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# THE ORIGIN OF UNUSUAL CHROMOSOME CONSTITUTIONS AMONG NEWLY FORMED ALLOPOLYPLOIDS[1]

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- *Premise of the study:* Wide hybridization followed by spontaneous chromosome doubling of the resulting hybrids plays an important role in plant speciation. Such chromosome doubling is usually accomplished via unreduced gametes produced by altered meiosis, the so-called 'meiotic restitution'. Unreduced gametes are expected to carry somatic chromosome numbers and constitutions. However, it has been shown recently that new allopolyploids often carry unusual chromosome constitutions which include compensating and noncompensating nulli-tetrasomies and monotrisomies, and translocations of homoeologues.
- *Methods:* We have reanalyzed meiotic divisions in a wheat-rye hybrid by in situ probing with labeled DNA focusing on deviations from the standard pattern of meiotic restitution.
- *Key results:* In a typical first division restitution in a wide hybrid, there is no chromosome pairing, univalents separate sister chromatids in anaphase I, and there is no meiosis II. Here we illustrate that occasional pairing of homoeologous chromosomes in metaphase I, combined with separation of sister chromatids of univalents, generates diads with compensating nulli-disomies and associated translocations of homoeologues. Similarly, precocious metaphase I migration to the poles of some undivided univalents generates a wide range of noncompensating simple and complex nulli-disomies in the gametes.
- *Conclusions:* Both alterations to the standard pattern of meiotic restitution tend to maintain the somatic chromosome numbers in the gametes; chromosome constitutions are variable but mostly genetically balanced. This source of variation among progeny may be an important factor contributing to greater success of natural allopolyploids.

**Key words:** allopolyploidy; chromosome constitution; meiotic restitution; wide hybridization.

Many plant species, especially in the temperate and polar regions, are polyploids. Polyploidization is a ubiquitous event in life histories of essentially all higher organisms (Masterson, 1994; Wood et al., 2009; Jiao et al., 2011). Among polyploids, allopolyploids usually originate by interspecific or intergeneric hybridization followed by spontaneous doubling of the chromosome numbers of the $F_1$ hybrids. This chromosome doubling is usually accomplished by formation of unreduced gametes, and these in turn are produced via modified meiosis, the so-called "meiotic restitution". In meiotic restitution events of wide hybrids, standard meiosis, with its two rounds of cell division for one round of DNA replication, is abbreviated to a single division. Various terms have been proposed to describe such abbreviated meiosis, such as "unreductional meiotic cell division" (UMCD), "single division meiosis" (SDM), "haploid meiotic restitution" (HMR) or, when things get difficult, "indeterminate meiotic restitution" (IMR) (Lim et al., 2001; Cai et al., 2010; Wang et al., 2010; Kynast et al., 2012; for review see Ramanna and Jacobsen, 2003). Instead of four products, each with *n* chromosomes, such modified meiosis generates only two products that carry (restitute) the somatic number of chromosomes. Fusion of unreduced gametes generates $F_2$ progeny with twice the number of chromosomes of the $F_1$ hybrid, and these usually are fertile. At times meiocytes undergo no divisions at all and the resulting spores carry twice the somatic number of chromosomes (4*n*) (Xu and Joppa, 2000). There is, however, no clear evidence that such gametes participate in reproduction.

In standard meiosis (for a detailed description see Dawe, 1998; for color illustrations see Lukaszewski et al., 2011) homologues pair in meiotic prophase, bivalents congregate on the metaphase plate in metaphase I (MI) and in anaphase I (AI) homologues separate to the poles of the karyokinetic spindle, forming two daughter nuclei each with only one homologue from each pair present. In meiosis II sister chromatids are separated, producing a tetrad of four nuclei with *n* number of chromosomes each. With the exception of achiasmate meiosis, this process is entirely dependent on the formation of crossovers which form chiasmata when combined with cohesion of sister chromatids along entire lengths of chromosomes. Chiasmata are released when cohesion of sister chromatids is released along the chromosome arms. Cohesion of sister chromatids in the centromeres is released in meiosis II, permitting their separation. Standard meiosis thus depends entirely on chromosome pairing and properly timed two-step dissolution of sister chromatid cohesion. The timing of dissolution is often disturbed in chromosomes that fail to pair and enter meiosis I as univalents. Univalents may migrate randomly to the poles in meiosis I, lag on the metaphase plate, separate sister chromatids in AI, or be broken across the centromeres (Lukaszewski, 2010, 2013). In a wide hybrid there may be no chromosome pairing at all, and all chromosomes enter meiosis I as univalents. Under such circumstances, and with proper genetic mechanisms present, the meiocytes may enter one of the meiotic restitution pathways.

Many variants of meiotic restitution have been described recently in a broad range of wide hybrids (Bretagnolle and Thompson, 1995; Jauhar, 2007; Cai et al., 2010; Kynast et al., 2012;

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Silkova et al., 2011b, 2013; for review see Ramanna and Jacobsen, 2003). A variant common in wide hybrids of many Triticeae is the first division restitution (FDR) or single division meiosis (SDM). In this version of the process, univalents line up on the metaphase plate in MI in a bipolar attachment to the karyokinetic spindle, separate sister chromatids in AI, and there is no meiosis II (Fig. 1). SDM is very similar to mitosis, save for differences in chromosome condensation and sister centromere/kinetochore arrangement. Because FDR/SDM is so similar to mitosis, it has always been assumed that entire constituent genomes are passed on to the gametes which were therefore assumed to carry the same numbers and sets of chromosomes as the somatic tissues of the hybrid undergoing meiotic restitution. Minor deviations from this rule were already observed by Karpechenko (1928) and attributed to lagging univalents, but were assumed to be removed by strong gametic selection. It was, therefore, cause for considerable surprise when frequent unusual chromosome constitutions were identified, first among *Tragopogon miscellus* Ownbey (Chester et al., 2012), and soon thereafter in a range of wide hybrids in Triticeae (Zhang et al., 2013). *Tragopogon miscellus* is a recent allopolyploid generated by interspecific hybridization of two diploid progenitors (Ownbey, 1950; Lim et al., 2008; Tate et al., 2009). Its documented unusual chromosome constitutions include compensating nulli-tetrasomies or monotrisomies, often accompanied by translocations of the homoeologues involved in the numerical chromosome aberration itself. In a survey of a large sample of new polyploids replicating the origin of polyploid wheat, Zhang et al. (2013) observed a similar pattern of variation: euploid chromosome numbers but abnormal chromosome constitutions such as noncompensating nulli-tetrasomies and monotrisomies. These two examples alone suggest that the phenomenon may be widespread, and perhaps even common, among new allopolyploids. It remained undetected because of low resolution of older observational techniques, usually limited to chromosome counts. Deviously, the chromosome constitution variants tend to have the number of chromosomes expected for a simple process of chromosome doubling.

Variable chromosome constitutions among progenies of a single wide hybridization event greatly expand the range of genetic variation among progeny and may contribute to the evolutionary success of allopolyploids. Deviations from the expected chromosome constitutions are not random; most of them are genetically balanced, where the absence of one or a pair of chromosomes is compensated by an appropriately increased dose of its homoeologue, or, in case of translocations, by corresponding equivalent segments of homoeologues. Owing to genetic equivalency of homoeologues (which tend to carry the same genetic loci) these changes in chromosome constitution do not alter gene dosages. Thus, the new allopolyploids with altered chromosome constitutions do not suffer any immediate penalty that would be a consequence of random numerical aneuploidy (i.e., absence or extra chromosomes), but may benefit from altered dosages of homoeoalleles. This study was undertaken to illustrate the mechanisms that generate unusual chromosome constitutions among progenies derived via restitution of the first meiotic division.

## MATERIALS AND METHODS

$F_1$ hybrids were generated by intercrossing line Do1 of tetraploid wheat (*Triticum turgidum* L.) with diploid rye *Secale cereale* L. 'MAD510'. The Do1 line was selected by Dr. B. Lapinski, then at the Institute of Plant Genetics, Poznan, Poland, from a hybrid between *T. turgidum subsp. dicoccoides* × *T. turgidum subsp. persicum,* for its ability to produce self-fertile $F_1$ hybrids with rye. Later observations have shown that self-fertility manifests itself in all tested wide hybrids (Lukaszewski, unpublished; Rezaei et al., 2010). Rye population 'MAD510' is a winter rye homozygous for an introgression of a segment of wheat chromosome 1D on the long arm of rye chromosomes 1R (Lukaszewski et al., 2000). Otherwise, this rye is typical.

Wheat-rye pollinations of previously emasculated wheat heads were done by hand. Developing embryos were rescued on Orchid Agar (Difco) supplemented with sucrose 14 to 17 d post pollination. Plants with 2–3 leaves were potted and grown to maturity. Tillers with spikes suspected to be at meiosis were cut and one anther from each spikelet was fresh-squashed in a drop of acetocarmine. If desired stages of meiosis were present, the remaining two anthers were fixed in a mixture of 3 parts of absolute alcohol to 1 part glacial acetic acid for one week at 37°C and stored at −18°C until used. Flowering

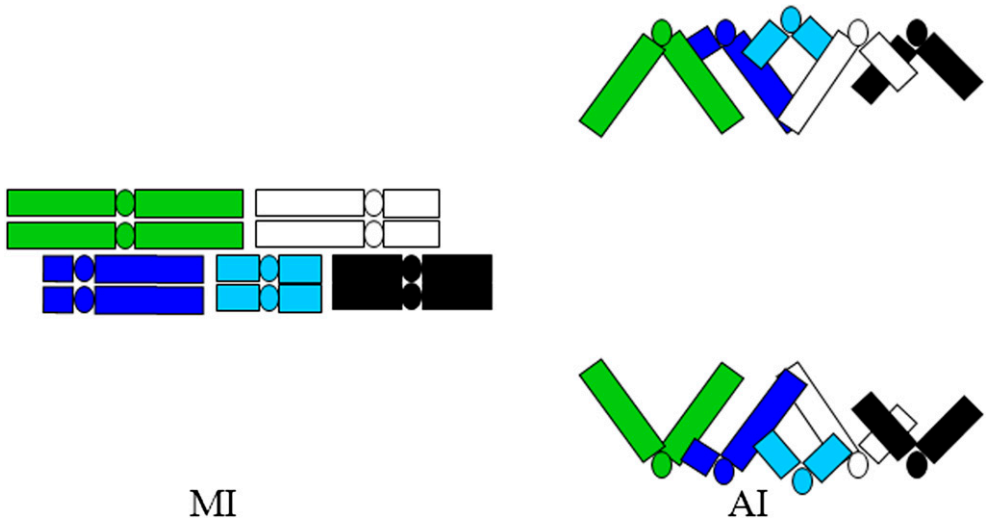


Fig. 1. Schematic representation of the first division restitution in a wide hybrid. In metaphase I all univalents line up on the metaphase plate in a bipolar attachment to the karyokinetic spindle, and in anaphase I they separate sister chromatids, creating two daughter nuclei with somatic chromosome numbers. This is equivalent to a single division meiosis; all gametes have somatic number of chromosome and standard chromosome constitution. MI = metaphase I; AI = anaphase I.

heads were bagged to assure self-pollination. A testcross was made to a standard hexaploid triticale with known chromosome constitution (cv. Presto), using self-fertile Do1 × 'MAD510' $F_1$ hybrids as male. Samples of $F_2$ and $BC_1$ progenies were karyotyped in root-tips by sequential C-banding and in situ probing with labeled total genomic DNA of rye or the A-genome specific DNA probe based on BAC 676D4 (Zhang et al., 2004), kindly provided by Dr. B. Friebe, Kansas State University, Manhattan KS, USA.

All observations were done on standard squash preparations made from individual anthers or root-tips. C-banding was done according to Giraldez et al. (1979). In situ probing with labeled DNA was done according to Masoudi-Nejad et al. (2002). Various combinations of probes, blocks and fluorochromes were tested on meiotic preparations, but the best resolution was consistently observed when the rye centromere sequence (Francki, 2001) labeled with dig-oxygenin was used as a probe and detected with anti-DIG-FITC; herring DNA was used as a block, usually in about 50× excess relative to the probe. Counterstaining was done with 0.3% propidium iodide in standard antifade solution.

All observations were made under a Zeiss Axioscope 20 (Carl Zeiss Group, Oberkochen, Germany) equipped with epi-fluorescence, recorded with a SPOT RT Color digital camera (SPOT Imaging Solutions, division of Diagnostic Instruments, Sterling Heights, Michigan, USA). All images presented here were manipulated as needed, using the SPOT Advanced (SPOT Imaging Solutions, Sterling Heights, Michigan, USA) and Adobe Photoshop CS (Adobe Systems, San Jose, California, USA) software to enhance contrast, remove debris if present, properly orient images on the N-S axis, and tone down background distortion.

## RESULTS

All twelve $F_1$ hybrids examined had 21 chromosomes with expected ABR genomic constitution. Among them, eight had meiosis typical for a wide hybrid, had indehiscent anthers, and did not produce progeny. The remaining four showed clear evidence of FDR/SDM in at least some pollen mother cells (PMCs) of the analyzed anthers, had some dehiscent anthers, and produced seed under self-pollination. The term FDR is used here following Xu and Joppa (2000) and Jauhar (2007), but the process appears to be the same as the single division meiosis (SDM) of Wang et al. (2010) or the "unreductional meiotic cell division" (UMCD) of Cai et al. (2010).

Meiosis in PMCs in nonrestitution plants was typical for a wide hybrid: there was little chromosome pairing and most chromosomes were present as univalents. At the stage equivalent to MI, univalents were scattered throughout the volume of the PMC while bivalents, if present at all, were positioned on the metaphase plate (Fig. 2A). The average pairing frequency was 0.62 chromosome arms paired per PMC (1184 PMCs scored at MI). Pairing frequencies of individual wheat homoeologues was scored in a sample of 52 bivalents, 25 of which involved group-1 chromosomes while the remaining were group 2, 3, 5, 6 and 7 bivalents with 4–6 cases each. No pairing was observed for group 4 homoeologues. Engineered rye chromosome 1R was paired only once in the sample of 25 group 1 bivalents (with chromosome 1A). Its pairing in the entire sample was 0.7%. In all instances, 1R appeared to be paired in the introgressed segment of 1D. Only three trivalents were observed in the entire study, one involving a rye chromosome with two wheat chromosomes, presumably group-1 homoeologues, and two wheat trivalents. No other rye chromosomes were involved in MI pairing.

Most univalents in standard meiosis were in monopolar orientation with fused sister centromeres (Fig. 2B). After AI, PMCs formed typical diads, occasionally with micronuclei present, and the second division proceeded normally forming tetrads, at times with nuclei of uneven sizes, and often with micronuclei present. Based on the numbers of labeled rye centromeres present, segregation of univalents to diads was random with 3:4 and 2:5 for rye chromosomes being the most common.

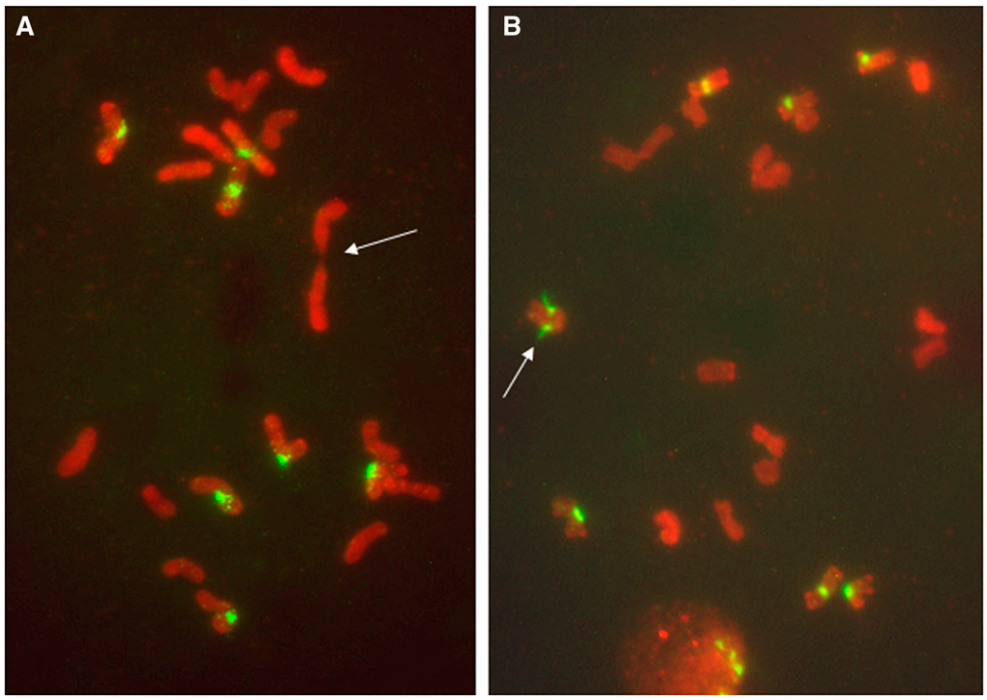


Fig. 2. Metaphase I in pollen mother cells in the nonrestitution pathway, in a wheat-rye $F_1$ hybrid. A, one homoeologous bivalent (arrowed); nine univalents on one pole and ten on the other. B, 21 univalents, one (arrowed, green centromeres) in a bipolar attachment to the karyokinetic spindle on the metaphase plate. Rye chromosomes are marked by green signals of a rye centromere specific DNA probe.

In anthers with FDR, there was usually a mixture of PMCs undergoing standard meiotic division (as described earlier in this section), or FDR, with variable proportions. In one plant, a small proportion of PMCs appeared to undergo no nuclear division at all, eventually forming giant microspores, or attempting to undergo cytokinesis across a field of separated sister chromatids. In the FDR pathway, all or most univalents congregated on the MI plate in bipolar orientation but not necessarily with separated sister centromeres (Fig. 3A, B). There appeared to be an inverse relation between chromosome pairing and restitution: the average number of chromosome arms paired per PMCs in plants without FDR was 0.68 arms versus 0.28 arms paired per PMCs in plants with FDR, respectively ($\chi^2 = 30.51$, df = 1, $p > 0.01$). The actual ratio was probably even more skewed toward low pairing in the FDR meiocytes as FDR plants always had a mixture of PMCs in both pathways, and the FDR pathway could not be easily predicted for PMCs in early MI.

Among MI PMCs with FDR, most or all univalents congregated on the metaphase plate while others were already at the poles. The distribution patterns of such precocious univalents at the poles appeared to be random, from 0 : 1, 2 : 1, 2 : 0, 2 : 2, to 3 : 1 and so on (Fig. 3C–F), and were probably accomplished by random movement of univalents in unstable monopolar interactions with the karyokinetic spindle. In rare instances when a bivalent was present in a restitution PMC, the presence of chiasmata indicated that sister chromatid cohesion had not yet been released. The PMC was therefore still in MI and univalents at the poles could not have been delivered there by precocious separation from bivalents. Most univalents congregating on the plate were in bipolar attachment to the karyokinetic spindle (Fig. 3A–F). In AI, univalents on the metaphase plates separated sister chromatids and these migrated to the poles, as did chromosomes in bivalents (no trivalent was observed in the FDR PMCs). Hence, the chromosome constitution of the daughter nuclei after AI depended on the mode of chromosome behavior in MI: chromosomes involved in pairing and precocious univalents delivered both sister chromatids to the pole toward which they were oriented; univalents congregating on the metaphase plate in bipolar attachment to the spindle and separating sister centromeres in AI contributed one sister chromatid to each pole. In most cases observed this produced 21-chromosome daughter nuclei, but with variable chromosome constitutions.

A sample of 92 progeny from self-pollination of $F_1$ hybrids with FDR was karyotyped by a combination of C-banding and in situ probing with total genomic DNA of rye *(Secale cereale)* and A-genome probes. Of these, to the extent that the techniques used permitted verification, it was found that 89 had normal chromosome constitutions, that is 42 chromosomes and AABBRR genomes with seven pairs of homologues in each genome. Of the three exceptions, one was monosomic (41 chromosomes) for rye chromosome 3R. The other two had 42 chromosomes each, but one was a compensating nullisomic-1-R-tetrasomic-1B heterozygous for a deficiency of approximately one half of the long arm of chromosome 2B (Fig. 4), and the other was nullisomic-1R-trisomic-2A-trisomic-1B. Meiosis in plants with standard chromosome number and constitution was normal for triticale, with some univalents present in a proportion of PMCs. However, four of the $F_2$ plants tested had high pollen mortality and reduced seed set. Chromosome counts in the first pollen mitosis showed mostly 21 chromosomes, ranging from 17 to 22. Among 25 $F_3$ progeny of karyotypically normal and stable $F_2$ plants, three showed clear deviation from standard morphology and heading date. All had 42 chromosomes with complete, normal AABBRR genomic constitution so the deviations could not be explained by altered chromosome constitution. Among 32 karyotyped progeny from a backcross of $F_1$ to standard triticale, all had 42 chromosomes with AABBRR constitution but two deviated from standard morphology including one with much delayed heading, suggesting incomplete vernalization. When checked at meiosis, this plant was found frequently to form a trivalent, most likely of group 5 chromosomes, suggesting the presence of a translocation. Genetic loci responsible for the vernalization response (*Vrn*) of wheat are located on the long arms of group 5 chromosomes (McIntosh et al., 2008) and a translocation could have changed dosages of individual genes. However, in situ probing with total rye genomic and wheat A-genome specific DNA probes, as well as C-banding of all plants with deviant morphology, did not detect any obvious chromosome translocations.

## DISCUSSION

Production of unreduced gametes in wide hybrids by modifications of meiosis has been observed and known for many decades (Karpechenko, 1928; Liljefors, 1936, 2010), but the subject has recently attracted renewed interest. Several restitution pathways have been described, discussed in considerable detail, and named or renamed, in different crops and different contexts (Karpechenko, 1928; Xu and Joppa, 2000; Jauhar, 2007; Cai et al., 2010; Kynast et al., 2012; Silkova et al., 2011a, b, 2013; for review see Ramanna and Jacobsen, 2003). Given the sheer number of detailed studies, it is somewhat surprising that we have learned only recently of unusual chromosome constitutions among newly formed allopolyploids. These unusual karyotypes were detected in *Tragopogon miscellus* (Chester et al., 2012) and in 16 lineages of new poplyploids created to replicate the origin of hexaploid wheat (Zhang et al., 2013). Major genomic rearrangements have also been observed among progenies of wide hybrids in *Brassica* (Szadkowski et al., 2010; Xiong et al., 2011), but the responsible mechanisms may be quite different from those observed here, probably representing a version of the pivotal-differential concept of genome evolution proposed by Zohary and Feldman (1962). The deviant karyotypes detected in *Tragopogon* formed specific patterns: there were compensating nulli-tetrasomic and monotrisomics, at times accompanied by translocations of homoeologues (Chester et al., 2012). In the Triticeae they also included noncompensating nulli-tetrasomics and monotrisomics (Zhang et al., 2013). Using a wheat-rye hybrid with a known tendency to produce unreduced gametes we illustrate the mechanisms generating such chromosome constitutions.

The standard pathway of generating unreduced gametes in this wheat-rye hybrid (and many similar hybrids) was outlined in Results above (see Fig. 1): univalents congregate on the metaphase plate, in AI they separate sister chromatids generating two daughter nuclei, each with a complete set of somatic chromosomes composed of single chromatids. There is no second division, and diads eventually produce functional sperm nuclei. In most examples of the restitution of the first meiotic division, even in cases where designated pairs of homologues were present (Wang et al., 2010; Silkova et al., 2011a) no other scenarios for chromosome segregation were mentioned. Fortuitously, the hybrids in this study offered a complete range of behaviors, from standard meiosis in a wide hybrid (no or very little chromosome pairing, random segregation of univalents in

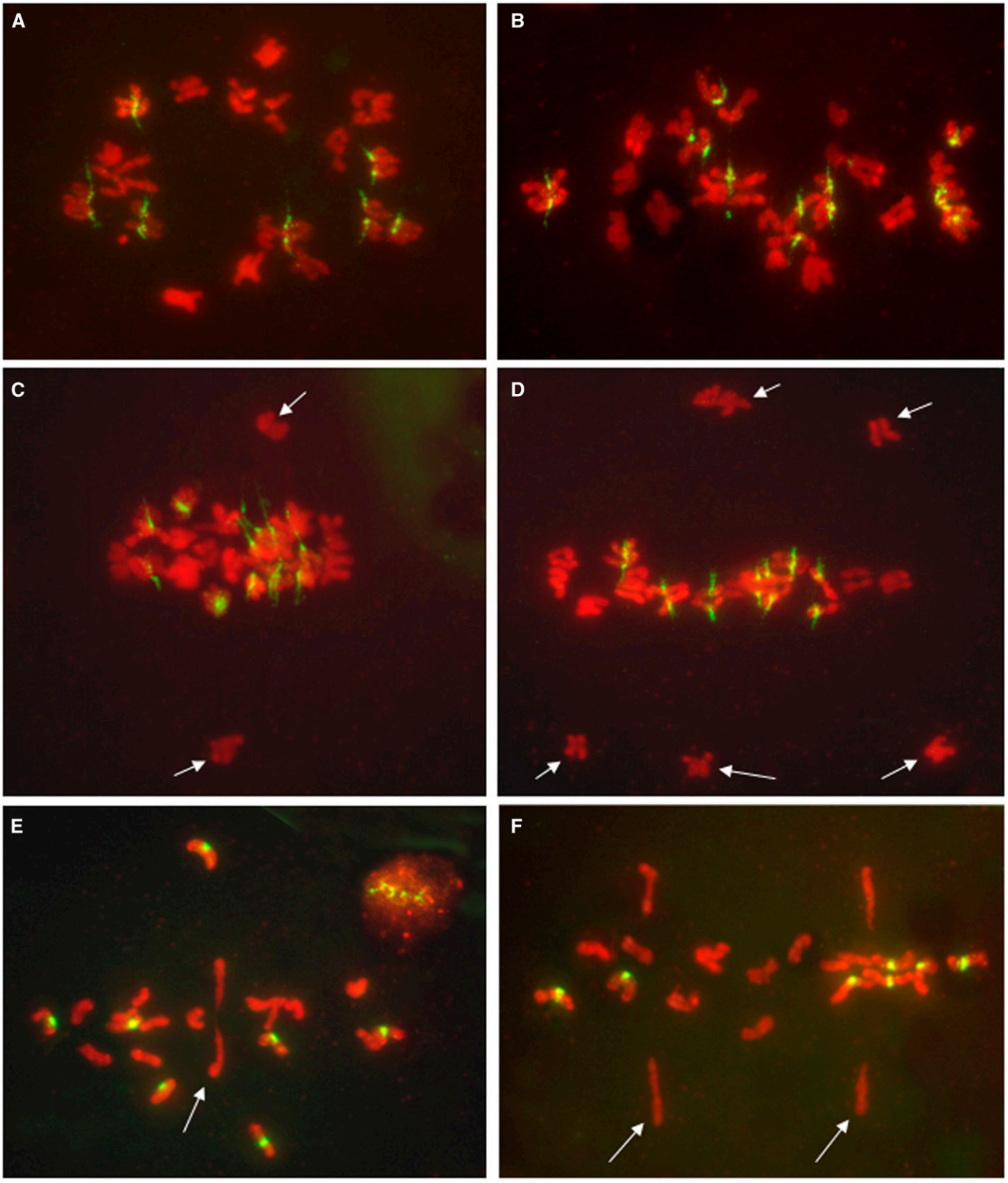


Fig. 3. Metaphase I in pollen mother cells in the restitution pathway, in a wheat-rye hybrid. Polar (A) and equatorial (B) views of metaphase I with all 21 univalents congregating on the metaphase plate. Two univalents (C, arrowed) and six univalents (D, arrowed), each with both sister chromatids, have already migrated to the poles while remaining univalents congregate on the metaphase plate in bipolar attachment to the karyokinetic spindle and will contribute single chromatids to each pole. Meiocytes with one (E) and two (F) homoeologous bivalents present (arrowed). Most univalents still congregate on the metaphase plate in bipolar attachment to the spindle. Rye chromosomes are marked by green signals of a rye centromere specific DNA probe.

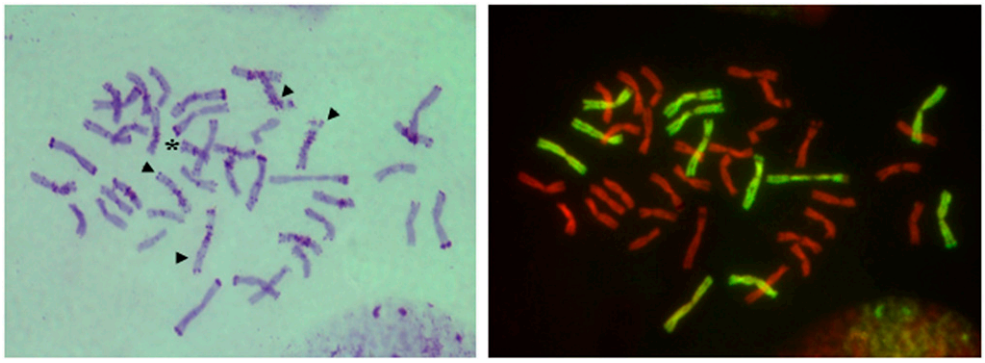

Fig. 4. Nulli-tetrasomic progeny from the restitution of the first meiotic division in a hybrid of wheat with rye. A, C-banding, four chromosomes 1B labeled with arrowheads; no identifiable rye chromosome 1R. B, In situ probing with total genomic DNA of rye: 12 rye chromosomes present (green). Chromosome 2B with a deficiency in the long arm marked by * on photo A.

AI, unbalanced chromosome numbers in microspores and sterility) through various combinations of normal segregation of univalents with restitution, to perfect restitution. This range even included a case with no meiotic division at all, but with cytokinesis attempting to cut across replicated but undivided nuclei of PMCs. However, it is the chromosome segregation patterns between the two extremes (i.e., normal meiosis and restitution) that offer an explanation for the unusual karyotypes of *Tragopogon* and wheat alloploids (Chester et al., 2012; Zhang et al., 2013).

The pattern of chromosome segregation in standard FDR/SDM is disturbed when homoeologues engage in pairing: while univalents deliver one sister chromatid each to each pole, paired chromosomes deliver both sister chromatids of one homoeologue to one pole and none to the other while their pairing partner does the exact opposite, delivering to the other pole (Fig. 5). Unlike the illustration provided by Ramanna and Jacobsen (2003) for "restitution with recombination", this pattern of chromosome behavior in fact produces gametes with a proscribed number of chromosomes (21 in for the hybrids studied here) but nullisomic for one homoeologue and disomic for the other. When such gametes fuse in fertilization, either a nulli-tetrasomic or monotrisomic will be formed, both with compensating chromosome constitutions. Pairing frequencies in individual homoeologous groups may create specific patterns of chromosome constitutions in the gametes; frequently pairing homoeologues will be frequently involved in aberrant constitutions while absence of pairing assures single dose of a chromosome in the gamete. If pairing affinity across all homoeologous groups is similar, a wide range of gametes can be produced, mostly compensating nulli-disomics, in one or more homoeologous groups, but always with a standard somatic number of chromosomes. Fusion of such gametes will generate progeny with the expected number of chromosomes, but mostly monotrisomics (or multiple monotrisomics). Moreover, since the delivery of both sister chromatids of a homoeologue to the same pole is by segregation from a bivalent, and bivalent formation is based on homoeologous crossing over and chiasmata, at least one of the two sister chromatids in each chromosome will be recombined, consisting of segments from both homoeologues. Since crossing over is responsible for generation of such translocated chromosomes, and assuming colinearity of the homoeologues involved, the gametes carrying them should be genetically complete, hence viable. These were exactly the types of progeny observed among interspecific hybrids of *Tragopogon miscellus* (Chester et al., 2012): compensating nulli-tetrasomies or monotrisomies accompanied by translocations between homoeologues involved in numerical aberrations, always with the standard number of chromosomes present.

Different patterns of chromosome constitutions are generated by precocious MI migration of intact univalents to the poles, at the time when remaining univalents congregate on the metaphase plate and will eventually deliver single chromatids to the poles. This may generate deviations from the somatic chromosome number. Each precocious univalent present at the pole at the onset of AI contributes two sister chromatids to a nucleus of a diad and guarantees that no copy of it will be present in the other nucleus (Fig. 6). Chromosome numbers in individual nuclei of diads depend on the distribution patterns of univalents: as long as they are balanced, all resulting diad nuclei are guaranteed 21 chromosomes but they are noncompensating nulli-disomics. However, since the distribution of such univalents is apparently random (there is no pairing of homoeologues involved), the resulting gametes do not have compensating chromosome constitutions except in situations when, by chance, two homoeologues land at the opposite poles of a dividing PMC. Because the numbers of migrating univalents are not controlled, their distribution to the poles need not be even (proportional) and hence, the resulting microspores may differ in chromosome number as well as constitution. Fusion of gametes produced in this fashion may create progeny with noncompensating single and multiple monotrisomies and different chromosome numbers. Combination of the restitution pathway in which there is MI homoeologous pairing with the pathway involving random precocious migration of univalents only increases the range of chromosome constitutions possible among progeny.

In PMCs without any chromosome pairing it is not always possible to ascertain how a given two-chromatid chromosome was delivered to the pole (see Fig. 3C, D) by segregation from a bivalent or by precocious random migration of a univalent. Separation of sister chromatids along the arms' length indicates AI and segregation from bivalents cannot be entirely excluded, at least in cases where the same numbers are present at each of the poles (as in Fig. 3C, D). However, uneven segregation, such as 1 : 0, 1 : 2, 2 : 3 and so on, was observed as well as 1 : 1 with one rye chromosome present at each pole. It is, therefore, more likely that these precocious chromosomes are delivered to the poles by random migration of univalents with no homoeologous pairing involved, and hence that the resulting gametes are

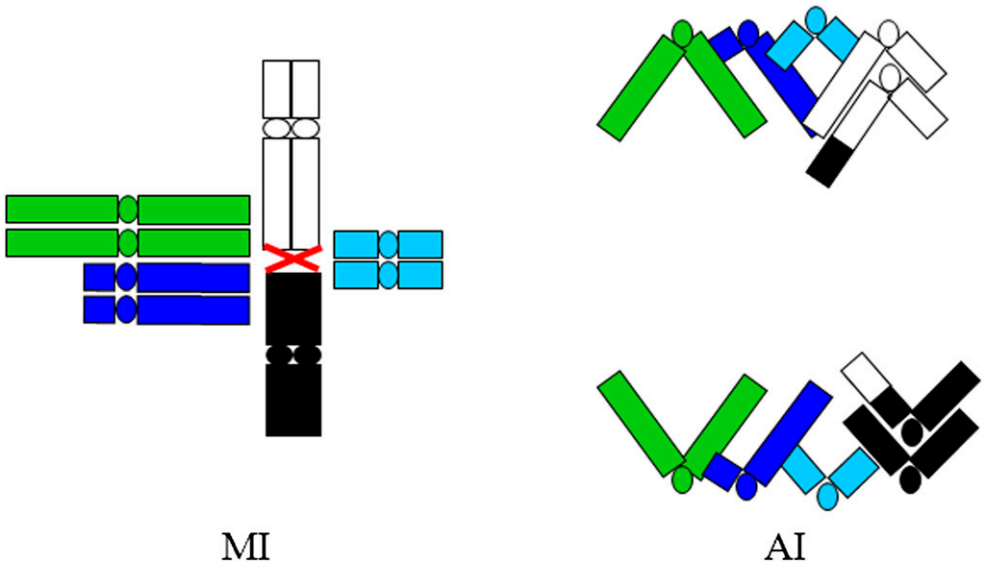


Fig. 5. Schematic representation of the first division restitution with one homoeologous bivalent: In anaphase I the bivalent contributes both sister chromatids of one homoeologue to one pole and both sister chromatids of the other homoeologue to the other pole, while univalents separate sister chromatids. Sister chromatids of pairing homoeologues can be recombined. In the absence of the second division, pairing of homoeologues produces gametes disomic for one of the two pairing homologues and nullisomic for the other, with accompanying translocations. MI = metaphase I; AI = anaphase I.

unlikely to have balanced chromosome constitutions. Various combinations of chromosome delivery to the poles in AI, by means of pairing or precocious migration of univalents with both sister chromatids attached, appear responsible for the non-compensating chromosome substitutions with proper chromosome numbers observed by Zhang et al. (2013).

The tendency for meiotic restitution appears to be genetically controlled, at least in wheat (Zhang et al., 2007), but it takes place preferentially in the absence of chromosome pairing (Wang et al., 2010; Silkova et al., 2011a, 2013) even though the relationship is not absolute. When designated pairs of homologues were present in a wide hybrid, restitution preferentially occurred in meiocytes where homologues failed to pair (Silkova et al., 2013). A similar relationship between pairing and restitution was observed here, but it is far from clear if it is the presence of one or more bivalents that directs a predisposed cell away from the restitution pathway, or if it is predisposition to the restitution pathway that reduces the chances for homoeologous pairing. In the materials studied here homoeologous pairing was most frequent among group 1 chromosomes (almost one half of

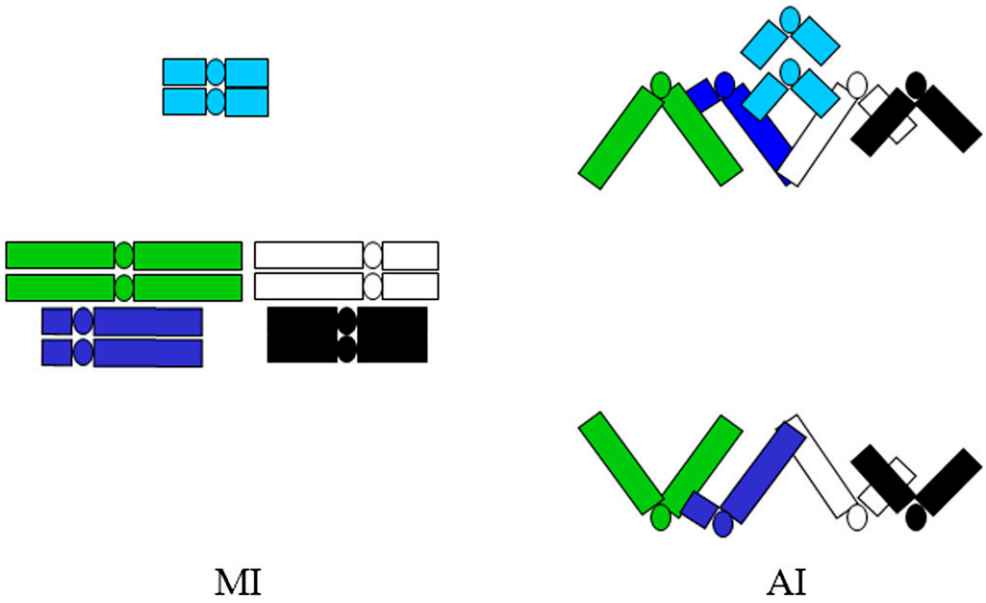


Fig. 6. Schematic representation of the first division restitution with precocious migration of a univalent to one pole in metaphase I. In anaphase I, precocious univalent delivers both sister chromatids to one pole and none to the other; remaining univalents uniformly separate sister chromatids to the poles. This produces gametes disomic-nullisomic for a specific chromosome. MI = metaphase I; AI = anaphase I.

all bivalents observed). The next five pairs of homoeologues paired with similarly low frequencies (4–9% each of all bivalents observed) and group 4 homoeologues and rye chromosomes did not pair at all (with the exception of the engineered 1R, which appeared to pair via the introgressed segment of wheat 1D, rye chromosomes did not engage in pairing). Two of four abnormal chromosome constitutions among progeny involved group 1 homoeologues.

Based on the frequencies of different pathways and configurations in MI and AI, a very wide range of gametic chromosome numbers and constitutions must have been produced by the Do1 × 'MAD510' hybrids studied here. However, in a sample of 215 gametes surveyed, only six deviations from normal were observed and as far as the backcross progeny are concerned, there is no guarantee that aneuploidy was not contributed by the tester. This suggests that in this material, gametic or zygotic selection strongly favored standard chromosome constitutions. However, the patterns of chromosome behavior in the restitution pathway and the intensity of gametic selection may vary in different hybrids, and under different conditions. Line Do1 always produced self-fertile $F_1$ hybrids with rye and other species, and no unusual variation was observed among the $F_2$ hybrids. In this study, perhaps by more careful observation, morphological variation was observed among progenies where none was expected, and it could not be explained by standard karyotyping. Cytology might not have been sensitive enough to detect recombination events of homoeologues. All homoeologous bivalents observed here had distal chiasmata so the translocation breakpoints, if present, must have been distal. There are few diagnostic C-bands in these regions of wheat homoeologues, and the A-genome specific probe used produces little signal close to the telomeres (Zhang et al., 2007). However, high resolution techniques used by Chester et al. (2012) and Zhang et al. (2013) clearly show that such translocations do take place and are frequent. If the model proposed here is correct, all incidents of tetrasomy and trisomy generated by segregation of chromosomes from homologous bivalents should be accompanied by homoeologous translocation of one of the chromosomes involved.

The exercise demonstrates that minor deviations from the standard pattern of chromosome behavior during meiotic restitution, such as relatively infrequent homoeologous pairing or precocious migration of univalents to poles, are capable of generating unusual chromosome constitutions such as those observed by Chester et al. (2012) and Zhang et al. (2013), and may explain at least some patterns of linked loci/chromosome loss in allopolyploids (Tate et al., 2009; Buggs et al., 2012). The patterns of chromosome segregation observed in this material create the potential for far more varied chromosome constitutions among progeny than standard "unreduced gametes" imply, including compensating and noncompensating nulli-tetrasomies (single and multiple) as well as deviations from the standard chromosome numbers. These in turn create the potential for additional chromosome rearrangements in subsequent generations. Rather than lineages of pure lines of amphiploids, swarms of different chromosome constitutions are generated. This greatly increases the genetic variation among such newly created species from which natural selection can select the best fit combinations. It has been argued that, historically, spontaneous chromosome doubling via unreduced gametes was far more successful than artificial chromosome doubling by colchicine (Ramanna and Jacobsen, 2003). Leaving aside the question of how valid such comparisons may be, it is clear that minor deviations in the patterns of abbreviated meiosis typical of wide hybrids vastly expand the range of possible chromosome constitutions among progeny, more often than not in a genetically balanced manner, and this may significantly contribute to fitness of spontaneously generated allopolyploids.

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