Theor Appl Genet (2004) 109: 1115–1124
DOI 10.1007/s00122-004-1727-4

ORIGINAL PAPER

**J. Thomas · E. Riedel · A. Benabdelmouna · K. Armstrong**

# A cytogenetic method for stacking gene pairs in common wheat

Received: 19 May 2003 / Accepted: 13 May 2004 / Published online: 27 August 2004
© Springer-Verlag 2004

**Abstract** The potential for non-reciprocal Robertsonian translocations of wheat (*Triticum aestivum* L.) to assist in the stacking of genes was assessed from a study of their cytological and genetic behaviour. To obtain translocations, a double monosomic (3B+5A; 2*n*=40=19ii+2i) was crossed reciprocally with a contrasting disomic. Individuals inheriting a broken monosome were identified from the loss of one arm-specific DNA marker coupled with retention of a marker for the opposite arm. No double breaks (potential translocations) were found in 180 cross progeny recovered from pollen of the double monosomic but two instances (loss of 5AL plus 3BS; loss of 5AL plus 3BL) were found in 251 progeny recovered from ovules. Meiotic pairing and multi-color genome-specific fluorescence in situ hybridization (mcGISH) showed that each plant with a double break contained one translocated chromosome between the A and B genomes that had rejoined at the centromere and that formed a trivalent (19ii + 1iii) in about 83% of PMC. Most trivalents (approximately 92%) aligned at metaphase in a ‘V’ configuration (alternate disjunction) while the rest aligned in linear ‘I’ (adjacent disjunction) or ambiguous ‘L’ configurations. Genetic analysis of a testcross of these ‘fusion monosomics’ showed that this preferential co-orientation of the trivalent influenced the assortment of the chromosome arms involved. Loci that were located in the hemizygous ends of the ‘V’ trivalent showed strong quasi-linkage in that most ovules from the female testcross carried relevant DNA markers either from both standard chromosomes or from neither. This shows that, in most cases, the two standard chromosomes assorted to the same pole while the fused monosome segregated to the opposite pole. For heterozygous loci (present both on the fusion monosome and the standard chromosomes) assortment was either independent or showed partial linkage to the hemizygous arm depending on the reported recombination distance from centromere. Marker assortment was further distorted in male testcrosses and in doubled haploids (made from the fusion monosomics by the maize method) by the strong selective advantage of pollen or haploids that inherited the standard chromosomes rather than the deficiencies. This genetic data shows that under the combined influence of alternate disjunction and natural selection, progeny of fusion monosomics will revert to the standard disomic arrangement, fixing the gene content of both hemizygous arms in the process. Thus, any pair of genes could be targeted for joint fixation by isolating the fusion monosome that will link them temporarily in a segregating population.

Communicated by J.W. Snape

J. Thomas (✉) · E. Riedel
Cereal Research Centre, Agriculture and AgriFood Canada,
195 Dafoe Road,
Winnipeg, MB, Canada, R3T 2M9
e-mail: jthomas@em.gc.ca
Tel.: +1-204-9443505
Fax: +1-204-9834604

A. Benabdelmouna · K. Armstrong
Eastern Cereal and Oilseed Research Centre, Agriculture and AgriFood Canada,
Ottawa, ON, Canada, K1A 0C6

## Introduction

The need to replace one gene with an allelic alternative is a common problem in plant breeding. To do this without constricting the working gene pool (i.e. without reducing variability for other desirable traits) requires that new crosses must segregate and undergo selection for the new trait and that this must continue for long enough to fill the pipeline of the breeding program with altered germplasm. Even for just one locus this requires a substantial commitment of resources.

Due to its polyploid nature, wheat will tolerate the loss of substantial segments of its genome especially where the deficiency is hemizygous (only one dose is missing). Even so, over several generations, hemizygous aneuploids (monosomics, telosomics etc.) will tend to revert to the disomic condition and fix the gene content of their hemizygous chromosome segments in the process. Rever-

sion from hemizygosity to a standard chromosome complement is driven by selection against pollen and plants that carry the deficiency in competition with individuals that carry the standard arrangement. For instance, hemizygous reversion of the short arm of chromosome 2BS was shown to occur rapidly (≅99% by $F_6$) in plants monotelodisomic for 2BL (Thomas et al. 2003). This telo (2BL) is now being introduced into $F_1$ hybrids with genotypes resistant to wheat midge (*Sitodiplosis mosellana*) in order to accelerate introgression of the midge resistance gene *Sm*1 that is located on 2BS (Thomas et al. 2001) .

While this method should work with any monogenic trait, its potential impact on the introgression of polygenic traits will obviously be less. This impact would be increased if two deficiencies could be combined. One straightforward way to do this is to combine two appropriate telocentrics in an elite breeding line and use these in crosses with the trait donor. While such dual telocentrics will assort independently at the first meiotic metaphase (MI), as we shall see there is evidence that joining the two deficiencies into one by joining the telocentrics at the centromere can create a pseudo- or quasi-linkage (Rieger et al. 1976) between the hemizygous arms. Translocations with their breakpoint at the centromere are called Robertsonian translocations (Rieger et al. 1976). Among common wheat cultivars, reciprocal Robertsonian translocations exist in the cultivars 'Capelle Desprez' (5BS/7BS and 5BL/7BL; Riley et al. 1967; Law and Worland 1996) and 'Alcedo' (3BS/6BS and 3BL/6BL; Kazman and Lein 1996; Friebe and Gill 1994). Our hypothetical case however represents one non-reciprocal Robertsonian translocated chromosome plus the two standard chromosomes from which it was derived. Non-reciprocal Robertsonian translocations are more commonly encountered as alien introgressions (Zeller 1973; Thomas et al. 1998) in which case there is a partial substitution. In our case the reciprocal of the translocated chromosome is missing. The genetic deficiency of these plants is the same as the combined deficiency of a corresponding dual monotelodisomic plant but it is arranged differently. No simple term exists to describe these plants. Technically they are non-reciprocal Robertsonian translocation heterozygotes ($2n$=41=19ii+1iii). As a shorter alternative, we propose the term 'fusion monosomic' since they are hemizygous for two half chromosomes, which is a deficiency comparable to that of a monosomic but with the two remaining arms fused at the centromere into a single translocated chromosome.

Evidence that this chromosome configuration might generate a quasi-linkage arose during the isolation ('extraction') of 'tetra-Prelude' ($2n$=4$x$=AABB) from 'Prelude' ($2n$=6$x$=AABBDD) (Kaltsikes et al. 1968, 1970). Such derived tetraploids are obtained by backcrossing a hybrid between common wheat and durum (*Triticum turgidum* L.; AABB) with common wheat and selecting for 14 chromosome gametes from the pentaploid ($2n$=35=AABBD=14ii+7i; Kerber 1964). After selfing to recover 'tetra-Prelude', a study of the pentaploid hybrid between 'Prelude' and 'tetra-Prelude' revealed a chain trivalent (1iii+13ii+6i) at meiosis suggesting that a translocation between the D genome and the A or B genome had occurred during this process. Presumably, the trivalent represents a translocated chromosome lying in the centre with two standard non-homologous chromosomes lying on either side. In the photograph that was presented, the trivalent was shown co-oriented as a 'V'. Disjunction of the trivalent from this 'V' configuration will assort the two standard chromosomes together into gametes which have a minimum count of 15 while the central, translocated chromosome will segregate into gametes with a minimum count of 14. While no numerical data were presented on the co-orientation of the trivalent, an attempt was made to eliminate the translocation from 'tetra-Prelude' by backcrossing to 'Prelude' (P.J. Kaltsikes, personal communication). This did not succeed. Since 14 chromosome gametes without the translocation were not recovered from the pentaploid, 'V' configurations may have predominated to create an association (quasi-linkage) between the two standard chromosomes which were then discarded together based on the chromosome counts.

Our present study will show that introducing an appropriate non-reciprocal Robertsonian translocation (fusion monosome) into an $F_1$ does create a quasi-linkage between genes from different chromosomes and alters their assortment in a way that enforces the fixation of desirable two-gene stacks. Details are presented on the isolation and cytology of two fusion monosomics in common wheat and their effect on the genetics of loci that were rendered hemizygous by the rearrangement.

## Materials and methods

### Understanding the trivalent

To illustrate the link between the meiotic behaviour of a Robertsonian trivalent and its genetics, Fig. 1 shows how the translocated chromosome synapses with the two standard chromosomes from which it was derived while Fig. 2 shows how the resulting trivalent disjoins at MI. In Fig. 1, the translocated chromosome occupies the centre of the assemblage and synapses with the two standard chromosomes on either side in an open trivalent. During diplotene and diakinesis this trivalent condenses and is then stretched and co-oriented on the metaphase spindle (Fig. 2). As Fig. 2 shows, assortment of the two standard chromosomes at first anaphase depends on the way that the trivalent co-orients at first metaphase. Two stable co-orientations are possible. If the trivalent co-orients into a 'V' configuration, the two standard chromosomes will assort to the same pole while if the trivalent co-orients into an 'I', the two standard chromosomes will segregate to opposite poles (Fig. 2). The extent to which the two standard chromosomes assort together or segregate thus depends on whether 'V's or 'I's predominate and the genetics of the trivalent is best understood if this is kept in mind.

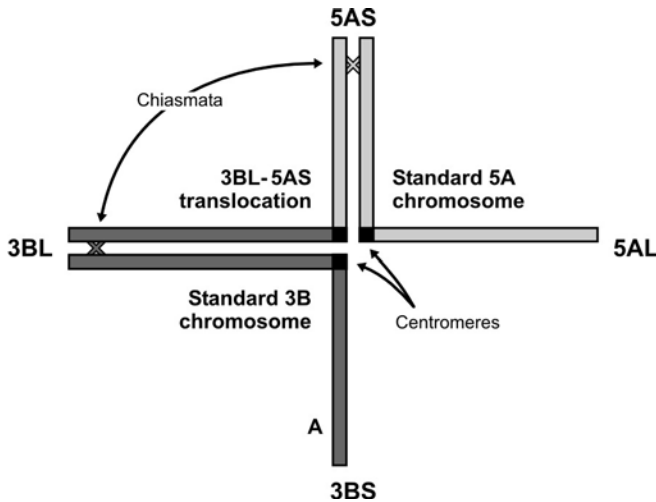


**Fig. 1** Pachytene schematic of the open trivalent that is expected from synapsis between a non-reciprocal Robertsonian translocation (centric fusion) involving the long arm of chromosome 3B and the short arm of chromosome 5A with standard versions of chromosomes 3B and 5A

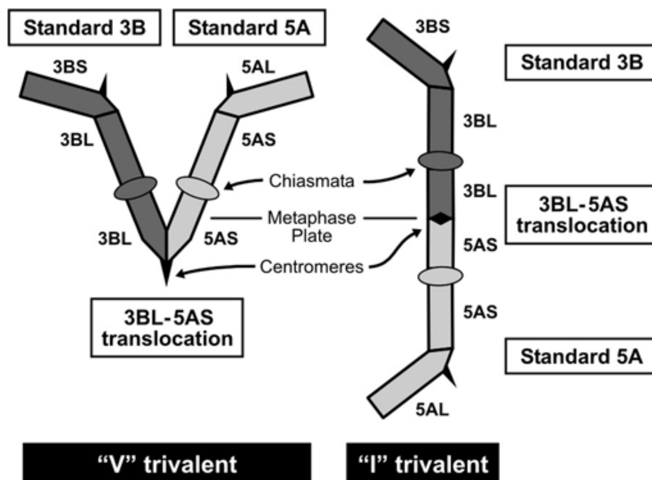


**Fig. 2** First metaphase schematic of stable co-orientations expected at first MI for the open trivalent of a non-reciprocal Robertsonian translocation 3BL-5AS pairing with standard versions of these two chromosomes. These metaphase trivalents are derived from the pachytene trivalent depicted in Fig. 1

## Isolation of translocations

Where necessary, additional plants were obtained from individuals of particular interest by sub-dividing their crowns (Thomas et al. 1997). Fusion monosomics ($2n$=41=1iii+19ii) of common wheat (*T. aestivum* L.) were isolated from crosses between a double monosomic ($2n$=40=19ii+2i) and a disomic. DNA markers (microsatellites) were used to identify double breaks while meiotic analysis and multi-color fluorescence in situ hybridization with genomic DNA (mcGISH) demonstrated that reunion of the breaks had created a translocation between the monosomes.

A speltoid triple-monosomic, (plant A1080) obtained by pollinating a haploid plant from the cross of 'Augusta'/'Hard White Alpha' with 'Superb' (Thomas et al. 2001) and lacking chromosomes 3B, 4B and 5A, was crossed with 'Snowbird'. Two speltoid plants (A2675 and B47), monosomic for 3B plus 5A were identified in the progeny based on pairing data and DNA markers. These were crossed with 'Superb'. No polymorphic microsatellites were found on the short arm of chromosome 3B due to common parentage between 'Snowbird' and 'Superb' (i.e. 'AC Domain'). Additional speltoid double monosomics (5A and 3B) were identified among the progeny of A2675 × 'Superb' and B47 × 'Superb', based on microsatellites for 5A, absence of a SCAR marker from 3BL of 'Snowbird' and meiotic pairing (19ii+2i). Meanwhile other cultivars were screened for an informative suite of microsatellite alleles on 3B and 5A. 'AC Cadillac' was distinguishable from 'Superb' by four microsatellite alleles specific to the four chromosome arms (3BS, 3BL, 5AS and 5AL). Egg and pollen mother cells containing the monosomes passed through meiosis under fluorescent 'Cool White' light plus supplemental tungsten lamping on a 16/8 h, 18/16°C cycle. Eight double monosomics (3B and 5A) with monosomes derived from 'Superb' were hybridized as male and as female with 'AC Cadillac' and the testcross was screened for double breaks using appropriate markers.

## DNA markers

DNA was extracted following the CTAB method with addition of 50 µl of 20 mg/ml proteinase K to 20 ml of the extraction buffer and replacement of 400 µl phenol-chloroform with 400 µl isoamyl alcohol-chloroform. For leaf discs, DNA was resuspended in 100 µl 0.1× TE buffer plus RNAse while for crushed half-seeds this volume was 50 µl. Amplification of microsatellite loci was as described by Röder et al. (1998) using 2 µl of resuspended DNA. PCR cycles were uniform (30 cycles—95°C for 1 min, 50, 55 or 60°C for 1 min, 73°C for 1 min and back to 95°C ramping at 0.5°C/s). Fragments were separated at 85 W in a 96-lane polyacrylamide gel (500 mm long × 0.4 mm thick) and were stained with silver (Promega, Madison, Wis., USA).

## Cytology

Preparation of mitotic metaphase chromosomes for in situ hybridization was as described by Benabdelmouna et al. (2001). Three diploid species were used for genomic probes and/or DNA blocking [*T. urartu* ($2n$=$2x$=14, AA); *T. tauschii* ($2n$=$2x$=14, DD) and *Aegilops speltoides* ($2n$=$2x$=14, BB)]. mcGISH of the chromosomes was obtained as described by Han et al. (2003). After stringent washings, a digoxigenin-labeled A genome probe was revealed in tan or yellow to aqua with anti-dig-FITC (Roche) while a biotinylated D genome probe was revealed in pink to red with Avidin-Texas red (Vector Laboratories). Under these conditions, chromosomes of

the B genome fluoresced grey to purplish blue. Chromosomes were counter-stained with 1 μg/ml DAPI (4′,6-diamidino-2-phenylindole) and were mounted in Vectashield (Vector Laboratories). Chromosome pairing of putative translocations was assessed from acetocarmine squashes of pollen mother cells (PMC) obtained from young spikes fixed for 24 h in >20 ml of cold, fresh fixative (six parts 95% ethanol, three parts chloroform, one part acetic acid) and leached with two changes of cold 70% ethanol for 4–7 days. Anthers in metaphase 1 were placed on a slide, blotted to remove excess ethanol and were macerated in 45% acetocarmine with the back of a no. 11 scalpel. Anther remains were removed, the slide was covered and briefly heated and PMC were spread with light pressure on the coverslip. Slides were made semi-permanent by irrigating with one part glycerine to nine parts 45% acetic acid. About 100 PMC in first metaphase were analyzed per translocation. Trivalents were classified as ‘V’s (standard or end chromosomes oriented to one pole and the translocation oriented to the other), as ‘I’s (end chromosomes oriented to opposite poles with the translocation un-oriented in the middle) or as ‘L’s, (one end chromosome unoriented). Pairing of the remainder of the complement was scored as univalents, bivalents (ring or rod) plus a few other multivalents.

## Genetics of the translocation

The genetic effects of the translocation were assessed from the segregation of polymorphic microsatellite loci in a testcross of the fusion monosomic as male and female with ‘Superb’. Inheritance of the same loci was also studied in doubled haploid populations obtained from ovules of the fusion monosomics by corn pollination (Thomas et al. 1997). Particular microsatellites that were used are listed in the tables. Chromosome locations of markers listed as

**Table 1** Chromosome constitution of gametes recovered from double monosomic 3B-5A testcrossed with ‘AC Cadillac’ as revealed by presence of microsatellite alleles specific to the four chromosome arms (3BS, 3BL, 5AS and 5AL)

| Chromosome arms present as deduced from microsatellites[a] | | | | Number of gametes in group | Number of gametes tested for break[b] | | Number of centromeres tested for break[c] | | Number of breaks detected[d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| 3BS | 3BL | 5AS | 5AL | | In 3B | In 5A | In 3B | In 5A | In 3B | In 5A |
| Male testcross | | | | | | | | | | |
| + | + | + | + | 65 | 65 | 65 | 65 | 65 | 0 | 0 |
| + | + | + | ? | 52 | 52 | 0 | 52 | 0 | 0 | 0 |
| + | + | ? | ? | 8 | 8 | 0 | 8 | 0 | 0 | 0 |
| + | + | − | + | 4 | 4 | 4 | 4 | 4 | 0 | 4 |
| + | − | + | + | 5 | 5 | 5 | 5 | 5 | 5 | 0 |
| − | + | + | + | 3 | 3 | 3 | 3 | 3 | 3 | 0 |
| − | − | + | + | 17 | 17 | 17 | 0 | 17 | 0 | 0 |
| − | − | + | ? | 24 | 24 | 0 | 0 | 0 | 0 | 0 |
| − | − | ? | ? | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| − | − | ? | + | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Male totals | | | | 180 | 180 | 94 | 137 | 94 | 8 | 4 |
| Female testcross | | | | | | | | | | |
| + | + | ? | ? | 18 | 18 | 0 | 18 | 0 | 0 | 0 |
| + | + | − | + | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| + | + | − | − | 18 | 18 | 18 | 18 | 0 | 0 | 0 |
| + | − | + | − | 1[e] | 1 | 1 | 1 | 1 | 1 | 1 |
| + | − | − | − | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| − | − | ? | ? | 68 | 68 | 0 | 0 | 0 | 0 | 0 |
| − | − | − | ? | 114 | 114 | 0 | 0 | 0 | 0 | 0 |
| − | − | − | − | 5 | 5 | 5 | 0 | 0 | 0 | 0 |
| − | − | + | + | 15 | 15 | 15 | 0 | 15 | 0 | 0 |
| − | − | − | + | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| − | + | + | + | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| − | + | + | − | 1[f] | 1 | 1 | 1 | 1 | 1 | 1 |
| − | + | − | − | 6 | 6 | 6 | 6 | 0 | 6 | 0 |
| − | + | ? | ? | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| − | ? | − | ? | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| ? | − | − | ? | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| ? | ? | ? | ? | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Female totals | | | | 258 | 251 | 50 | 48 | 20 | 11 | 4 |

[a] 3BS is scored from *gwm533*, 3BL from *gwm340*, 5AS from *gwm205* and 5AL from *gwm595*. Alleles present in the monosomes are derived from ‘Superb’: viz. High, High, High and Low respectively while ‘AC Cadillac’ alleles are Low, Low, Low and High respectively. Plants which inherit a particular arm from the monosome (+) are dimorphic (High/Low). Plants which lack the arm from the monosome (–) show the ‘AC Cadillac’ allele only. ? indicates no data

[b] A gamete is tested for a break where loci from both the long and the short arm are tested

[c] A centromere is absent if both arms are absent

[d] A break is detected where the tests for the long and short arms differ

[e,f] Double breaks and translocations: [a]3BS-5AS, plant C1783; [b]3BL-5AS, plant C845

tentative in Röder et al. (1998) have been confirmed (D.J. Somers, unpublished).

## Results

### Chromosome breakage

Breaks were detected by screening seedlings or seeds for the presence of one arm of a monosome but not the other based on recovery of specific microsatellite alleles from the monosomes. In the male test cross, 180 seedlings were tested (Table 1). Break rates were calculated both per gamete and in terms of how many of the recovered centromeres had in fact broken (see Table 1). While most of the male gametes that were recovered in the male testcross inherited chromosome 3B intact, the entire deficiency (i.e. monosomic 3B) as well as breakage products with 3BS and 3BL were also recovered from pollen. About 4.5% of male gametes (8/180; cf. Table 1) showed a break in 3B (loss of one arm only). Per centromere, the break rate was about 6% (8/137). Since individuals without a break in 3B cannot carry a non-reciprocal translocation, many of these plants were not tested further and so the number of cases tested for 5A was smaller than for 3B. Here, 4 of 94 individuals showed a break in 5A (4.5%). No plant with a break in both 3B and 5A was recovered nor were any 5A monosomics or 5AS breakage products recovered in this group (i.e. 5AL deficiency was not recovered through the pollen). Consequently the male break rate calculated per 5A centromere is the same as the rate calculated per male gamete (4/94=4.5%). In the female test cross, 258 plants were extracted and 251 yielded useful data (Table 1). For 3B, 11 breaks were detected out of the 251 tested individuals (4.5%) while for 5A the corresponding number was 4 of 50 (8%). Per centromere, the break rates were 11 of 48 (23%) and 4 of 20 (20%) for 3B and 5A, respectively (Table 1). Two plants (C845 and C1783) were recovered with a break in both 3B and 5A. (Note that one individual with a break in 3B was not successfully tested for a break in 5A.) On the basis of microsatellites, plant C845 inherited the short arm of 5A of 'Superb' but not the long arm and the long arm of 3B of 'Superb' but not the short arm. Plant C1783 contained microsatellite alleles from the short arms of chromosomes 3B and 5A of 'Superb' while alleles from both long arms were absent. As expected, C845 and C1783, with a single dose of 5AL, were both speltoid.

### Cytology of double breaks

Multi-color genome-specific fluorescence of root-tip chromosomes (Fig. 3) from C845 and C1783 demonstrated the presence of a translocation between an A and a B genome chromosome fused at the centromere in both plants. From Fig. 3 the translocation in C845 (3BL/5AS) is subterminal while in C1783 (3BS/5AS) it is submedian (see Gill et al. 1991). Note that the C1783 translocation was stained with mcGISH in appropriate testcross progeny (i.e. individuals of C1783 × 'Superb' lacking microsatellite alleles from 'AC Cadillac' on 3BL and 5AL) rather than in C1783 itself. About 100 PMC each from C845 and C1783 were analyzed for first metaphase pairing. Pairing trends of the two plants were similar (Table 2). For both plants, most first metaphase PMC contained a chain trivalent (Fig. 3; Table 2). In two PMC of C845, the 5AS-3BL trivalent paired up with two other chromosomes to form an alternately disjoined linear pentavalent. Otherwise both translocations paired conventionally with 79–85% of PMC containing the trivalent (Table 2). About 73–77% of cells contained a 'V' type trivalent, 3–9% of cells contained an 'I' trivalent (end chromosomes pulling in opposite directions) and 2% contained an 'L' trivalent (one end chromosome unoriented). Among cells in which the trivalent was seen, 90–94% were oriented as a 'V'. (The two pentavalents seen in C845 were disjoined alternately, so for this calculation these cells were counted as 'V's). For five cells out of 203 (2.5%) it was not possible to logically infer the state of the translocation (i.e. cells with no trivalent plus one or more rods plus three univalents). In these cells it is more likely the translocation paired as a rod plus a univalent and less likely that all three univalents derived from the translocation; however this latter case creates a rare possibility for complete non-disjunction of the trivalent (see next paragraph).

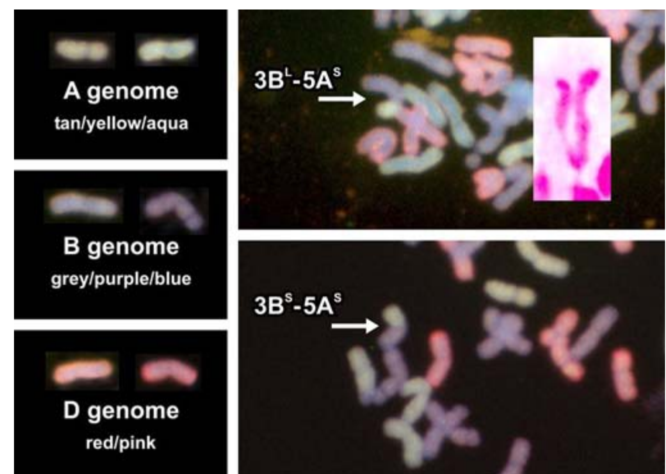


**Fig. 3** Mitotic and meiotic chromosomes of translocation lines. Mitotic chromosomes show mcGISH as described in Materials and methods. Specimen chromosomes from each genome (one per metaphase) are shown in the three frames on the *left*. *Tan/yellow/aqua* chromosomes are from the A genome, *blue/grey/purple* chromosomes are from the B genome and *red/pink* chromosomes are from the D genome. The *top right* frame shows the large sub-terminal Robertsonian translocation from C845 involving a short arm from the A genome (5AS) and a long arm from the B genome (3BL) while the *insert* shows this chromosome (stained with acetocarmine) paired as a 'V' trivalent with its two standard partners at metaphase 1. The *lower right* frame shows the sub-median translocation between the A genome (5AS) and the B genome (3BS) from a progeny of C1783

**Table 2** Meiotic chromosome pairing in two fusion monosomics of wheat

| Plant | V[a] Open | IV Ring | IV Open | III 'V'[b] | III 'I' | III 'L' | III Total | III 'V'/total | II Ring | II Rod | I Unpaired | 2*n* | Number of cells |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C845 | 0.02[c] | 0.05 | 0.07 | 0.76 | 0.07 | 0.02 | 0.87 | 0.90 | 16.74 | 2.02 | 0.35 | 41 | 100 |
| C1783 | 0.00 | 0.09 | 0.11 | 0.74 | 0.03 | 0.02 | 0.79 | 0.94 | 16.54 | 2.19 | 0.39 | 41 | 103 |
| Average | 0.01 | 0.07 | 0.09 | 0.75 | 0.05 | 0.02 | 0.83 | 0.92 | 16.64 | 2.11 | 0.37 | 41 | – |

[a] *V* Pentavalent, *IV* quadrivalent, *III* trivalent, *II* bivalent, *I* univalent
[b] '*V*', '*I*' and '*L*' indicates co-orientation of the trivalent; see Materials and methods for explanation
[c] Average number observed per PMC

## Genetics of hemizygous loci

Since no recombination is expected in the hemizygous arms involved in these rearrangements, all loci present on these arms are linked to the centromere and act as a marker for the standard chromosome. Reciprocal absence of a marker however does not necessarily mark the translocation. The genetic segregation recorded in Tables 3 and 4 stems from pairing, recombination and segregation of a fusion monosome derived from chromosomes of 'Superb' with standard chromosomes from 'AC Cadillac' in a testcross with 'Superb'. Gametes which inherit none out of

**Table 3** Genetic segregation of microsatellite markers on chromosomes 3B and 5A in ovules and pollen of fusion monosomic 3BL-5AS (plant C845 testcrossed with 'Superb'). Chromosome arms are listed in the headings in the order in which they are found in the trivalent while *high* and *low* indicates the relative molecular size of the alleles

| Type of disjunction[a] | Chromosome 3B | | Chromosome 5A | | Parental (P) or recombinant (R) | | Number of individuals | |
|---|---|---|---|---|---|---|---|---|
| | 3BS *gwm389* | 3BL *gwm340* | 5AS *gwm205* | 5AL *gwm595* | On 3BL[c] | On 5AS[b] | Ovules | Pollen |
| 'V' (alternate) | | | | | | | | |
| 3BS+5AL | High/low | High/low | High/low | High/low | P | P | 20 | 45 |
| 3BS+5AL | High/low | High/low | High | High/low | P | R | 8 | 6 |
| 3BS+5AL | High/low | High | High/low | High/low | R | P | 19 | 29 |
| 3BS+5AL | High/low | High | High | High/low | R | R | 6 | 4 |
| Total | | | | | | | 53 | 84 |
| Null+null | Low | High | High | Low | P | P | 23 | 0 |
| Null+null | Low | High | High/low | Low | P | R | 12 | 0 |
| Null+null | Low | High/low | High | Low | R | P | 19 | 0 |
| Null+null | Low | High/low | High/low | Low | R | R | 14 | 1 |
| Total | | | | | | | 68 | 1 |
| 'I' (adjacent) | | | | | | | | |
| 3BS+null | High/low | High/low | High | Low | P | P | 4 | 1 |
| 3BS+null | High/low | High/low | High/low | Low | P | R | 1 | 0 |
| 3BS+null | High/low | High | High | Low | R | P | 3 | 1 |
| 3BS+null | High/low | High | High/low | Low | R | R | 1 | 0 |
| Total | | | | | | | 9 | 2 |
| Null+5AL | Low | High | High/low | High/low | P | P | 1 | 4 |
| Null+5AL | Low | High | High | High/low | P | R | 0 | 1 |
| Null+5AL | Low | High/low | High/low | High/low | R | P | 1 | 0 |
| Null+5AL | Low | High/low | High | High/low | R | R | 0 | 1 |
| Total | | | | | | | 2 | 6 |
| C845 | High | High/low | High/low | High | Overall totals | | 132 | 93 |
| 'Superb' | Low | High | High | Low | | | | |
| 'AC Cadillac' | High | Low | Low | High | | | | |

[a] 'V' type ovules [(3BS+5AL)+(null+null)=53+68=121] exceed 'I' types [(3BS+null)+(null+5AL)=9+2=11]; [$\chi^2(1:1)=91.7$, $P<0.001$]; (3BS+5AL, $n=53$) and (null +null, $n=68$) appear equally frequent [$\chi^2(1:1)=1.86$, n.s.]. For pollen the non-deficient class (3BS+5AL) was more frequent ($n=84$), than the other three classes combined [(null+null)+(3BS+null)+(null+5AL)=1+2+6=9] [$\chi^2(1:1)=60.5$, $P<0.001$]. Percentages of 'V' types: for ovules=121/132=92%; for pollen=85/93=91%
[b] Recombination between *gwm340* and the centromere (*gwm389*) was homogeneous (for ovules=69/132; for pollen=36/93; $\chi^2=4.0$, 3 *df*, $0.1<P<0.5$) and showed no linkage [105/225=0.47; $\chi^2(1:1)=1.0$, 1 *df*, $P>0.05$]
[c] Recombination between *gwm205* and the centromere (*gwm595*): for ovules=44/132=33% [$\chi^2(1:1)=14.7$, $P<0.001$]; for pollen=14/93=15%; [$\chi^2(1:1)=45.4$, $P<0.001$]. These rates are heterogeneous ($\chi^2=9.5$, 3 *df*, $P<0.025$)

three chromosomes from the trivalent will be indistinguishable from gametes with a non-recombinant translocation since both will display only markers from 'Superb'. Likewise, those gametes which inherit all three chromosomes from the trivalent are indistinguishable from standard karyotypes since for these any combination of markers is possible. However both of these categories (i.e. no chromosomes or three chromosomes) require non-disjunction of all three chromosomes involved in the trivalent. Based on the meiotic data, complete non-disjunction of the trivalent is expected in a tiny minority of PMC and therefore such misclassification should be rare (see previous paragraph).

Since the markers under discussion are normally present on separate chromosomes (Röder et al. 1998) they are normally expected to assort independently (25% of gametes to inherit both). However, the presence of the 3BL-5AS fusion monosome in C845 created an association between loci on 3BS and 5AL (Table 3) with 53 of 132 ovules (40%) inheriting both markers. Likewise, with the 3BS-5AS fusion monosome in C1783, 47 of 92 gametes (51%) inherited markers on 3BL and 5AL (Table 4). A corresponding class inherited neither marker from the translocation stock (null-null ovules) in comparable proportions [52% and 41% for C845 (Table 3) and C1783 (Table 4), respectively]. Plus-plus ovules represent the joint disjunction of the two standard chromosomes that make up the sides of the 'V' trivalent while null-null ovules arise from opposed segregation of the translocation from the base of the 'V' (Fig. 2). Thus for both translocations, about 92% of ovules appeared to derive from 'V' type (alternate) disjunctions. The remaining 8% (plus-null and null-plus ovules) arose from 'I' type disjunction.

When the fusion monosomics were used as male, gametic frequencies were further distorted by selection for

**Table 4** Genetic segregation of microsatellite markers on chromosomes 3B and 5A in ovules and pollen of fusion monosomic 3BS-5AS (plant C1783 testcrossed with 'Superb'). Chromosome arms are listed in the headings in the order in which they are found in the trivalent while *high* or *low* indicates the relative molecular size of the alleles

| Type of disjunction[a] | Chromosome 3B | | Chromosome 5A | | Parental (P) or recombinant (R) | | Number of individuals | |
|---|---|---|---|---|---|---|---|---|
| | 3BL *gwm340* | 3BS *gwm389* | 5AS *gwm205* | 5AL *gwm595* | On 3BS[c] | On 5AS[b] | Ovules | Pollen |
| 'V' (alternate) | | | | | | | | |
| 3BL+5AL | High/low | High/low | High/low | High/low | P | P | 23 | 44 |
| 3BL+5AL | High/low | High/low | High | High/low | P | R | 1 | 14 |
| 3BL+5AL | High/low | Low | High/low | High/low | R | P | 21 | 22 |
| 3BL+5AL | High/low | Low | High | High/low | R | R | 2 | 12 |
| Total | | | | | | | 47 | 92 |
| Null+null | High | Low | High | Low | P | P | 22 | 0 |
| Null+null | High | Low | High/low | Low | P | R | 0 | 0 |
| Null+null | High | High/low | High | Low | R | P | 15 | 0 |
| Null+null | High | High/low | High/low | Low | R | R | 1 | 0 |
| Total | | | | | | | 38 | 0 |
| 'I' (adjacent) | | | | | | | | |
| 3BL+null | High/low | High/low | High | Low | P | P | 3 | 1 |
| 3BL+null | High/low | High/low | High/low | Low | P | R | 0 | 0 |
| 3BL+null | High/low | Low | High | Low | R | P | 0 | 0 |
| 3BL+null | High/low | Low | High/low | Low | R | R | 0 | 0 |
| Total | | | | | | | 3 | 1 |
| Null+5AL | High | Low | High/low | High/low | P | P | 3 | 0 |
| Null+5AL | High | Low | High | High/low | P | R | 0 | 0 |
| Null+5AL | High | High/low | High/low | High/low | R | P | 1 | 0 |
| Null+5AL | High | High/low | High | High/low | R | R | 0 | 0 |
| Total | | | | | | | 4 | 0 |
| C1783 | Low | High/low | High/low | High | Overall totals | | 92 | 93 |
| 'Superb' | High | Low | High | Low | | | | |
| 'AC Cadillac' | Low | High | Low | High | | | | |

[a]'V' type ovules [(3BL+5AL)+(null+null)=47+38=85] exceeded 'I' types [(3BL+null)+(null+5AL)=3+4=7]; [$\chi^2$(1:1)=66.1, $P$<0.001]; (3BL+5AL=47) and (null+null) types ($n$=38) were equally frequent [$\chi^2$(1:1)=1.0, n.s.]. For pollen the non-deficient class (3BL+5AL=92) was more frequent than the other three classes combined [(null+null)+(3BL+null)+(null+5AL)=0+1+0=1] [$\chi^2$(1:1)=89.0, $P$<0.001]. Percentages of 'V' types: for ovules=85/92=92%; for pollen=92/93=99%

[b]Recombination between *gwm389* and the centromere (*gwm340*) was homogeneous for ovules (40/92) and pollen (34/93) ($\chi^2$=1.0, 3 *df*, $P$>0.05) and suggested slight linkage [74/185=0.40; $\chi^2$(1:1)=7.4, 1 *df*, $P$>0.01]

[c]Recombination between *gwm205* and the centromere (*gwm595*): for ovules=4/92=4% [$\chi^2$(1:1)=80.7, $P$<0.001]; for pollen=26/93=28% [$\chi^2$(1:1)=45.4; $P$<0.001]. These rates are heterogeneous ($\chi^2$=27.7, 3 *df*, $P$<0.001)

euploid pollen (certation). The principal sign of this was a shortage of null-null pollen compared to the frequency of null-null ovules (Tables 3, 4). The stringency of this certation may vary since only one pollen grain with one deficiency was recovered from C1783 out of 93 examined while for C845, 9 of 93 plants were recovered with one or two deficiencies (heterogeneity $\chi^2$=6.76, 3 *df*, 0.05<*P*<0.10). Distorted inheritance was also noted when the two fusion monosomics were used to produce doubled haploid lines (DHL) by pollination with corn (Table 5). Microsatellite markers indicated that about 75% of haploids inherited the standard karyotype. (This compares to the source population of ovules in which the frequency of standard karyotypes was about 45%; see Tables 3, 4 ). Of these standard haploids, about 86% (119/157) set seed following colchicine treatment. Recovery of seed following colchicine treatment of null-plus or plus-null haploids (one chromosome arm missing; see Table 5) was less successful (4/12 plants set seed) while no seed was recovered from any null-null haploids (two chromosome arms missing; 0/26 plants set seed, see Table 5). These data demonstrate that doubled haploids in which one or more deficiencies were fixed were less viable and more sterile than their standard counterparts.

To summarize, compared to a conventional digenic segregation where one quarter of gametes will contain both genes, our data suggests that about 45% of ovules and 95% of pollen from a fusion monosome will be plus/plus (i.e. contain both genes) while most of the residue will be null/null (i.e. contain neither). Based on the simplifying assumption that all residual gametes are null/null, calculation suggests that 90% of segregates will have fixed the targeted genes by $F_6$ (Fig. 4) while most of the remainder will have fixed the deficiency. This compares with a conventional segregation where the asymptote is 25%. In reality, additional hemizygous karyotypes will emerge from the segregation of fusion monosomics at low but non-negligible frequencies. These are near-disomics (a standard pair, a standard monosome and a translocation) and regular monosomics; these will also revert to disomics over time. Eventually, inbreeding will eliminate all hemizygous karyotype genotypes leaving only standard karyotypes (disomics) and fixed non-standard karyotypes with their deficiencies exposed to selection. Data from the doubled haploids confirms these latter are at a reproductive disadvantage which will tend to leave only disomics. In practice, the rate at which these changes occur needs to be measured for useful translocations and in real populations.

## Genetics of heterozygous loci

Recombination within the arms of the translocation (Tables 3, 4) was consistent with the reported map positions of the loci that were scored (Röder et al. 1998). In each case any locus present on the hemizygous arm serves as a marker for the centromere. As expected, *gwm205* (located sub-proximally on 5AS) was linked to the centromere. All four recombination estimates were significantly lower than 50% with a mean value of 20%; however both translocations (C845 and C1783) showed significant heterogeneity for recombination in this interval between male and female gametes (Tables 3, 4). *Gwm340* (located distally on 3BL) showed no linkage to the centromere (recombination was 47%; Table 3) while *gwm389* (distal on 3BS) showed slight linkage (recombination was 40%; Table 4). Recombination in both intervals on 3B was homogeneous between male and female gametes.

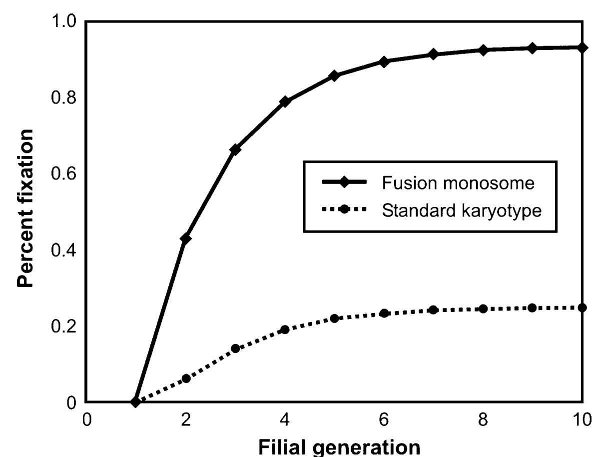


**Fig. 4** Effect of a non-reciprocal Robertsonian translocation heterozygote (fusion monosome) on the fixation of targeted genes present on the hemizygous arms of the translocation predicted from the segregations observed in Tables 3 and 4 (see the discussion in Results section) compared with the fixation of the same genes expected from segregation under standard Mendelian conditions

**Table 5** Karyotype[a] and doubling[b] of haploids obtained from fusion monosomics by corn pollination

| Plant | Spikes pollinated | Florets pollinated | Caryopses dissected | Embryos | Transplants | Haploids with indicated karyotype[a] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 3B+5A | 3B+5AS | 3BL+5A | 3BL+5AS |
| C845 | 31 | 731 | 637 | 168 | 68 | 52 (45) | 3 (0) | 2 (1) | 11 (0) |
| | | | | | | 3B+5A | 3B+5AS | 3BS+5A | 3BS+5AS |
| C1783 | 75 | 1416 | 1133 | 211 | 89 | 67 (59) | 6 (3) | 1(0) | 15 (0) |

[a]Karyotype inferred from presence/absence of an arm-specific microsatellite (for 3BS, *gwm533* or *gwm389*; for 3BL, *gwm340*; for 5AS, *gwm205* and for 5AL, *gwm295*). Plants with all four arms present contain both standard chromosomes. Plants with one arm missing contain a standard chromosome plus the translocation. Plants with two arms missing contain only the translocation. One DHL from C1783 may have inherited two standard chromosomes plus the translocation

[b]Figure in parenthesis is the number of plants in the group that set one or more seed following colchicine treatment

## Discussion

### Chromosome breakage and repair

Translocation may occur in the progeny of a double monosome only if chromosome breaks occur often enough to coincide in the same cell and close enough to serve as partners in repair. Even where the two chromosomes break, presumably the most likely candidates for rejoining are the original chromosome arms and even one such repair will preclude the possibility of translocation. Conversely, rejoining non-homologous arms (translocation) precludes restoration of the normal structure. Out of 431 testcross individuals, two seeds showed microsatellite constitutions indicative of breaks in both chromosomes (5A and 3B). Since both double breaks proved to be a Robertsonian translocation rather than two telocentrics, this suggests either that few breaks fail to rejoin or that many unrejoined breaks (i.e. nascent telocentrics) are lost.

### Trivalent co-orientation and the spindle checkpoint

The stability of the chromosome number in eukaryotes testifies to the accuracy of mitosis and meiosis. This accuracy is enforced by two factors. Metaphase is a state of dynamic equilibrium. Chromosomes are attached by their centromeres to a spindle that exists in a state of continuous and rapid turnover (Wittmann et al. 2001). Despite this turnover, these attachments are stabilized by tension between the spindle and the centromere, and if tension is removed the attachment dissolves (Nicklas 1987; Nicklas et al. 1995). A second factor ensuring stable chromosome numbers is the spindle checkpoint (Elledge 1996; Yu et al. 1999). Unoriented centromeres are detected in the cell through the chemistry of special proteins that bind to centromeres that have no active spindle attachment (i.e. are not under tension). One function of these bound proteins is to extend meiotic and mitotic metaphase until all the chromosomes are stably aligned. Readiness to extend or terminate metaphase is influenced by the presence of just one unattached centromere (Nicklas et al. 1995; Li and Nicklas 1995). While most of this evidence comes from animal cells, biochemical elements of the system have been detected in the mitosis and meiosis of corn (Yu et al. 1999). Existence of a spindle checkpoint in the meiosis of interspecific wheat hybrids is also suggested by the fact that pollen mother cells with many univalents are the last to disjoin (Wagenaar 1961a, b).

In a ‘V’ configuration, centromeres of the end or standard chromosomes of the Robertsonian trivalent pull together toward the same pole while the middle translocated chromosome pulls toward the opposite pole. Since ‘V’ configurations predominate, they must somehow be preferred. In the case of an ‘I’ trivalent, centromeres of the end chromosomes pull to opposite poles while the centromere of the middle chromosome is unoriented. If this unoriented centromere affects checkpoint biochemistry of the spindle, then these particular metaphases will be preferentially extended. Suppose the centromere of the middle chromosome of an ‘I’ trivalent now acquires a definite orientation toward either pole. The resulting reorganization will stabilize attachment of the opposed end chromosome but relax and detach the unopposed end chromosome. This will convert the ‘I’ trivalent to an ‘L’. The unopposed end chromosome is now free to reorient, placing the trivalent in the preferred ‘V’ configuration. In essence we suggest that ‘I’ and ‘L’ configurations are metastable to ‘V’s and that many metaphases are prolonged until this configuration appears.

### Fusion monosomics in breeding

While fusion monosomics provide a means to fix preselected genotypes without affecting segregation in the remainder of the genome their use in making crosses for wheat breeding will restrict the choice of parents to plants with the fusion monosome and plants carrying the target genes. Crosses therefore must be planned with this in mind. Fresh variability can be introduced into the background of the fusion monosome through pre-breeding. In a straight cross, new fusion monosomics should be identifiable using microsatellites but in a backcross, cytology will be required. A second complication exists for the trait donor. Since the fusion monosome will suppress crossover in the targeted chromosome arms, this guarantees the transfer of any unfavorable traits linked to the targeted gene if these are present in the trait donor. To provide opportunity for crossover and further upgrade the background, the trait donor may first be crossed with an elite line and its hybrid then crossed with hybrid fusion monosomic. These second round crosses are now segregating both for chromosome constitution and for recombination in the targeted arm. Fusion monosomic $F_1$s with crossover products carrying the desired genes can be identified based on the status of nearby markers and grown into large plants to generate $F_2$ seed. While some of these hemizygous $F_1$s may have crossed over next to the marker and thereby lose the gene, this is a general feature of marker use and not a particular fault of our method. In fact the hemizygous sampling aids in the elimination of unwanted recombinants. Only one or a few members of each $F_1$-derived family will need to be critically examined for adequate expression of the trait, since if one segregate is inappropriately recombinant then all of its sisters will also be the same.

### Fusion monosomics and haploidy

About four to six standard-karyotype DHL were recovered per hundred florets pollinated for the two fusion monosomics (Table 5). While we typically expect to recover many more DHL (say 15–25 per hundred) from a conventional $F_1$, only one quarter of these will share the genotype that was targeted by the fusion monosomic and

these would need to be identified. Therefore combining the use of fusion monosomics and doubled haploidy appears to be an effective option.

## Costs, benefits and applications

Since creation and use of fusion monosomics represents a significant complication in the conduct of wheat breeding, the benefit needs to be correspondingly large. The principal benefit is that once suitable $F_1$s are identified, fixation of the trait becomes more or less inevitable and further screening is theoretically unnecessary. This method is worth considering where it is desirable that most elite lines carry a problematic trait which they currently lack. For instance, a method to stack major disease resistance QTLs would assist breeding for quantitative resistance to diseases such as Fusarium Head Blight. As a second example, the stacking of race-specific genes for resistance to an obligate parasite such as leaf rust, has been suggested as a means to avoid sequential evolution of virulence. What has been lacking up to now is an efficient and reliable method to create and identify gene stacks. As a third example, fusion monosomics could be used to associate unrelated traits and transmit them as package where these depend on single genes.

## Conclusion

Fusion monosomics provide a means to accelerate production of particular two-locus genotypes. In a conventional cross, the frequency of desired genotypes converges on 25%. By contrast, those $F_1$s that combine desired alleles with the fusion monosome will yield a population in which the frequency of targeted genotypes approaches fixation (>90%). In plant breeding applications not only will the recovery of the desirable lines be increased but their recovery should be high enough that some of the resources expended on their screening could be reallocated to other traits.

**Acknowledgements** This idea originated from a conversation with P.J. Kaltsikes many years ago. The work was funded by Western Grains Research Foundation Saskatoon and by the Matching Investment Initiative of Agriculture and AgriFood Canada. J. Budzinski, D. Jones and L. Shead provided technical assistance.

## References

Benabdelmouna A, Shi Y, Abirached-Darmency M, Darmency H (2001) Genomic in situ hybridization (GISH) discriminates between the A and the B genomes in diploid and tetraploid *Setaria* species. Genome 44:685–690

Elledge SJ (1996) Cell cycle checkpoints; preventing an identity crisis. Science 274:1664–1672

Friebe B, Gill BS (1994) C-band polymorphism and structural rearrangements detected in common wheat (*Triticum aestivum*). Euphytica 78:1–5

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum* L.). Genome 34:830–839

Han FP, Fedak G, Benabdelmouna A, Armstrong K, Ouellet T (2003) Characterization of six wheat by *Thinopyrum intermedium* derivatives by GISH, RFLP and multicolor GISH. Genome 46:490–495

Kaltsikes PJ, Evans LE, Bushuk W (1968) Durum-type wheat with high bread-making quality. Science 159:211–213

Kaltsikes PJ, Evans LE, Larter EN (1970) Chromosome segregation in hybrids between extracted AABB tetraploids and common wheat varieties. Cytologia 35:242–251

Kazman ME, Lein V (1996) Cytological and SDS-PAGE characterization of 1994–95-grown European wheat cultivars. Annu Wheat Newsl 42, Items from Germany

Kerber ER (1964) Wheat: reconstitution of the tetraploid component (AABB) of hexaploids. Science 143:253–255

Law CN, Worland AJ (1996) Inter-varietal chromosome substitution lines in wheat revisited. Euphytica 89:1–10

Li X, Nicklas RB (1995) Mitotic forces control a cell cycle checkpoint. Nature 373:630–632

Nicklas RB (1997) How cells get the right chromosomes. Science 275:632–637

Nicklas RB, Ward S, Gorbsky GJ (1995) Kinetochore chemistry is sensitive to tension and may link miotic forces to a cell cycle checkpoint. J Cell Biol 130:929–939

Rieger R, Michealis A, Green MM (1976) Glossary of genetics and cytogenetics—classical and molecular, 4th edn. Springer, Berlin Heidelberg New York, pp 647

Riley R, Coucouli H, Chapman V (1967) Chromosomal interchanges and the phylogeny of wheat. Heredity 22:233–248

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Thomas J, Chen Q, Howes N (1997) Chromosome doubling of haploids of common wheat with caffeine. Genome 40:552–558

Thomas JB, Chen Q, Talbert L (1998) Genetic segregation and the detection of spontaneous wheat-alien translocations. Euphytica 100:261–267

Thomas JB, Riedel E, Penner G (2001) An efficient method for assigning traits to chromosomes. Euphytica 119:217–221

Thomas JB, Riedel E, Fox SJ (2003) An efficient method for breeding monogenic traits in wheat. In: Pogna NE, Romanò M, Pogna EA, Galterio G (eds) Proceedings of the 10th international wheat genetics symposium. Istituto Sperimantale per la Cerealicoltura, Rome, pp 171–174

Wagenaar EB (1961a) Cytological studies of the development of metaphase I in hybrids between *Triticum timopheevi* Zhuk. and *T. durum* Desf. Can J Bot 39:81–108

Wagenaar EB (1961b) Cytological studies of the development of metaphase I in *Triticum* hybrids. II. The behaviour of univalents in meiotic cell division. Can J Genet Cytol 3:204–225

Wittmann T, Hyman A, Desai A (2001) The spindle: a dynamic assembly of microtubules and motors. Nat Cell Biol 3:E28–E34

Yu HG, Muszynski MG, Dawe RK (1999) The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. J Cell Biol 145:425–435

Zeller FJ (1973) 1B/1R chromosome substitutions and translocations. In: Sears ER, Sears LMS (eds) Proceedings of the 4th international wheat genetics symposium. Agriculture Experiment Station, College of Agriculture, University of Missouri, pp 209–221