Maize Genetics Cooperation Newsletter vol 84 2010

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Metaphase I pairing of B-chromosomes of *Zea mays* L. in the alien genetic background of *Avena sativa* L.

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INTRODUCTION

Maize plants with native maize B-chromosomes (*Bs*) in their complements prevail with different frequencies in tested populations by balancing the impact of selfish drive (*i.e.* microgametophytic chromatid non-disjunction associated with preferential ovum fertilization) with that of counteracting factors (*i.e.* meiotic loss through lagging univalents with micronuclei formation). Driving as well as counteracting factors for *B* presence in maize plants were assigned to the *Bs* themselves and to the regular A-chromosomes (*As*) of the host genome with complex interactions among them (reviewed in: Jones *et al.* 2008, Cytogenet Genome Res 120, 265-280).

In order to study host species-specific influences on behavior and mode of inheritance of maize *Bs* in a very remotely alien genetic background, we transferred native *Bs* of maize (*Zea mays* L. ssp. *mays* cv. *Black Mexican Sweet*) into common oat (*Avena sativa* L. ssp. *sativa* cv. *Starter*) (Kynast *et al.* 2007, Maize Genet Coop Newsletter 81, 17-19) and proved their maternal and paternal transmission to successive offspring generations (Kynast *et al.* 2008, Maize Genet Coop Newsletter 82, 19-21).

Native maize *Bs* are among the first-discovered (Kuwada 1925, Bot Mag Tokyo 39, 227-234) and presumably molecularly and cytogenetically best-described *Bs* in the plant realm (Jones and Diez 2004, The B chromosome database, http://www.bchromosomes.org/bdb/). The extensive experimental data on behavior and mode of inheritance of maize *Bs* in their native host species can serve as excellent references when evaluated and compared with those data on behavior and mode of inheritance of maize *Bs* in an alien host species.

Native oat *Bs* have not been reported to exist in wild and cultivated species of hexaploid oats. These species apparently have not been exposed to native *Bs* during their evolution. Therefore, the oat genomes are not likely to have developed and/or to have been selected for genetic factors that control behavior and mode of inheritance of *Bs*.

Hybridization experiments involving different hexaploid oat lines (*2n = 6x = 42*) and the maize line $B73^{B}$ (*2n = 2x + 6 Bs = 20 + 6 =26*) produced two fertile inter-species F_1-plants (5811_1 and 5845_1) carrying maize *Bs* in shoot and root tissues. PCR assays of their genomic DNAs using two maize *B*-specific markers and a selected set of maize *A*-specific SSR markers proved presence of maize *Bs* and absence of maize *As* in the leaf tissues of both F_1-plants. GISH assays using fluorophore-labeled genomic maize DNA as probe on root meristems proved that the F_1-plant 5811_1 retained three maize *Bs* along with the complete haploid set of 21 oat chromosomes ($2n_i - n_j$ *= 3x + 3 Bs = 21 + 3 = 24; Bs are named B.1*), and that one maize *B* was retained along with the complete haploid set of 21 oat chromosomes ($2n_i - n_j$ *= 3x + 1 B = 21 + 1 = 22; B is named B.2*) in the F_1-plant 5845_1. Controlled self-pollination of the F_1-plants 5811_1 and 5845_1 produced 59 and 73 seeds (F_2 1188_1 … 1188_59, and F_2 1190_1 … 1190_73), respectively. Cytological and molecular analyses of 36 F_2-offspring of the F_1-plant 5811_1 revealed 27 F_2-plants with different numbers of *Bs* and nine F_2-plants without a *B* (Figure 1), whereas in 36 F_2-offspring of the F_1-plant 5845_1 no F_2-plant had a *B*, neither in its shoots nor in its roots based on PCR and GISH assays.

We addressed the question in our research objectives: **How do maize *Bs* pair in microsporocytes during meiosis I after being transferred from maize into oats, *i.e.* after being converted from native *Bs* to alien *Bs*?**

MATERIALS AND METHODS

Oat inbred lines *Starter-1*, *Sun II-1*, oat cultivar *Paul* and maize inbred line $B73^B$, were grown and crossed, hybrids *in vitro* cultivated and molecularly and cytologically tested, and consecutive offspring produced and analyzed as described elsewhere (Kynast *et al.* 2007, 2008). The oat inbred lines *Starter-1* and *Sun II-1* are single plant descendents from the oat cultivars *Starter* and *Sun II*, respectively; *Starter*, *Sun II* and *Paul* were from the oat stock collection at the University of Minnesota Saint Paul. The line $B73^B$ is a dent corn inbred *B73* derivative that carries six *Bs* of the sweet corn cultivar *Black Mexican Sweet* (*2n = 2x + 6 Bs = 20 + 6 = 26*); seed of $B73^B$ was generously provided by JA Birchler, University of Missouri Columbia.

We selected oat genotypes with different numbers of added *Bs* in their complements among 36 tested and cultivated F_2-offspring of the F_1-plant 5811_1 based on estimated chromosome numbers and histological stability/instability of their root meristems by using the Feulgen-staining technique and GISH assay of ice water-pretreated and fixed root tips. The following four genotypes with stable karyotypes without any indication of mosaic meristems in their roots (F_2-plants 1188_3, 1188_7, 1188_20 and 1188_8) were selected from the total of 27 maize *B*-positive F_2-plants (Figure 1) in order to develop an oat-maize addition (OMA) series of alien maize *B* addition lines of hexaploid oats (*OMAmB.1*, *OMAdB.1*, *OMAtB.1,* and *OMAqB.1* for monosomic, disomic, trisomic, and tetrasomic addition, respectively) for seed production (Table 1) and for the analyses of metaphase I pairing performance of the alien maize *Bs* (Table 2).

We germinated eight F_3-seeds from each of the four offspring populations (F_3 1374_10 … 1374_18, F_3 1378_1 … 1378_8, F_3 1391_1 … 1391_8, and F_3 1379_1 … 1379_8) of these four different F_2-plants and verified chromosome numbers and histological stability/instability of their root meristems as described for the 36 analyzed F_2-plants. F_3-seed production was by controlled self-fertilization of individual F_2-panicles (a-, b-, and c-panicles) from the physiologically first three F_2-tillers (a-, b-, and c-tillers, correspondingly) with flag leaves, of which their genomic DNAs tested *B*-positive in PCR assays using two maize *B*-specific markers. For each of the four lines, we selected one out of the eight F_3-plants with appropriate stable karyotypes for meiosis analyses. Because the panicles of the F_3-plants' a-tillers were reserved for controlled F_4-seed production, we used the b- and c-panicles for meiosis assays (Table 2).

In order to increase the conclusive force of our data generated from F_3-plants, we also observed *B* pairing performance in plants of the successive offspring generation with appropriate stable karyotypes. We analyzed meioses from b- and c-panicles of one out of six cultivated F_4-

plants for each line (F_4 000769_1 … 000769_6, F_4 000805_1 … 000805_6, F_4 000837_1 … 000837_6, and F_4 000853_1 … 000853_6) grown under environmental conditions equal to those used for F_3-plant cultivation (Table 2).

Anthers of an appropriate physiological age to have the majority of microsporocytes at metaphase I (tested using aceto-carmine quick squash technique) were fixed in a mixture of 1.5 volume parts of methanol, 1.5 volume parts of ethanol and 1 volume part of glacial acetic acid at room temperature (RT) for two days. For slide preparation, fixed anthers were rinsed in 45%$_{v/v}$ acetic acid at RT for about 15 min. The two thecae of an anther were split and separately processed further. Theca ends were cut open and microsporocytes gently stroked out into a droplet of 45%$_{v/v}$ acetic acid onto a glass slide. Pressing a cover slip firmly upon the cell area, freezing the slide in liquid N_2, and flicking off the cover slip accomplished squash preparation. Slides – dried in absolute ethanol at RT for about 30 min followed by air-drying at RT for about 1 h – were processed through a regular GISH procedure at a stringency of ≥85% using fluorophore-labeled genomic maize DNA as hybridization probe. Microsporocytes were observed and pairing scored by the use of an epifluorescence microscope equipped with a CCD camera for picture documentation.

RESULTS AND CONCLUSIONS

Table 3 summarizes the data of chromosome pairing performance for the different numbers of *Bs* observed in microsporocytes at metaphase I of the four different addition lines *OMAmB.1* (monosomic *B* addition), *OMAdB.1* (disomic *B* addition), *OMAtB.1* (trisomic *B* addition), and *OMAqB.1* (tetrasomic *B* addition). We screened 30 microsporocytes for each line from four panicles of the selected F_3- and F_4-plants of the addition line. In the monosomic *B* addition line *OMAmB.1*, 30 sporocytes had one *B* univalent, and no pairing between a *B* and any of the oat *As*. In the disomic *B* addition line *OMAdB.1*, 18 sporocytes had two *B* univalents, and 12 sporocytes had one *B* bivalent. Again, no sporocytes showed pairing between a *B* and any of the oat *As*. In the trisomic *B* addition line *OMAtB.1*, 19 sporocytes had three *B* univalents, and 11 sporocytes had one *B* univalent plus one *B* bivalent. None of the 30 sporocytes had a *B* trivalent, and none of the 30 sporocytes showed pairing between a *B* and any of the oat *As*. In the tetrasomic *B* addition line *OMAqB.1*, 12 sporocytes had four *B* univalents, and 13 sporocytes had two *B* univalents plus

one *B* bivalent, and 5 sporocytes had two *B* bivalents. None of the 30 sporocytes had a *B* quadrivalent, and none of the 30 sporocytes had a *B* trivalent plus a *B* univalent configuration. Again, the line did not show pairing between a *B* and any of the oat *As*. Taken all data together (Table 4), there are four main results and conclusions from our experiments:

First, there were no configurations of pairing between maize *Bs* and oat *As* in any sporocytes, even when the maize *B* was a monosomic addition only, and thus, had not a homolog to pair with as it was the case, for instance, in F_3-plant 1374_13 and F_4-plant 000769_2 (monosomic *B* addition line *OMAmB.1*). There was no sporocyte with chiasmatic conjugation of any maize *B* with an oat *A*. Moreover, the maize *B* univalents did not show any spatial proximity indicative of secondary end-to-end, end-to-side or side-by-side alignments of achiasmatic association with oat *As*. In contrast, maize *Bs* in any configuration appeared physically well separated from oat *As* even while being perfectly co-orientated with the complement of oat *A* bivalents across the equatorial plate (Figure 2).

Second, univalents of maize *Bs* frequently scattered across the spindle figure off the equator (Figure 2a). Nonetheless, every so often *B* univalents were remarkably well integrated into the heterotypic cell equator (Figure 2b). Although at an overall higher frequency (20/30 non-equatorial positions *versus* 10/30 equatorial positions), the variable positions were of no recognizable pattern for the distance to equator or pole, and therefore, more likely resulting from the impact of the methodology of squashing the 3D structure of the sporocyte to a flat plate by the slide preparation technique. True 3D analysis of the sporocytes by using, for instance, confocal microscopy will deliver data that are more reflective of the genuine position pattern of univalent *Bs*. Further research experiments are in progress to investigate the spatial organization of alien maize *B* univalents and bivalents in oat sporocytes. Yet, our observations point to an amazing stability of *B* univalents in the alien oat background probably contributing to high frequencies of maternal as well as paternal transmission to offspring (Kynast *et al.* 2008). The overall harmonic performance is leaving us with the impression that the alien maize *Bs* behave quite normally in the oat genome with a good fit into the oat timing for chromosome formation, pairing and orientation during prophase I and metaphase I.

Third, when a *B* had a homologous partner for potential pairing, bivalents were formed, notwithstanding univalent formation at a modest frequency, *e.g.* in the disomic *B* addition line *OMAdB.1* (F_3-plant 1378_4 and F_4-plant 000805_2). The significant number of sporocytes

showing two *B* univalents instead of one *B* bivalent ($\bar{x}$ *per* sporocyte: 1.2^{I} *versus* 0.4^{II}) distinguishes pairing performance of alien maize *Bs* from that of native maize *Bs* (*i.e.* maize *Bs* in maize) and of native *Bs* in other plant species (*e.g.* rye) too (Figures 2c and 2d). Also, the alien maize *B* pairing differs from that of alien regular *As*. In general, homologous *As* in disomic condition hosted by an alien genome almost always pair and, hence, form bivalents instead of two univalents setting aside major structural rearrangements between homologs by chromosome mutations and plant mutants with alleles for asynaptic behavior of individual chromosomes and whole genomes. However, we cannot exclude in our conclusions drawn from metaphase I pairing data alone that the enlarged number of sporocytes with *B* univalents could result from (during zygotene) perfectly synapsed, but (during diplotene) prematurely desynapsed chromosome pairs due to (during pachytene) achiasmatic conjugation caused by, for instance, failure or extreme reduction of crossing over. Further molecular cytogenetic analyses of *Bs* in sporocytes at zygotene, pachytene, and diplotene are in progress and will address the question on whether the increased *B* univalent frequency in disomic *B* additions does result from asynapsis and/or premature desynapsis due to achiasmatic chromosome conjugation.

Fourth, in all observed sporocytes, the trisomic addition of homologous maize *Bs* in the line *OMAtB.1* (F_3-plant 1391_8 and F_4-plant 000837_4) did not result in the formation of *B* trivalents (Figures 2e and 2f). Likewise, the sporocytes with tetrasomic addition of homologous maize *Bs* in the line *OMAqB.1* (F_3-plant 1379_4 and F_4-plant 000853_3) did not form one *B* quadrivalent or one *B* trivalent plus one *B* univalent either (Figures 2g and 2h). All maize *Bs* appeared just to form univalents and bivalents only in oat background with a modest preference for univalents ($\bar{x}$ *per* sporocyte: 2.3^{I} *versus* 0.4^{II} in *OMAtB.1* and 2.5^{I} *versus* 0.8^{II} in *OMAqB.1*, respectively) though more than two homologous pairing partners were present in the sporocytes of both genotypes. This obvious restraint on trivalents and quadrivalents seemed intriguing particularly owing to all *Bs* in the plant materials tested were descended from the same origin of maize germ plasm *Black Mexican Sweet* and go back to one defined (*Starter-one* oat × $B73^{B}$ maize) hybridization event (F_1-plant 5811_1). Hence, all the maize *Bs* represent from a cytogenetic view multiple copies of the same chromosome. It seems feasible to exclude significant structural differences among the individual *Bs* as a cause for the multivalent suppression. In addition, *B* bivalent formation is not suppressed though modestly reduced. The restraint on multivalent formation causes an additional intriguing feature to the *B* behavior in metaphase I sporocytes.

When the disomic, trisomic and tetrasomic addition lines are compared, the frequency distributions of sporocytes with *B* univalents are more polynomial than normal (Figure 3). The average bivalent frequencies ($\bar{x}$ *per* sporocyte: 0.4^{II} in *OMAdB.1*, 0.4^{II} *OMAtB.1*, and 0.8^{II} in *OMAqB.1*) appear remarkably constant (Table 4).

In order to track down potential genetic factors for the multivalent suppression that interact between the maize *Bs* and the host species we are trying to transfer the *Bs* from *Starter-one* oat into further different genetic backgrounds. We have recently started a backcross program of the two lines *OMAtB.1* and *OMAqB.1* to the hexaploid oats *A. fatua* L., *A. sterilis* L., and *A. sativa* L. subspp. *byzantina* and *nuda*.

Table 1: Selected F_1-plant and a-panicles of F_2-plants for addition line development; OMA = oat-maize chromosome addition, m = monosomic, d = disomic, t = trisomic, q = tetrasomic, *B.1* = *B* that is descended from the first recovery in one individual (oat × maize) plant 5811_1

Table 2: Selected b- and c-panicles of F_3- and F_4-plants for meiosis assays

Table 3: Maize *B* pairing performance in metaphase I microsporocytes (n = 30 per plant line) of four oat-maize *B* addition lines, I = *B* univalents, II = *B* bivalents, III = *B* trivalents, IV = *B* quadrivalents

Table 4: Minimal, maximal and mean frequencies, ranges, standard deviations and coefficients of variation of maize *B* pairing configurations in metaphase I microsporocytes (n = 30 per plant line) of four oat-maize *B* addition (OMA) lines; m = monosomic, d = disomic, t = trisomic, q = tetrasomic, *B.1* = *B* of first recovery, I = *B* univalents, II = *B* bivalents, III = *B* trivalents, IV = *B* quadrivalents

Figure 1: Numbers of 36 tested F_2-offspring without and with maize *B* additions reflecting the transmission frequencies of added maize *Bs* from F_1 to F_2 through doubled haploid formation by unreduced gametes of the aneuhaploid F_1-plant 5811_1 ($2n_i - n_j = 3x + 3\ Bs = 21 + 3 = 24$) after self-fertilization; presentation includes data from Kynast *et al.* 2007 (20 tested F_2-offspring) and Kynast *et al.* 2008 (10 tested F_2-offspring)

Figure 2: Metaphase I microsporocytes of *OMAxB.1* plants with different numbers (*x*) of added maize *Bs* labeled by GISH (green fluorescing, univalent marked with one arrow head, bivalent marked with one double arrow head, pairs of thin arrows indicate direction of chromosome migration towards the poles); (2a) *OMAmB.1*, one *B* univalent scattered across the spindle figure outside the cell equator; (2b) *OMAmB.1*, one *B* univalent well integrated into the cell equator; (2c) *OMAdB.1*, two *B* univalents scattered across the spindle figure outside the cell equator; (2d) *OMAdB.1*, one *B* bivalent well integrated into the cell equator; (2e) *OMAtB.1*, three *B* univalents scattered across the spindle figure outside the cell equator; (2f) *OMAtB.1*, one *B* univalent plus one *B* bivalent both well integrated into the cell equator; (2g) *OMAqB.1*, four *B* univalents scattered across the spindle figure outside the cell equator; (2h) *OMAqB.1*, two *B* univalents scattered across the spindle figure outside the cell equator plus one *B* bivalent well integrated into the cell equator; (2i) *OMAqB.1*, two *B* bivalents well integrated into the cell equator

Figure 3: Polynomial frequency distributions of metaphase I sporocytes with *B* univalents in the addition lines *OMAdB.1*, *OMAtB.1*, and *OMAqB.1*

Table 1: Selected F_1-plant and a-panicles of F_2-plants for addition line development; OMA = oat-maize chromosome addition, m = monosomic, d = disomic, t = trisomic, q = tetrasomic, *B.1 = B* that is descended from the first recovery in one individual (oat × maize) plant 5811_1

Line	**Genotype/Karyotype**
	F_1 5811_1 (aneuhaploid hybrid) with three *Bs* ($2n_i - n_j = 3x + 3\ Bs = 21 + 3 = 24$)
OMAmB.1	F_2 1188_3a (*aka* 5811_1_3a) with one *B* ($2n = 6x + 1\ B = 42 + 1 = 43$)

OMAdB.1	F_2 1188_7a (*aka* 5811_1_7a) with two *Bs* (*2n = 6x + 2 Bs = 42 + 2 = 44*)
OMAtB.1	F_2 1188_20a (*aka* 5811_1_20a) with three *Bs* (*2n = 6x + 3 Bs = 42 + 3 = 45*)
OMAqB.1	F_2 1188_8a (*aka* 5811_1_8a) with four *Bs* (*2n = 6x + 4 Bs = 42 + 4 = 46*)

Table 2: Selected b- and c-panicles of F_3- and F_4-plants for meiosis assays

Line	**Genotype/Karyotype**
OMAmB.1	F_3 1374_13b (*aka* 5811_1_3a_13b) with one *B* (*2n = 6x + 1 B = 42 + 1 = 43*)
	F_3 1374_13c (*aka* 5811_1_3a_13c) with one *B* (*2n = 6x + 1 B = 42 + 1 = 43*)
	F_4 000769_2b (*aka* 5811_1_3a_13a_2b) with one *B* (*2n = 6x + 1 B = 42 + 1 = 43*)
	F_4 000769_2c (*aka* 5811_1_3a_13a_2c) with one *B* (*2n = 6x + 1 B = 42 + 1 = 43*)
OMAdB.1	F_3 1378_4b (*aka* 5811_1_7a_4b) with two *Bs* (*2n = 6x + 2 Bs = 42 + 2 = 44*)
	F_3 1378_4c (*aka* 5811_1_7a_4c) with two *Bs* (*2n = 6x + 2 Bs = 42 + 2 = 44*)
	F_4 000805_2b (*aka* 5811_1_7a_4a_2b) with two *Bs* (*2n = 6x + 2 Bs = 42 + 2 = 44*)
	F_4 000805_2c (*aka* 5811_1_7a_4a_2c) with two *Bs* (*2n = 6x + 2 Bs = 42 + 2 = 44*)
OMAtB.1	F_3 1391_8b (*aka* 5811_1_20a_8b) with three *Bs* (*2n = 6x + 3 Bs = 42 + 3 = 45*)
	F_3 1391_8c (*aka* 5811_1_20a_8c) with three *Bs* (*2n = 6x + 3 Bs = 42 + 3 = 45*)
	F_4 000837_4b (*aka* 5811_1_20a_8a_4b) with three *Bs* (*2n = 6x + 3 Bs = 42 + 3 = 45*)
	F_4 000837_4c (*aka* 5811_1_20a_8a_4c) with three *Bs* (*2n = 6x + 3 Bs = 42 + 3 = 45*)
OMAqB.1	F_3 1379_4b (*aka* 5811_1_8a_4b) with four *Bs* (*2n = 6x + 4 Bs = 42 + 4 = 46*)
	F_3 1379_4c (*aka* 5811_1_8a_4c) with four *Bs* (*2n = 6x + 4 Bs = 42 + 4 = 46*)
	F_4 000853_3b (*aka* 5811_1_8a_4a_3b) with four *Bs* (*2n = 6x + 4 Bs = 42 + 4 = 46*)
	F_4 000853_3c (*aka* 5811_1_8a_4a_3c) with four *Bs* (*2n = 6x + 4 Bs = 42 + 4 = 46*)

Table 3: Maize *B* pairing performance in metaphase I microsporocytes (n = 30 per plant line) of four oat-maize *B* addition lines, I = *B* univalents, II = *B* bivalents, III = *B* trivalents, IV = *B*

quadrivalents

Line	Cell number per line	M I Configuration	Cell number per Configuration
OMAmB.1	30	1^{I}	30
OMAdB.1	30	2^{I}	18
		1^{II}	12
OMAtB.1	30	3^{I}	19
		$1^{I} + 1^{II}$	11
		1^{III}	0
OMAqB.1	30	4^{I}	12
		$2^{I} + 1^{II}$	13
		2^{II}	5
		$1^{I} + 1^{III}$	0
		1^{IV}	0

Table 4: Minimal, maximal and mean frequencies, ranges, standard deviations and coefficients of variation of maize *B* pairing configurations in metaphase I microsporocytes (n = 30 per plant line) of four oat-maize *B* addition (OMA) lines; m = monosomic, d = disomic, t = trisomic, q = tetrasomic, *B.1* = *B* of first recovery, I = *B* univalents, II = *B* bivalents, III = *B* trivalents, IV = *B* quadrivalents

Genotypes	*OMAmB.1*				*OMAdB.1*				*OMAtB.1*				*OMAqB.1*			
Pairing figures	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
$\sum_{j=1}^{n=30} x_j$	30	n/a	n/a	n/a	36	12	n/a	n/a	68	11	0	n/a	74	23	0	0
x_{min} *per cell*	1	n/a	n/a	n/a	0	0	n/a	n/a	1	0	0	n/a	0	0	0	0

x_{max} *per cell*	1	n/a	n/a	n/a	2	1	n/a	n/a	3	1	0	n/a	4	2	0	0
$\bar{x}$ *per cell* $= \frac{\sum_{j=1}^{n=30} x_j}{n}$	1	n/a	n/a	n/a	1.2	0.4	n/a	n/a	2.3	0.4	0	n/a	2.5	0.8	0	0
$\bar{v} = x_{max} - x_{min}$	0	n/a	n/a	n/a	2	1	n/a	n/a	2	1	0	n/a	4	2	0	0
$s = \sqrt{\frac{\sum_{j=1}^{n=30}(x_j - \bar{x})^2}{n-1}}$	0	n/a	n/a	n/a	1.0	0.5	n/a	n/a	1.0	0.5	0	n/a	1.5	0.7	0	0
$s\% = \frac{s}{\bar{x}} \times 100$	0	n/a	n/a	n/a	83	125	n/a	n/a	43	125	n/d	n/a	60	88	n/d	n/d

Figure 1: Numbers of 36 tested F_2-offspring without and with maize *B* additions reflecting the transmission frequencies of added maize *Bs* from F_1 to F_2 through doubled haploid formation by unreduced gametes of the aneuhaploid F_1-plant 5811_1 ($2n_i - n_j = 3x + 3\ Bs = 21 + 3 = 24$) after self-fertilization; presentation includes data from Kynast *et al.* 2007 (20 tested F_2-offspring) and Kynast *et al.* 2008 (10 tested F_2-offspring)

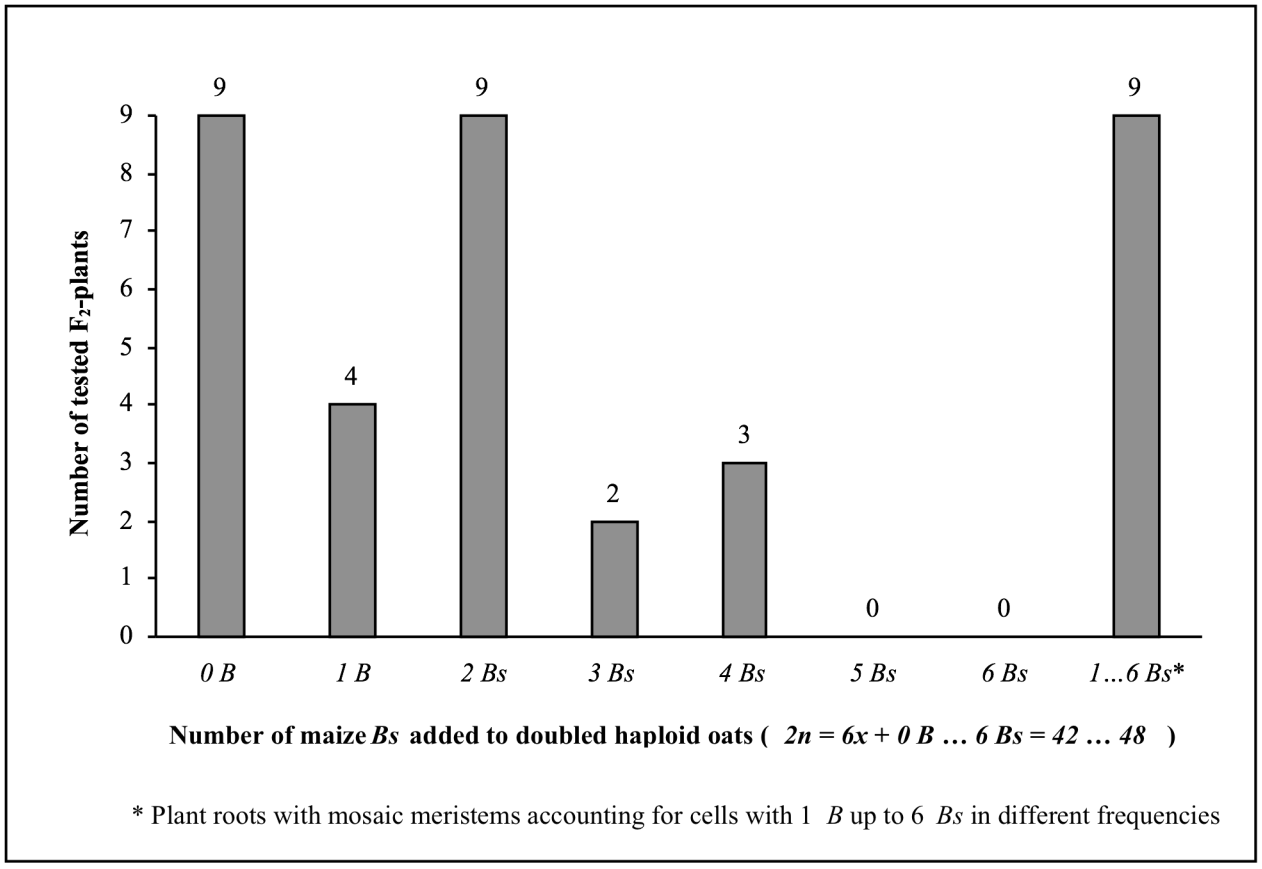

Figure 2

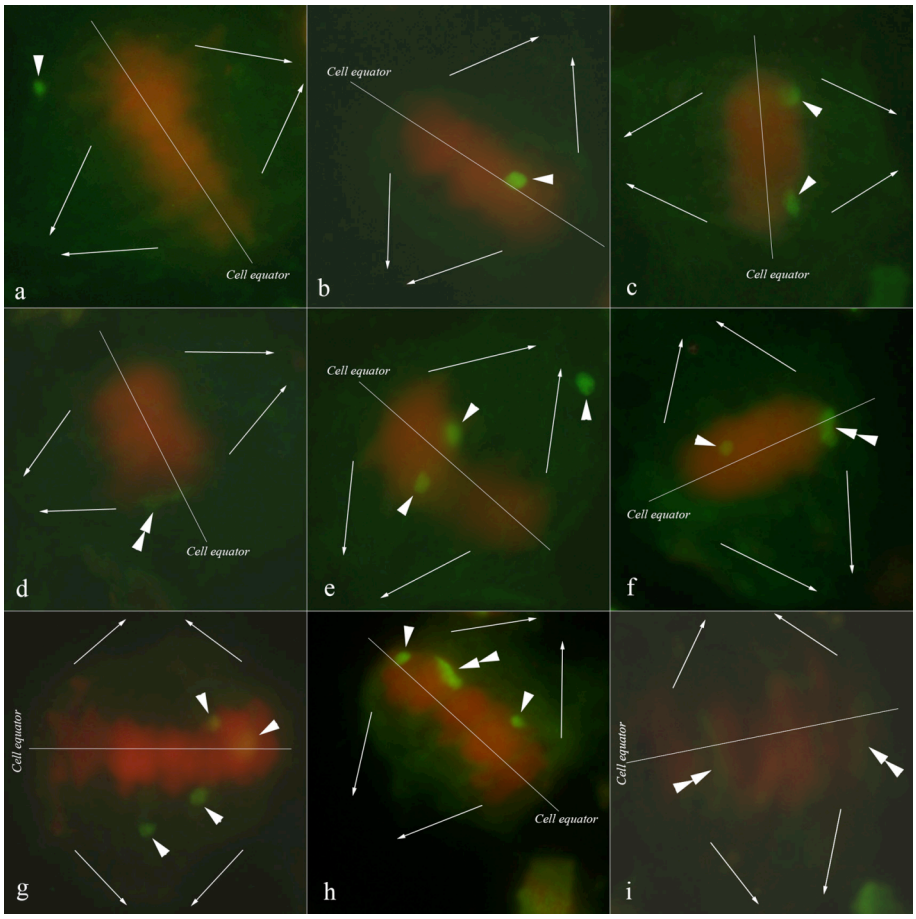
a
Cell equator
b
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Figure 3: Polynomial frequency distributions of metaphase I sporocytes with *B* univalents in the addition lines *OMAdB.1*, *OMAtB.1*, and *OMAqB.1*