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Accumulation of Multiple Copies of Maize Minichromosomes

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B chromosome · Engineered minichromosome · Maize

Abstract
Multiple copies of B chromosomes in maize *(Zea mays)* can accumulate in the genome using the B chromosome's accumulation mechanism, specifically nondisjunction at the second pollen mitosis and preferential fertilization of the egg. Using this mechanism, we accumulated 4 different-sized minichromosomes derived from the B chromosome to test the chromosome limits of the cell. The accumulation of normal B chromosomes is associated with multiple phenotypes including white stripes and asymmetric leaf blades, but when minichromosomes are accumulated these symptoms are absent. We also found that multiple B chromosome-derived minichromosomes can coexist with A chromosome-derived minichromosomes. During the years that these experiments were conducted, we found many B chromosome rearrangements and fragments, 2 recoverable A chromosome fragments, and observed a minichromosome breakage-fusion-bridge cycle in roots.

B chromosomes (B's) are supernumerary chromosomes found in many species and proliferate using species-specific methods of meiotic or mitotic drive. The maize B chromosome accumulates by surviving as a univalent in meiosis, nondisjoining at the second pollen mitosis, and by the B-containing sperm preferentially fertilizing the egg [Longley, 1927; Roman, 1948; Carlson, 1969; Carlson and Roseman, 1992]. Although no identified genes have been localized to the maize B chromosome, it can increase recombination in the normal karyotype (A chromosomes) and, in certain cultivars, can trigger breaks in knobbed chromosomes at the second pollen mitosis [Randolph, 1941; Rhoades et al., 1967; Hanson, 1969; Nel, 1973].

The survival mechanism of the B chromosome can be manipulated to accumulate multiple B's in the genome. Although vigor and fertility are affected when numbers are significantly increased, by selecting for plants with the highest number of B's in successive generations, plants with 34 B chromosomes have been recovered [Randolph, 1941]. The first phenotype of maize plants with multiple B chromosomes is a longitudinal white striping on leaves, which appears sporadically when 5 copies are present, and is increasingly frequent as B chromosomes are accumulated [Staub, 1987]. Obtaining plants with more than 22 B's was increasingly difficult, as reductions in vigor and fertility hindered further accumulation [Randolph, 1941].

Successive studies have been undertaken to dissect properties of the maize B chromosome using breakage derivatives and translocations of the B. There are several

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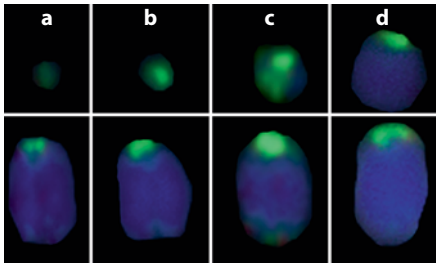

Fig. 1. The different relative sizes of B-derived minichromosomes (top) in comparison to the B chromosome (bottom). Each cell has differential levels of condensation and thus requires a standard B chromosome for a comparison between minichromosomes. **a** Minichromosome 9 is the smallest chromosome derived from the BFB cycle. **b** Minichromosome 20 is the second smallest chromosome derived from the BFB cycle. **c** Minichromosome 86-74 is the second largest minichromosome derived from telomere truncation. **d** Minichromosome 76-15a is the largest minichromosome derived from telomere truncation. Green signals: B repeat.

essential regions of the B chromosome necessary for nondisjunction at the second pollen mitosis. The distal euchromatin and a segment in the proximal euchromatin of the B are *trans*-acting and must be present in the cell for the 2 *cis*-acting sites in the proximal euchromatin and proximal heterochromatin to initiate nondisjunction [Roman, 1949; Rhoades et al., 1967; Rhoades and Dempsey, 1972; Carlson, 1973; Ward, 1973; Lin, 1978; Carlson and Chou, 1981]. The short arm of the B chromosome can also act in *trans* to increase the frequency of B chromosome nondisjunction, but is not essential [Carlson, 1978; Lin, 1979].

In this study we used 4 different-sized minichromosomes to test the accumulation limits of B chromosome derivatives. The first 2 minichromosomes, 86-74 and 76-15a, are derived from the bombardment of telomere repeats into maize embryos, resulting in truncations of the B chromosome [Yu et al., 2007]. Minichromosome 76-15a retains approximately half of the distal heterochromatin of the B, resulting in a minichromosome half the size of a normal B chromosome (fig. 1d). Minichromosome 86-74 is a truncation of the B that removed all of the distal heterochromatin, but appears to have all of the proximal euchromatin intact (fig. 1c) [Yu et al., 2007]. The smallest minichromosomes, mini 9 and 20 (fig. 1a, b), are derived from a breakage-fusion-bridge (BFB) cycle. Minichromosome 20 is missing all chromatin distal to the proximal knob of the B, and minichromosome 9 appears to be missing this knob that is a prerequisite for nondisjunction at the second pollen mitosis. Both minichromosomes were produced by the same method, which started with an inter-chromatid exchange on TB-9SbDp9, a B centromere translocated to an inverted duplication of chromosome arm 9S. Crossovers in this orientation result in the formation of a dicentric chromosome transmitted to the next generation. A series of breaks and fusions removed the intervening chromatin, and a small chromosome, equivalent in size to a B chromosome centromere, eventually stabilized. The *trans*-acting factor of a normal B chromosome in the genome enabled the accumulation of these minichromosomes using the mitotic drive of the B chromosome [Han et al., 2007].

Materials and Methods

Plant Materials

Minichromosome 9 and minichromosome 20 were both derived from the BFB cycle in a mixed background [Kato et al., 2005; Han et al., 2007]. Minichromosomes 86-74 and 76-15a were obtained by bombarding telomere-containing constructs into HI-II maize embryos containing B chromosomes [Yu et al., 2007]. The breeding program for accumulation kept each minichromosome line isolated with constant self and sib pollinations. To obtain higher frequencies of minichromosomes in the next generation, a male was selected that had the highest number of minichromosomes, while keeping at least 1 B chromosome in the background to supply the *trans*-acting factors for nondisjunction.

Mitotic in situ Hybridization

Root tip chromosome screens were performed as previously described in detail [Masonbrink and Birchler, 2010] with a slight modification. Distilled water was substituted for all ethanol washes, which kept cell wall enzymatic digestion times predictable. The fluorescently labeled probes consisted of a microsatellite TAG repeat, the nucleolar organizer repeat (45S rDNA), a telomere repeat oligonucleotide that strongly cross-hybridizes to the B repeat, and a CentC oligonucleotide that hybridizes to the centromere of all chromosomes as well as the B chromosome long arm [Alfenito and Birchler, 1993; Lamb et al., 2005].

Probe Development

Probes were designed as previously described [Danilova and Birchler, 2008]. Online supplement table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000339615) lists the details to single gene probe development.

PCR Conditions

BAC DNA was used as a template for PCR reactions (online suppl. table 1). The reactions included JumpStart REDTaq ReadyMix (Sigma, cat. P0982), 5 μM of each primer and 0.5–4 ng/μl of template DNA. PCR cycles consisted of an initial denaturation at 95°C for 5 min; 35 cycles with 95°C for 30 s, annealing temperature 57°C for 30 s, 72°C extension time, 1 min for each 1,000 bp; and a final extension of 3× extension time.

Results

The B chromosome was accumulated to 21 copies in a single plant of the B73 inbred line which was sterile (fig. 2). The 2 largest minichromosomes, 76-15a and 86-74, were accumulated to 19 copies, while mini 9 and 20 were accumulated to 9 and 17 copies, respectively; all cells also contain 1–5 B chromosomes (fig. 3). All plants with the highest number of minichromosomes failed to produce seeds, although these plants did produce silks and some pollen. From generation to generation the increase in the number of minichromosomes was progressively less, while plants with lower numbers were frequently observed (table 1). In nearly every generation a new maximum number of minichromosomes was found, while a generational increase in the average number of minichromosomes per plant was much less pronounced. The nondisjunction of minichromosomes between cells of the same root tip may have contributed to the maximal chromosome counts. This was apparent when the maximal number in a cell was not the most frequently found number of minichromosomes in the root squash. In addition, B repeat chromatin was commonly diffuse and outstretched between multiple mini and B chromosomes that frequently clumped together in metaphase spreads (fig. 4).

Plants with at least 4 B chromosomes displayed white longitudinal leaf stripes, while plants that had more than 10 copies had additional severe phenotypes, including reduced fertility, reduced vigor, a zigzag pattern of internode growth, white stripes on the leaves, and asymmetric leaf blades (online suppl. figs. 1, 2). Other than reduced fertility, plants with minichromosomes did not show B chromosome accumulation-related phenotypes, even at high copy numbers.

An A-Derived Minichromosome in Combination with B-Derived Minis

The origin of a chromosome (86B-136) carrying a nucleolar organizer region (NOR) derived from telomere truncation experiments was clarified by combining 3 gene-specific FISH probes to the proximal end of the long arm of chromosome 6 as well as to the NOR (45S rDNA) (fig. 5a, online suppl. table 1) [Yu et al., 2007]. This truncation was thought to be a fission of the centromere, but as the gene-specific probes indicate, the proximal end of 6L is still present on the 86B-136 minichromosome (fig. 5a). During its characterization, another stably inherited fragment was found (fig. 5a). We were able to accumulate 2 copies of this fragment (fig. 5b), but could not clarify its origin. B chromosomes were added to the genome with these minichromosomes, but neither A-derived minichromosome could increase beyond 2 copies (fig. 5b, c). An 86B-136 containing plant was crossed to the 86-74 minichromosome line and multiple numbers of the 86-74 mini as well as two 86B-136 minichromosomes were found in the next generation (fig. 5c).

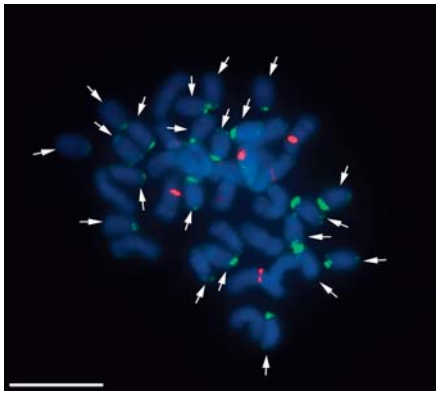

Fig. 2. A maize root cell with 21 B chromosomes accumulated in the B73 inbred line. Each arrow denotes a B chromosome. Typically the average number of B chromosomes per cell is slightly lower among cells of the same root due to somatic nondisjunction of the B chromosome in roots [Masonbrink and Birchler, 2010]. Differential signal strength on the B chromosomes is a result of background signal reduction in Adobe Photoshop 5.0. Telomere probes, which cross-hybridize to the B repeat, are green, CentC probes are red. The scale bar is 10 μm.

Other Abnormalities Observed

Aneuploidy of the normal karyotype was seen occasionally, although most screens did not involve a count of the A chromosomes. The progeny of one 76-15a minichromosome plant had high levels of aneuploidy (53% of seeds) to varying degrees (21–25 A's). In addition, 2 B/A translocations were found. The first B/A translocation is a complex rearrangement between one chromosome 4 homologue, an unidentifiable A chromosome, and 2 minichromosomes (fig. 6a). The second translocation involved chromosome 4, identified by the telomere probe cross-hybridization to Cent4 (fig. 6b). Two A chromosome fragments of unknown origin were observed, one with a CentC signal (fig. 6d) and one without (fig. 6c). The fragment without a centromere signal differed from the commonly distended NOR by having 2 sets of telomere signals. B chromosome-derived abnormalities were more commonly found due to chromosome breaks in the 86-74 mini, the 76-15a mini, or in normal B chromosomes. One

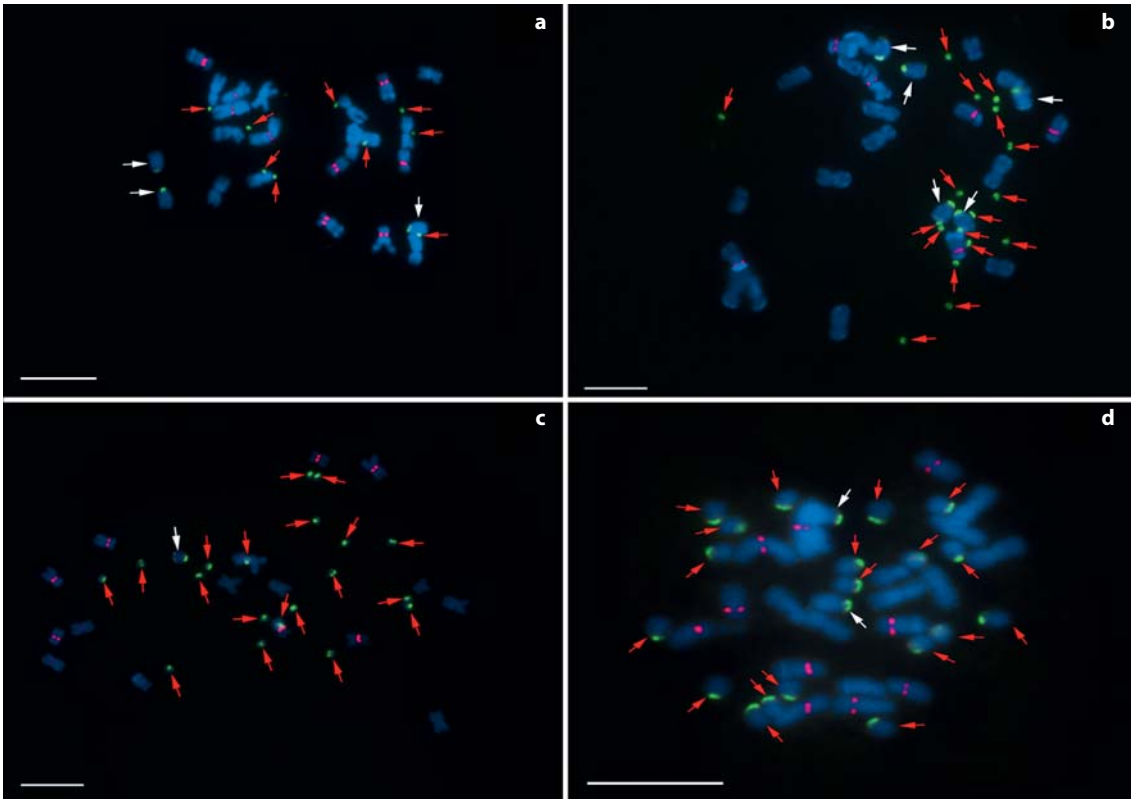

Fig. 3. Cells from each minichromosome line showing the maximum number of minichromosomes observed throughout the study. Red arrows denote minichromosomes, and white arrows denote normal B's. **a** A cell showing the maximum number of mini 9's at 9 copies and 3 B chromosomes. **b** A cell showing the maximum number of mini 20's at 17 copies and 5 B chromosomes. **c** A cell showing the maximum number of the 86-74 minichromosome at 19 copies and 1 B chromosome. **d** A cell showing the maximum number of the 76-15a minichromosome at 19 copies and 2 B chromosomes. Typically the average number of minichromosomes and B chromosomes per cell is slightly lower among cells of the same root due to somatic nondisjunction of B chromosomes and minichromosomes in roots. Telomere probes, which cross-hybridize to the B repeat, are green, CentC probes are red. The scale bar is 10 μm.

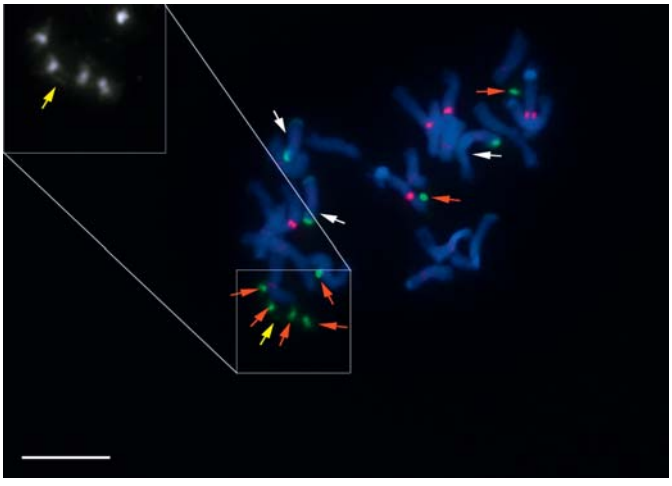

Fig. 4. A cell from a maize plant with 7 mini 20's and 3 B's showing diffuse B repeat chromatin. White arrows denote B chromosomes, red arrows denote minichromosomes, and the yellow arrow denotes diffuse B repeat chromatin. **Inset** shows diffuse B repeat chromatin in the green channel. Telomere probes, which cross-hybridize to the B repeat, are green, CentC probes are red. The scale bar is 10 μm.

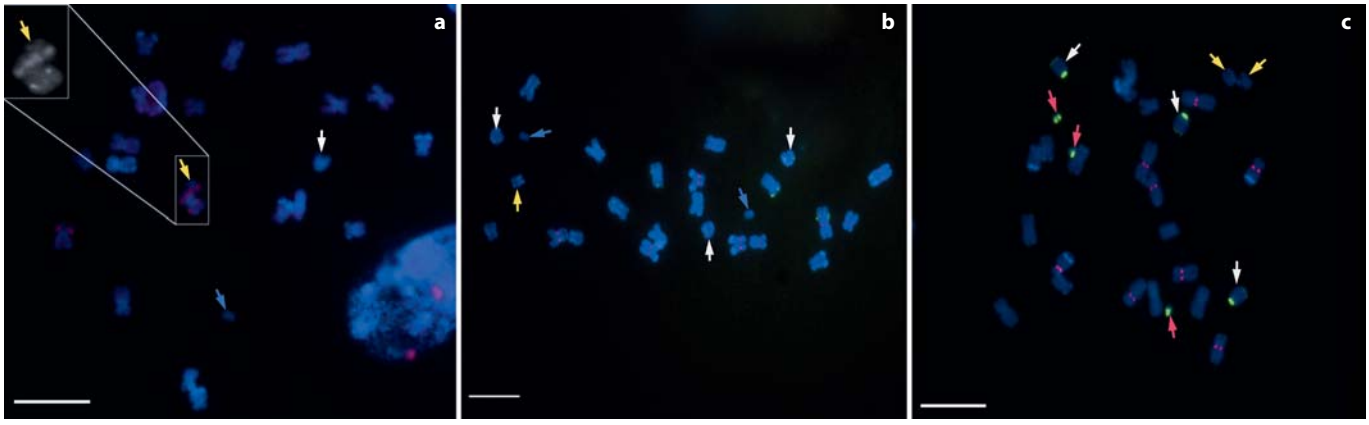

Fig. 5. Cells from 3 maize plants characterizing minichromosome 86B-136 and another heritable fragment of unknown origin. White arrows denote B chromosomes, blue arrows denote the fragment of unknown origin. Yellow arrows denote the 86B-136 minichromosomes and red arrows the 86-74 minichromosomes. **a** A cell with a 86B-136 minichromosome, a B chromosome, and a minichromosome of unknown origin probed with 3 gene-specific red probes on chromosome arm 6L, and a red NOR probe. **Inset** shows red channel only with normal chromosome 6 (lower) adjacent to the 86B-136 minichromosome (upper). **b** A cell with a 86B-136 minichromosome, 2 minichromosomes of unknown origin, and 3 B chromosomes. CentC probes are red, TAG probes are green. **c** A cell with two 86B-136 minichromosomes, three 86-74 minichromosomes, and 3 B chromosomes showing 2 types of minichromosomes with different inheritance mechanisms. Telomere probes, which cross-hybridize to the B repeat, are green, CentC probes are red. All scale bars are 10 μm.

Table 1. Accumulation of each minichromosome type in the last 4–6 generations

Type	Gen	Number of minichromosomes in a plant																				n	Avg
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
Mini	1	3	3	9	9	**14**	16	7	2	2	*1*	0	0	0	0	0	0	0	0	0	0	66	4.1
76-15a	2	0	0	1	2	4	2	11	**10**	6	5	*2*	0	0	0	0	0	0	0	0	0	43	6.6
	3	0	0	0	1	1	0	4	4	**7**	7	1	1	1	2	0	*1*	0	0	0	0	30	8.4
	4	0	0	1	1	3	4	4	**1**	4	2	2	1	0	0	0	0	0	0	0	0	23	6.5
	5	0	0	0	3	3	1	13	7	**13**	3	4	2	3	2	0	1	2	0	0	*1*	58	8.2
	6	0	0	0	2	3	4	3	3	**7**	6	1	2	*1*	0	0	0	0	0	0	0	32	7.2
Mini	1	0	10	10	**19**	16	5	2	*1*	0	0	0	0	0	0	0	0	0	0	0	0	63	3.1
86-74	2	1	1	4	4	9	9	**10**	13	6	4	2	2	*1*	0	0	0	0	0	0	0	66	5.9
	3	0	4	10	14	13	**25**	16	13	7	1	2	3	0	*1*	0	0	0	0	0	0	109	5.1
	4	0	0	2	1	2	6	8	9	5	**14**	9	6	5	7	4	5	3	3	0	*2*	91	9.9
Mini 20	1	3	8	4	11	**9**	6	4	2	*8*	0	0	0	0	0	0	0	0	0	0	0	55	4.0
	2	3	10	18	14	**19**	12	10	8	2	0	2	*1*	0	0	0	0	0	0	0	0	99	3.9
	3	3	2	4	0	3	4	**3**	0	2	3	1	1	1	0	0	0	0	*1*	0	0	28	5.6
	4	0	2	1	2	4	7	3	3	**2**	2	2	1	5	4	1	0	1	*1*	0	0	41	7.9
	5	2	2	4	5	5	4	10	**6**	13	6	7	8	4	1	1	0	0	*1*	0	0	79	7.3
Mini 9	1	17	33	**25**	6	5	2	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	89	1.7
	2	9	12	13	**13**	11	4	3	1	0	*1*	0	0	0	0	0	0	0	0	0	0	67	2.7
	3	7	17	18	**16**	5	3	3	2	2	*2*	0	0	0	0	0	0	0	0	0	0	75	2.8
	4	19	13	24	**12**	10	9	5	3	*1*	0	0	0	0	0	0	0	0	0	0	0	96	2.7
	5	13	22	**18**	9	3	6	2	3	*1*	0	0	0	0	0	0	0	0	0	0	0	77	2.4

Avg = Average; Gen = generation; n = number of plants screened in each generation.

Bold numbers indicate frequency of plants with the average number of B's per plant in each generation. Italic numbers indicate highest minichromosome number found in each generation.

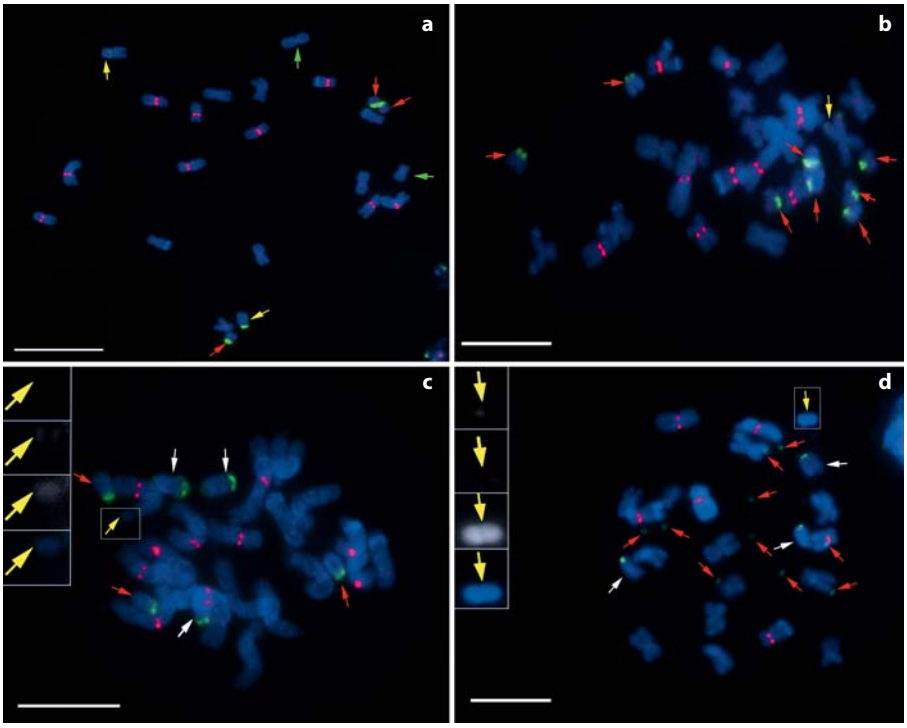

Fig. 6. Chromosomal aberrations encountered during the accumulation of minichromosomes, including B/A translocations and A chromosome fragments. White arrows denote normal B chromosomes, red arrows denote minichromosomes, and yellow arrows denote chromosomes of interest. **a** A reciprocal translocation of an unidentified A chromosome (yellow arrows), a B chromosome, and chromosome 4. A B chromosome centromere-sized B repeat signal is internal on the A/B chromosome, and 1 chromosome 4 homologue is significantly reduced in size (green arrows), indicating a complex rearrangement. **b** A B/A translocation (yellow arrow) of chromosome 4 without the reciprocal exchange present. **c** An A chromosome fragment that is missing the CentC centromere repeat and does not appear to have a constriction (yellow arrow). The 2 sets of telomere signals distinguish it from the NOR, which commonly appears distended. **d** An A chromosome fragment (yellow arrow) with 2 sets of telomere signals and a CentC signal in the primary constriction. Telomere probes, which cross-hybridize to the B repeat, are green and CentC probes are red. All scale bars are 10 μm.

frequently observed derivative in the 86-74 and 76-15a minichromosome lines was a small chromosome consisting of only the B centromere, which was still capable of nondisjunction (fig. 7a). Such a derivative was indistinguishable from mini 9 and 20 and therefore could not be scored if they formed in these 2 lineages. Another derivative appeared to be missing distal portions of the B chromosome long arm (fig. 7b). In addition, 2 derivatives from the B and 86-74 mini were found missing most of the B repeat at the centromere (fig. 7c, d).

Multiply rearranged minichromosomes were found with additional B repeat signals on both ends of the chromosome (fig. 8). Although multiple B repeat signals could indicate the presence of a dicentric chromosome, these rearranged minichromosomes were stable, possibly the result of centromere inactivation. Another indication of their stability was the constriction that was only seen at one of these B repeat signals. Even so, one of these derivatives was increased to 2 copies in a subsequent generation and remained stable (fig. 8c).

During the accumulation of the 76-15a minichromosomes, a plant was found that had chromosomes with B repeat at the distal ends of both arms, indicative of fusion between 2 broken chromosomes (fig. 9). The chromatin between these distal B repeat signals constantly increased and decreased in size, and frequently chromosomes undergoing BFB would appear at multiple different sizes with 1 B repeat signal. The frequent structural change in these chromosomes indicates the presence of 2 functional centromeres actively breaking and fusing among sister cells of the same root, suggesting the presence of a BFB cycle.

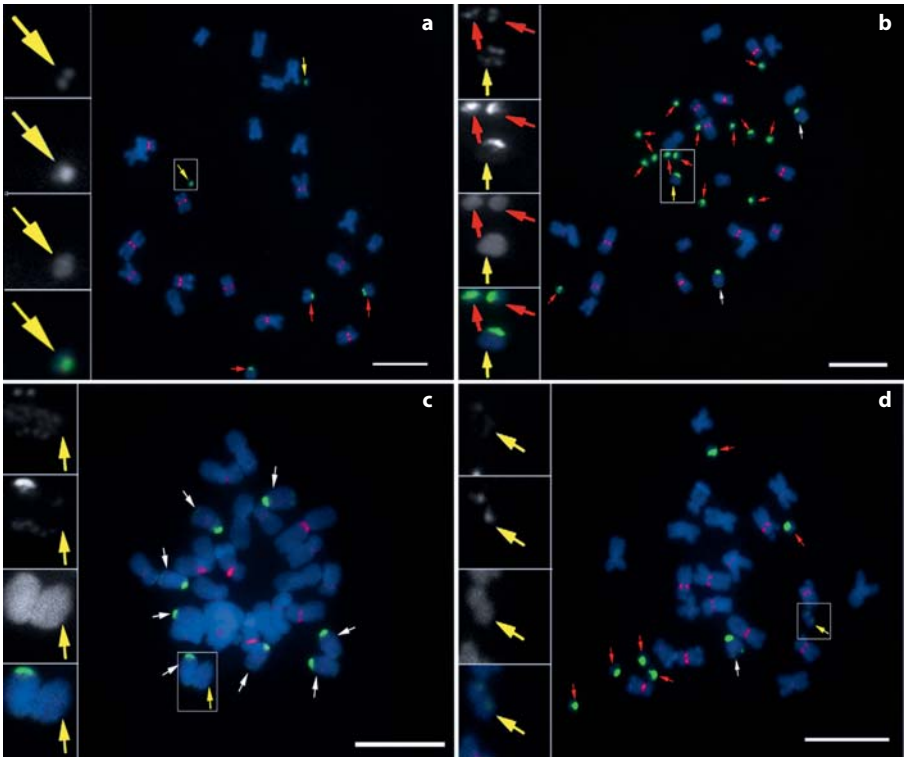

Fig. 7. Breakage derivatives of the B chromosome and/or minichromosomes. White arrows denote B chromosomes, red arrows denote minichromosomes, and yellow arrows denote the broken derivatives. **Insets**, in descending order, show the red (CentC), the green (B repeat), the blue (DAPI), and all channels. **a** A cell from a maize plant with three 86-74 minichromosomes and 2 centromere-only derivatives derived from a B chromosome or a minichromosome. **b** A cell from a maize plant with 1 B chromosome and fifteen 86-74 minichromosomes including a broken B derivative that is missing half of the long arm. **c** A cell from a maize plant with 9 B chromosomes including a B chromosome with reduced B repeat. **d** A cell from a plant with 1 B chromosome and six 86-74 minichromosomes and the 86-74 minichromosome with reduced B repeat signal at the centromere. CentC probes are red and telomere probes, which cross-hybridize to the B repeat, are green. All scale bars are 10 μm.

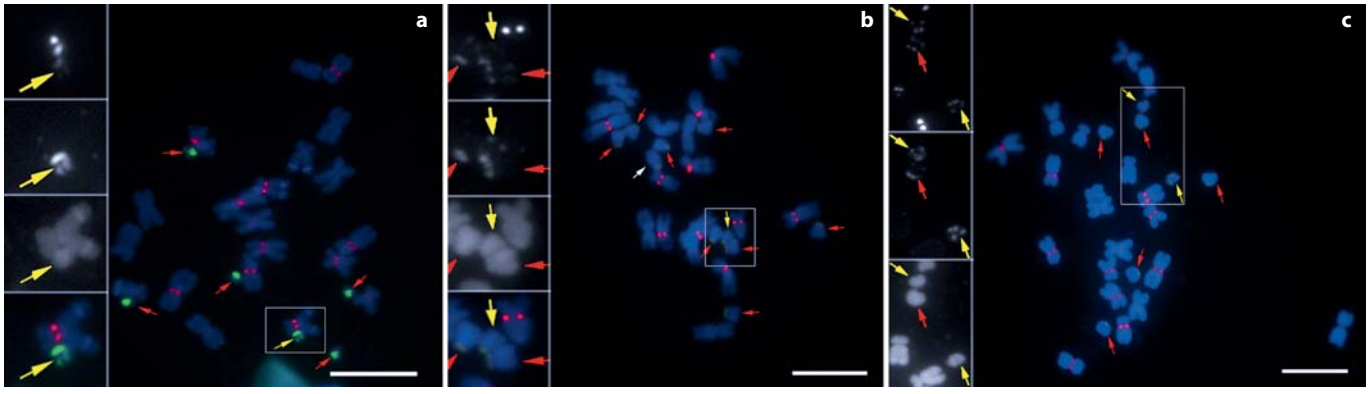

Fig. 8. Three B chromosome derivatives with similar rearrangements of B repeat on both arms of a chromosome. White arrows denote B chromosomes, red arrows denote minichromosomes, and yellow arrows denote the rearranged minichromosomes. **Insets**, in descending order, show the red (CentC), the green (B repeat), the blue (DAPI), and all channels. **a** A cell from a plant with five 86-74 minichromosomes and 1 rearranged minichromosome with unequal distal B repeat signals on both arms. **b** A cell from a plant with eight 76-15a minichromosomes, 1 rearranged minichromosome with B repeat in distal positions on both arms, and 1 B chromosome. **c** A cell from a plant with five 76–15a minichromosomes and 2 rearranged minichromosomes with B repeat signals in distal positions on both arms. CentC probes are red, telomere probes are green. Scale bars are 10 μm.

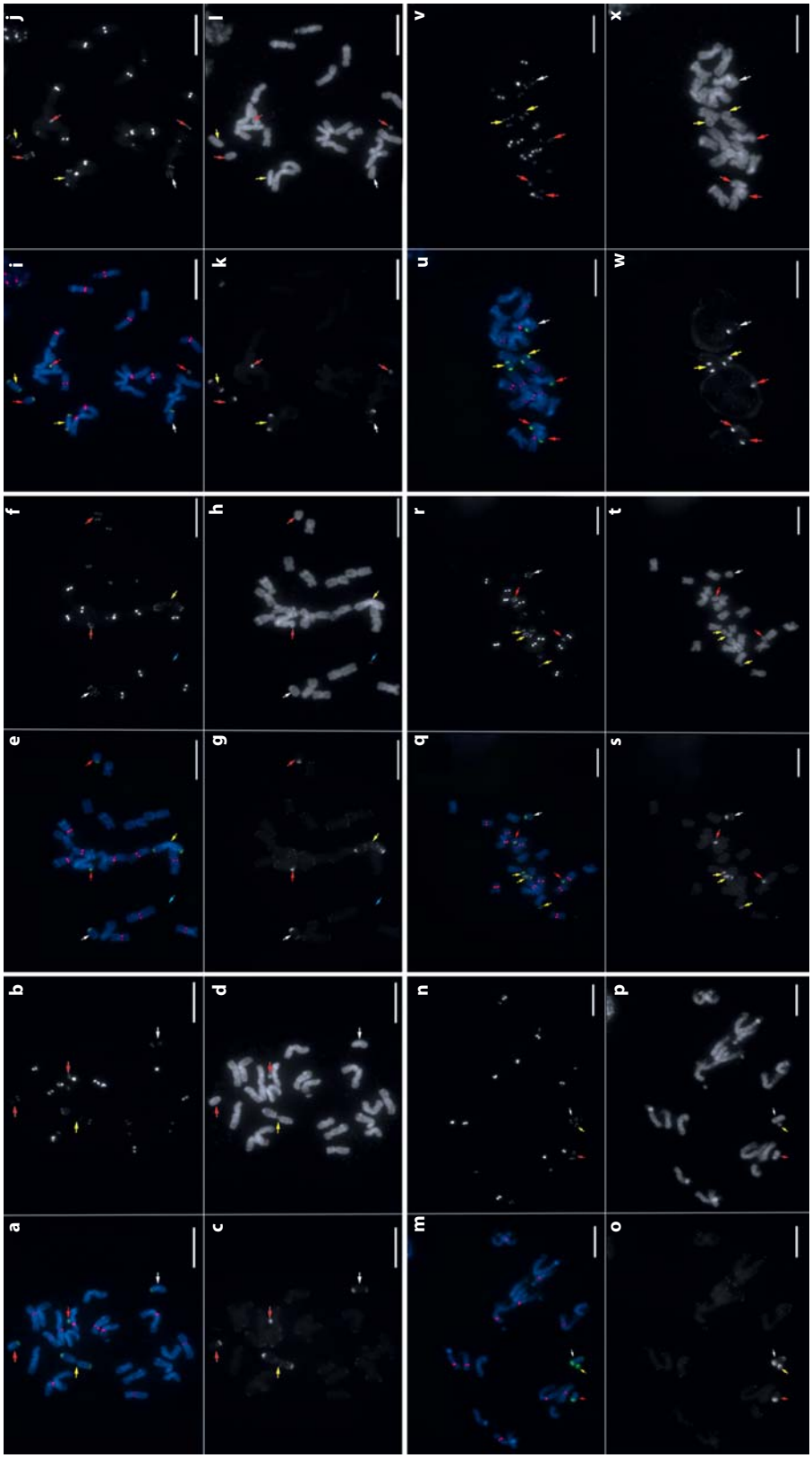

Fig. 9. Six cells from the same root tip showing 1 B chromosome and one 76-15a minichromosome that remains stable in all cells, while various numbers of rearranged minichromosomes participate in a BFB cycle. Telomere probes are green, CentC probes are red, and DAPI is blue. White arrows denote B chromosomes, red arrows denote minichromosomes that appear monocentric, blue arrows denote chromosome fragments, and yellow arrows denote possible dicentric chromosomes. **a–d** A cell with a large dicentric chromosome, 2 monocentric minichromosomes, and 1 B chromosome. **a** Merged image. **b** Red channel (CentC), showing an internal section of the dicentric missing CentC. **c** Green channel (B repeat). **d** Blue channel (DAPI), showing a metacentric constriction on the large dicentric. **e–h** A cell with 1 large dicentric chromosome, 2 monocentric minichromosomes, 1 B chromosome, and 1 chromosome fragment. **e** Merged image. **f** Red channel, showing the similar structure to the dicentric in **a** and a lack of CentC signal on the chromosome fragment. **g** Green channel. **h** Blue channel, showing the chromosome fragment. **i–l** A cell with 2 medium-sized dicentric chromosomes, 3 monocentric minichromosomes, and 1 B chromosome. This may be a descendent cell of the large dicentric, showing that broken ends may heal as shown by a similar pattern of CentC. **i** Merged image. **j** Red channel, showing both dicentrics have similar structure to half of the dicentric in **a** and **e**. **k** Green channel. **l** Blue channel. **m–p** A cell with 1 small dicentric, 1 monocentric minichromosome, and 1 B chromosome. **m** Merged image. **n** Red channel, showing 2 separate sets of CentC signals on the small dicentric chromosome. **o** Green channel, showing a separation of the signal in the dicentric chromosome. **p** Blue channel. **q–t** A cell with 1 very small dicentric chromosome, 2 small dicentrics, 2 monocentric minichromosomes, and 1 B chromosome. **q** Merged image. **r** Red channel, showing the distal centromere locations on the 3 dicentrics. **s** Green channel. **t** Blue channel. **u–x** A cell with 2 medium-sized dicentrics, 3 monocentric minichromosomes and 1 B chromosome. **u** Merged image. **v** Red channel, showing 1 dicentric with internal CentC and 1 without the internal signal, indicating a similar lineage to the dicentrics in **i**. **w** Green channel. **x** Blue channel. All scale bars are 10 μm.

Discussion

The highest reported number of B chromosomes ever accumulated in a maize plant was 34 [Randolph, 1941], and in our study the maximum number obtained was 21 (fig. 2). The discrepancy between the numbers could be from breeding plan differences. The previously reported number was obtained by maintaining hybrid vigor, while our maximum was achieved through consistent inbreeding.

The minichromosome maxima were obtained in the same manner as the B's accumulation, although the rate was somewhat slowed by the necessity of keeping 1–2 normal B's in the genome to provide the *trans*-acting nondisjunction factors. Accumulation of shortened B derivatives was also investigated in a previous study, although sterility was seen before more than 16 copies were found [Randolph, 1941]. The B chromosome is capable of somatic nondisjunction in roots, which also occurs for minichromosomes. Somatic nondisjunction played a role in achieving the maximum number, as it was the peak quantity seen in a range of cells. Because the minichromosomes could not be accumulated to higher numbers than B chromosomes, the limit is more likely based on B centromere quantities, rather than a chromatin mass limit.

Multiple phenotypes have been associated with increased numbers of B chromosomes in the genome, including reduced fertility, decreased vigor, defective seeds, scarred endosperm, pollen abortion, and white longitudinal stripes on leaves (online suppl. figs. 1 and 2) [Randolph, 1941; Staub, 1987]. The only phenotype connected to minichromosome accumulation was reduced fertility, which was seen previously with the accumulation of the shortened B derivatives [Randolph, 1941]. This suggests that all previously described B chromosome accumulation-associated symptoms may be caused by multiple copies of the long arm of the B chromosome.

The smallest B chromosome derivatives described by Randolph [1941], the F type, are similar to our centromere-only B derivatives. These derivatives with reduced centromeres are probably broken within the centromere, because most of the B repeat is missing. A less common break site may be present at the proximal end of the distal heterochromatin on the long arm of the B (fig. 7b). Randolph [1941] describes the frequencies of certain breakage sites along the B that are commonly recovered, suggesting hotspots for breakage along the B chromosome.

The various chromosomal rearrangements with B repeat at distal regions on both arms likely arise from breaks in the long arm of minichromosomes or B chromosomes followed by chromosome fusion. When the 2 centromeres go to opposite poles in a subsequent anaphase, the broken chromosomes would fuse in the telophase nucleus initiating a BFB cycle. This would account for the changes in size of the chromatin between the 2 centromeres. The chromosomes with B repeat at distal locations on both arms in figure 8 probably arose from a BFB cycle, but were stabilized with centromere inactivation, as previously described [Han et al., 2006]. Two of these stably rearranged chromosomes have differing amounts of B repeat (fig. 9a, b), and the constriction associated with centromere activity seems to follow the larger signal, which is in agreement with previous studies [Han et al., 2009].

The cross of the 86B-136 A chromosome truncation and 86-74 B truncation proves that 2 types of minichromosomes can coexist with one remaining at a stable number while another increases in quantity. Previous studies with telotrisomics have shown that significant reductions in vigor and fertility occur with increased doses of genes linked to the additional chromosome [Rhoades, 1940]. The same issues were apparent with the 6S minichromosome, so smaller A-derived minichromosomes would likely be better candidates.

The present study indicates that engineered minichromosomes derived from the B chromosome can be increased in copy number to amplify the output of the added genes. The amplification requires the presence of a full-sized B chromosome. The total number of extra chromosomes for the various cases was ~20. Based upon Randolph's [1941] result of achieving 34 B chromosomes in a hybrid, this might be an approach to surpass the limits we observed, but this would require first introgression into different inbred lines and then their subsequent amplification.

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