 6BL-14, and 6BL-15 (from left to right).

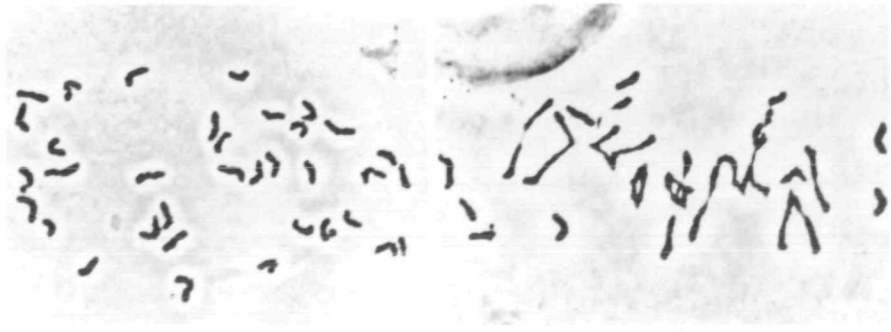

Figure 7. Meiotic chromosome configurations at metaphase I of hybrids between Chinese Spring and *A. variabilis*, including normal 5B (left) and deletion 5BL-1, respectively.

1DL1.2, and the central regions of 3DS and 4DL. Thus, the breakpoints of the deletions do not appear to be distributed randomly in wheat chromosomes and may be restricted to specific chromosome structures or DNA sequences. Also, some localized hot spots of chromosome breakage appear to be the sites of high recombination rates and gene densities.

Use of Deletion Stocks in Physical Genome Mapping

The deletion stocks have been used extensively in mapping genes controlling phenotypic traits (Endo et al. 1991; Mukai and Endo 1992) and in mapping in situ hybridization sites (Mukai et al. 1990, 1991; Werner et al. 1992b), biochemical markers (Yamamori et al. 1994), and DNA fragments (Delaney et al. 1995a,b; Gill et al. 1993a,b, in press; Hohmann et al. 1994; Kota et al. 1993; Mickelson-Young et al. 1995; Werner et al. 1992a) to subarm regions of individual chromosomes. These data have provided a wealth of information on the precise physical mapping of genes controlling phenotypic traits and many aspects of wheat chromosome structure and function, especially the distribution of genes and recombination along the length of chromosomes.

The deletion stocks should be useful for mapping genes controlling genes that are dominant in Chinese Spring and recessive in other cultivars. One has only to investigate the deletion homozygotes or the F_1 progeny of mutant gene stocks and appropriate deletion lines.

The deletion stocks are ideally suited for mapping codominant biochemical and DNA markers. Any missing marker can be allocated directly to the missing chromosome fragment. This obviates the need for marker polymorphism, which is low in wheat. Using genetically mapped DNA markers and the deletion lines, we constructed cytologically based physical maps of molecular markers of the 21 chromosomes of wheat (see the above references). These maps show that recombination is suppressed in the centromeric regions and is restricted to the distal regions of wheat chromosomes. Also, they show that most of the DNA markers are located in similar positions on all three homoeologous chromosomes. This fact enables the construction of a consensus map for each homoeologous group of wheat, a hypothetical map comprising all the DNA markers of the three homoeologues. We can calculate the average FL distance between the breakpoints in each arm of these consensus maps by dividing by the total number of the deletions of the three homoeologous arms (Table 1). For instance, the deletion breakpoints cover a hypothetical group 1 chromosome at an average FL distance of 3.1 in each of the arms. Since the DNA content of the haploid wheat genome (Bennett and Smith 1976) and relative size of each chromosome (Gill et al. 1991) are known, we can determine the DNA content per unit FL value. Furthermore, by aligning a consensus physical map with a corresponding genetic map, we can convert genetic distances into physical distances. Knowing the DNA content of a specific FL interval, its genetic length, and the number of markers in that interval allows us to calculate bp: cM ratio between any two markers in that interval. If this value is within the range of the resolution of pulsed-field gel electrophoresis, the next phase of long-range mapping becomes feasible. Eventually, such a strategy may lead to molecular cloning of a target gene in a specific FL interval region, like the *Ph* gene on 5BL (see "Results"). Thus, deletion stocks have proved to be important materials for identifying functionally important chromosome regions and have many possible uses in molecular manipulations.

Induction of Deletions in Other Genera

Endo et al. (1994) demonstrated that the *A. cylindrica* chromosome can induce breaks in a rye chromosome 1R as in wheat chromosomes. They produced a wheat line that had a disomic addition of 1R and a monosomic addition of the *A. triuncialis* chromosome (22″ +1′) and then isolated 1R deletions and translocations with wheat chromosomes from the progeny of this line. Breaks also are induced in other rye chromosomes (Friebe B, personal communication) and in barley chromosomes (Endo, unpublished data). Thus, it is possible to produce deletion stocks of rye and barley chromosomes. Because wheat-alien translocation also occurs, the system is also useful for introducing alien chromosome segments into wheat chromosomes.

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Received May 17, 1995
Accepted December 31, 1995

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