Introgression of *Aegilops speltoides* segments in *Triticum aestivum* and the effect of the gametocidal genes

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**INTRODUCTION**

Bread wheat (*Triticum aestivum*), one of the world’s leading sources of food, is an allopolyploid (6x = AABBDD = 42) composed of the genomes of three different species. The A genome is derived from *Triticum urartu* (Dvorak et al., 1993), the B from *Aegilops speltoides* or a closely related species (Sarkar and Stebbins, 1956; Riley et al., 1958; Dvorak and Zhang, 1990; Maestra and Naranjo, 1998; Marcussen et al., 2014) and the D genome from *Aegilops tauschii* (McFadden and Sears, 1946). The final hybridization event between tetraploid wheat (AABB) and *Ae. tauschii* (DD) is thought to have occurred only once or twice, ~8000–10 000 years ago. As a result, wheat went through a significant genetic bottleneck. Thus, the significant yield gains achieved by wheat breeders to date have been via the exploitation of genetic variation that has arisen via gene mutation over the last 8000–10 000 years and rare outcrossing events with tetraploid wheat. In many parts of the world wheat yields are now starting to plateau and this is thought to be a direct result of a lack of genetic variation compounded by the changing environment at a time of increasing demand for food due to the increasing global population (Charmet, 2011; Grassini et al., 2013; Ray et al., 2013). It is therefore essential that new sources of genetic variation be found that will enable breeders to generate the next generation of high-yielding environmentally adapted wheat varieties (Tanksley and McCouch, 1997; Haussmann et al., 2004; Tester and Langridge, 2010; McCouch et al., 2013; Warschefsksy et al., 2014; Brozynska et al., 2015; Zhang et al., 2017).

Unlike wheat, its progenitor species and wild relatives provide a vast and largely untapped source of genetic variation for most, if not all, traits of agronomic importance. Considerable efforts have been made to exploit the genetic variation within...
the progenitors and wild relatives, with some noticeable successes, for example the transfer of a segment of *Aegilops umbellulata* to wheat conferring resistance to leaf rust (Sears, 1955), the transfer of a segment from *Aegilops ventricosa* carrying resistance to eyespot (Maia, 1967; Doussinault et al., 1983) and its subsequent release in the variety Rendevouz, and the registration of ‘MACE’, a hard red winter wheat carrying a segment from *Thinopyrum intermedium* conferring resistance to wheat streak mosaic virus (Graybosch et al., 2009). Progress has been severely hindered, however, due to an inability to quickly and accurately identify and characterize interspecific transfers to wheat. However, with the advent of new molecular technologies coupled with specific crossing strategies, we can now systematically exploit the genetic variation available in the progenitors of wheat and its wild relatives. (e.g. Tiwari et al., 2014, 2015; King et al., 2016, 2017).

The transfer of genetic variation to wheat from its progenitors/wild relatives occurs via recombination between the chromosomes of wheat and those of its wild relative at meiosis in *F*₁ hybrids or in their derivatives, e.g. addition and substitution lines (King et al., 2016, 2017). This results in the generation of interspecific recombinant chromosomes. Once identified, interspecific recombinant chromosomes are then recurrently backcrossed with wheat. The ultimate objective is to generate lines of wheat carrying a small chromosome segment from a progenitor/wild relative with a target gene but few, if any, deleterious genes.

One of the wild relatives of wheat that is of particular interest to researchers is *Ae. speltoides*, a rich source of genetic variation for resistance to a range of diseases of importance in wheat (Jia et al., 1996; Mago et al., 2009; Klindworth et al., 2012; Deek and Distelfeld, 2014). *Aegilops speltoides*, or an extinct close relative, has been proposed as the donor of the B genome of wheat (Sarkar and Stebbins, 1956; Dvorak and Zhang, 1990; Friele and Gill, 1996; Tsunewaki, 1996; Dvorak and Akhunov, 2005; Marcusen et al., 2014). *Aegilops speltoides* is a facultative outbreeder, but can be readily inbred in the glasshouse.

Unlike most of the wild relatives of wheat, *Ae. speltoides* possesses the ability to suppress the wheat *Ph1* locus (Dvorak, 1972; Dvorak et al., 2006). Recombination is normally restricted to homologous chromosomes from the same genome, i.e. although the A, B and D genomes of wheat and chromosomes from wild relatives are related, recombination can only occur within genomes in the presence of *Ph1*. However, if *Ph1* is removed, related chromosomes (homoeologues) from different genomes can recombine at meiosis. *Aegilops speltoides* possesses genes on chromosomes 3S (*Su₁-Ph1*) and 7S (*Su₂-Ph1*) (Dvorak et al., 2006), with the ability to suppress *Ph1*. Thus, in *F₁* hybrids between wheat and *Ae. speltoides Su₁-Ph1* or *Su₂-Ph1* enables intergenomic homoeologous recombination to occur during meiosis.

*Aegilops speltoides* also carries gametocidal genes (*Gc*) (Tsujimoto and Tsunewaki, 1984, 1988; Oghihara et al., 1994), which are preferentially transmitted to the next generation. Individuals heterozygous for *Gc* gene(s) produce two types of gametes. Both male and female gametes that lack the *Gc* genes are generally not viable due to chromosome breakage (Finch et al., 1984). Gametes that carry the *Gc* genes, however, behave normally and thus it is only these gametes that are transmitted to the next generation. *Aegilops speltoides* carries two alleles of the gametocidal genes. *Gc1a* and *Gc1b*, both located on chromosome 2S, are transmitted at a very high frequency to the next generation and give rise to different morphological aberrations. *Gc1a* causes endosperm degeneration and chromosome aberrations while *Gc1b* results in the production of seeds that lack shoot primordia (Tsujimoto and Tsunewaki, 1988). The limitations of utilizing species with gametocidal genes for wild relative introgression are: (1) the fertility of hybrid/crossed grain is reduced by at least 50%; and (2) all introgressions from wild relatives with *Gc* genes will always carry them and any other genes closely linked to them. Hence it is very difficult, if not impossible, to transfer genes from other regions of the genome in the absence of the *Gc* genes.

In this paper we investigate (1) the ability of the *Ae. speltoides* suppressors of the *Ph1* locus located on chromosomes 3S and 7S to induce recombination between the chromosomes of wheat and those of *Ae. speltoides*, and (2) the frequency of transmission of the *Ae. speltoides* gametocidal chromosomes through the gametes in *F₁* hybrids and their derivatives.

**MATERIALS AND METHODS**

**Generation of introgressions**

The method used to induce introgressions between wheat and *Ae. speltoides* is the same as that described by King et al. (2017). In summary, hexaploid wheat (‘Paragon’, obtained from the Germplasm Resources Unit at the JIC; code W10074) was pollinated with *Ae. speltoides* (accession 2140066 obtained from the Germplasm Resources Unit at the JIC) to produce *F₁* interspecific hybrids. These hybrids were then grown to maturity and backcrossed as the female with the wheat parent to generate *BC₁* populations. The *BC₁* individuals and their resulting progenies were then recurrently pollinated to produce *BC₂*, *BC₃*, etc. populations (Fig. 1).

As a result of the presence of the suppressors of the *Ph1* locus, homoeologous recombination was expected to occur at meiosis in the *F₁* wheat/*Ae. speltoides* hybrids, leading to the production of interspecific recombinant chromosomes/introgressions. Subsequent recurrent backcrossing of any *BC₁* progeny to the wheat parent was undertaken to transfer any interspecific recombinant chromosomes generated into a wheat (Paragon) background.

**Detection of putative wheat/Ae. speltoides introgressions**

A 35K Axiom® Wheat-Relative Genotyping Array (Affymetrix, Santa Clara, CA) was used to detect the presence of putative wheat/*Ae. speltoides* introgressions in each of the backcross generations (King et al., 2017). In summary, the array is composed of single-nucleotide polymorphisms (SNPs), each showing polymorphism for the ten wild relatives (under study at the Nottingham/BBSRC Wheat Research Centre), including *Ae. speltoides* (King et al., 2017). All the SNPs incorporated in this array formed part of the Axiom® 820K SNP array (Winfield et al., 2016). The dataset for the Axiom® 820K SNP array is available from www.cerealsdb.uk.net (Wilkinson et al., 2012, 2016). Table 1 shows the number of putative SNPs between *Ae. speltoides* and each of the wheat genotypes included on the array. The array has been constructed in such a way that up to 384 lines could be screened at one time. Thus, the array facilitated the high-throughput, high-resolution
screening of wheat/Ae. speltoides introgressions. Genotyping was performed as described by King et al. (2017) with no modifications (see below).

**Genetic mapping of Ae. speltoides**

Genetic mapping was as described by King et al. (2017); in summary, DNA was extracted using a CTAB method (Zhang et al., 2013) from individuals of the back-crossed populations, BC1, BC2, BC3, BC4, and BC5 derived from the wheat/Ae. speltoides F1 hybrids. These populations were genotyped with the Axiom® Wheat-Relative Genotyping Array. Only Poly High Resolution (PHR) SNP markers, which were codominant and polymorphic between wheat and Ae. speltoides, were used for genetic mapping (King et al., 2017). The SNP markers that showed (1) heterozygous calls for either parent(s), (2) no polymorphism between the wheat parents and Ae. speltoides, and/or (3) no calls for either parent(s) were removed using Flapjack™ (Milne et al., 2010; v.1.14.09.24). The resulting markers were sorted into linkage groups (Fig. 2) in JoinMap® 4.1 (van Ooijen, 2011) with a LOD score of 20 and a recombination frequency threshold of 0.1 using the Haldane mapping function (Haldane, 1919). All markers that did not show any heterozygous call or were unlinked were ignored and only the highest-ranking linkage groups with >30 markers were selected for map construction. These were exported and assigned to chromosomes using information from the Axiom® Wheat HD Genotyping Array (Winfield et al., 2016).

**Comparative analysis**

Synteny analysis was carried out using sequence information of the markers located on the present map of Ae. speltoides. The sequences of the mapped markers were compared using

**Table 1. Number of polymorphic SNPs between Ae. speltoides and hexaploid wheat based on the Affymetrix 35K array. A comparison is made between all calls (all markers showing polymorphism between wheat and Ae. speltoides) and Poly High Resolution (PHR) calls, which are codominant and polymorphic between wheat and Ae. speltoides with at least two examples of the minor allele**

| Linkage group | Total |
|---------------|-------|
| All calls (% of total) | 2770 (12.4) | 3992 (17.9) | 3358 (15.1) | 2798 (12.6) | 3646 (16.4) | 2530 (11.4) | 3164 (14.2) | 22 258 |
| PHR calls (% of total) | 69 (12.7) | 78 (14.3) | 82 (15.1) | 85 (15.6) | 90 (16.5) | 46 (8.5) | 94 (17.3) | 544 |
Figure 2. Genetic linkage map of *Ae. speltoides*.

Cytogenetic analysis

The protocol for genomic in situ hybridization (GISH) was as described in Zhang *et al.* (2013), Kato *et al.* (2004) and King *et al.* (2017). In summary, genomic DNAs from young leaves of the three putative diploid progenitors of bread wheat, i.e. *T. urartu* (A genome), *Ae. speltoides* (B genome) and *Ae. tauschii* (D genome), were isolated using extraction buffer (0.1 M Tris–HCl pH 7.5, 0.05 M EDTA pH 8.0, 1.25% SDS). Samples were incubated at 65 °C for 1 h before being placed on ice and mixed with ice-cold 6 M NH₄C₃H₂O for 15 min. The samples were then spun down, the supernatant was mixed with
isopropanol to pellet the DNA and the isolated DNA was further purified with phenol/chloroform. The genomic DNAs of *Ae. speltoides* and *T. urartu* were fragmented to 300–500 bp in a heat block at 100 °C.

Preparation of chromosome spreads was as described in King et al. (2004) and King et al. (2017). Briefly, roots were excised from germinated seeds, treated with nitrous oxide gas at 10 bar for 2 h, fixed in 90 % acetic acid for 10 min and then washed three times in water on ice. Root tips were dissected and digested in 20 µL of 1 % pectolyase Y23 and 2 % cellulase Onozuka R-10 (Yakult Pharmaceutical, Tokyo) solution for 50 min at 37 °C and then washed three times in 70 % ethanol. Root tips were crushed in 70 % ethanol, cells collected by centrifugation at 2.5 g for 1 min, briefly dried and then re-suspended in 30–40 µL of 100 % acetic acid prior to being placed on ice. The cell suspension was dropped onto glass slides (6–7 µL per slide) in a moist box and dried slowly under cover.

Slides were initially probed using labelled genomic DNA of *Ae. speltoides* (100 ng) and fragmented genomic DNA of Chinese Spring (3000 ng) as blocker (in a ratio of 1:30 per slide) to detect the *Ae. speltoides* introgressions. The slides were bleached (dipped in 2 × saline–sodium citrate (SSC) to remove the coverslip, transferred to 4 × SSC for 5 min and air-dried in the light) and re-probed with labelled DNAs of *T. urartu* (100 ng) and *Ae. tauschi* (200 ng) and fragmented DNA of *Ae. speltoides* (3000 ng) as blocker in a ratio of 1:2:30 per slide.

### Aegilops speltoides linkage groups

| LG 5S | AX-94423707 AX-95094532  | AX-95161699 |
|-------|---------------------------|-------------|
| 0.2   | 25.1  | 26.6 | 29.9 | 32.8 | 33.7 | 34.1 |
| 0.7   | 4.3   | 13.9 | 15.8 | 17.9 | 19.6 | 20.1 |
| 0.9   | 27.8  | 28.3 | 28.6 | 29.5 | 30.2 | 30.6 |
| 1.2   | 31.5  | 32.6 | 33.8 | 34.8 | 35.4 | 36.0 |
| 1.5   | 39.0  | 40.6 | 41.6 | 42.4 | 43.1 | 44.8 |
| 1.8   | 48.8  | 49.2 | 49.3 | 50.1 | 50.9 | 51.6 |

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|-------|---------------------------|-------------|
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| 0.7   | 4.3   | 13.9 | 15.8 | 17.9 | 19.6 | 20.1 |
| 0.9   | 27.8  | 28.3 | 28.6 | 29.5 | 30.2 | 30.6 |
| 1.2   | 31.5  | 32.6 | 33.8 | 34.8 | 35.4 | 36.0 |
| 1.5   | 39.0  | 40.6 | 41.6 | 42.4 | 43.1 | 44.8 |
| 1.8   | 48.8  | 49.2 | 49.3 | 50.1 | 50.9 | 51.6 |

### Aegilops speltoides linkage groups

| LG 7S | AX-94423707 AX-95094532  | AX-95161699 |
|-------|---------------------------|-------------|
| 0.2   | 25.1  | 26.6 | 29.9 | 32.8 | 33.7 | 34.1 |
| 0.7   | 4.3   | 13.9 | 15.8 | 17.9 | 19.6 | 20.1 |
| 0.9   | 27.8  | 28.3 | 28.6 | 29.5 | 30.2 | 30.6 |
| 1.2   | 31.5  | 32.6 | 33.8 | 34.8 | 35.4 | 36.0 |
| 1.5   | 39.0  | 40.6 | 41.6 | 42.4 | 43.1 | 44.8 |
| 1.8   | 48.8  | 49.2 | 49.3 | 50.1 | 50.9 | 51.6 |

Fig. 2. Continued.
slide to detect the AABBDD genomes of wheat. In both cases, the hybridization mix was made up to 10 µL with 2 × SSC in 1 × Tris/EDTA buffer (TE). Slides were incubated initially at 75 °C for 5 min and then overnight at 55 °C in a closed box before counterstaining with Vectashield mounting medium with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI), and analysed using a high-throughput, fully automated Zeiss Axio Imager Z2 upright epifluorescence microscope (Carl Zeiss, BC 1 BC 2 BC 3 BC 4 BC 5 -5A BC 5 -5A LG 1s LG 1s LG 2s LG 2s LG 3s LG 3s LG 4s LG 4s LG 6s LG 6s LG 7s LG 7s LG 5s LG 5s LG 1s LG 2s LG 2s LG 3s LG 3s LG 4s LG 4s LG 6s LG 6s LG 7s LG 7s LG 5s BC 5-5A BC 5-5A 1S 2S 01 µm Fig. 3. (A) SNP characterization of *Ae. speltoides* introgressions in five consecutive generations, i.e. BC₁, BC₂, BC₃, BC₄, and BC₅. (B) Genomic in situ hybridization image of the BC₁ genotype (aneuploid 41-chromosome plant). In the SNP characterization red colour is used to represent the presence of an *Ae. speltoides* introgression and blue colour represents wheat. It should be noted that these diagrams cannot be used to assess which wheat chromosomes the *Ae. speltoides* segments have recombined with. The GISH image shows a metaphase spread of BC₅-5A probed with labelled genomic DNA of *Ae. speltoides*. Arrows show *Ae. speltoides* introgressions (green).
Oberkochen, Germany) with filters for DAPI (blue), Alexa Fluor 488 (green) and Alexa Fluor 594 (red). Photographs were taken using a MetaSystems Coolcube 1m CCD camera. Further slide analysis was carried out using Meta Systems ISIS and Metafer software (Metasystems, Altlussheim, Germany). This system enabled the fully automated capture of high- and low-power fluorescent images of root tip metaphase spreads. Slides with root tip preparations were automatically scanned and the images downloaded for analysis.

### RESULTS

**Generation of wheat/Ae. speltoides introgressions**

A total of 3890 crosses were made between wheat and *Ae. speltoides* and their derivatives, leading to the generation of 9953 crossed seeds and 2029 self-seeds (Fig. 1). The number of seeds germinated, plants crossed and seed set are shown in Table 2. Every ear produced by the *F*₁ was crossed with wheat. The *F*₁ hybrids generated between wheat and *Ae. speltoides* showed the lowest frequency of germination. Only 15 % germinated compared with 100, 73, 70 and 78 % in the *BC₂*, *BC₃*, *BC₄* and *BC₅* generations. The *F*₁ hybrids also showed the lowest fertility, with only 29 % of crossed ears producing any seeds compared with crossed ears from the *BC₂*, *BC₃*, *BC₄* and *BC₅* generations, which showed 58, 72, 83 and 84 % fertility, respectively.

Of the 20 *F*₁ seeds germinated, only three reached maturity and set seeds. Thus all of the wheat/*Ae. speltoides* introgressions generated in this programme were limited to these three viable *F*₁ hybrids. Table 2 summarizes the number of seeds germinated and seed set for each of the backcross generations. In each generation, the average seed set per crossed ear was very low, i.e. the lowest average seed set per ear was 0.6 (observed in the *F*₁ hybrid), while the highest average seed set per crossed ear was only 3.7 (observed in the *BC₅* hybrid). The fertility of plants that had previously been self-fertilized at least once with crossed ears from the *BC₁*, *BC₂*, *BC₃* and *BC₄* generations, which showed 58, 72, 83 and 84 % fertility, respectively.

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Detection of introgressions

Of the SNPs on the Axiom array, 22 258 were polymorphic between *Ae. speltoides* and wheat (Table 1). The Axiom array was used to screen genomic DNA prepared from 536 samples of *BC₁* to *BC₅* lines between wheat and *Ae. speltoides*. Genotype calls were generated, and the sample call rate (markers working in a particular genotype) ranged from 86.5 to 99.8 %, with an average of 98.8 % for the 536 samples. Affymetrix software classified the scores for each SNP into one of six cluster patterns. However, only those classified as Poly High Resolution (PHR) were used for genetic mapping as these are considered to be optimum quality. Linkage group 7 had the highest number of SNPs (17.3 %), while linkage group 6 had the lowest number (8.5 %).

JoinMap® (van Ooijen, 2011) was used to analyse the PHR SNPs and this led to the establishment of seven linkage groups. The genetic map was composed of 544 SNPs and represented the seven chromosomes of *Ae. speltoides*. Linkage groups 1–7 had 69, 78, 82, 85, 90, 46 and 94 SNPs respectively (Fig. 2). The lengths of linkage groups 1–7 were 70.8, 90.3, 98.5, 68, 110.5, 63.6 and 92.9 cM, respectively, with a total length of 594.6 cM and an average chromosome length of 84.9 cM.

Genomic in situ hybridization

In most cases the number of wheat/*Ae. speltoides* introgressions detected by genomic in situ hybridization (GISH) in *BC₄* and *BC₅* individuals corresponded exactly with the number detected via SNP analysis (Table 3 and Fig. 3). One exception was detected in the GISH analysis of *BC₅*-4A. The SNP analysis of this plant showed the only segment present to be a complete 2S chromosome. However, the GISH image showed the presence of a large *Ae. speltoides* 2S segment (one telomere was missing from the chromosome) and a very small *Ae. speltoides* segment recombined with a different wheat chromosome.

**Syntenic relationship**

Figure 4 shows the syntenic relationship between the seven linkage groups of *Ae. speltoides* and the three genomes of wheat, with large ribbons showing significant synteny. Some

| Table 2. Number of seed produced and germinated in relation to the number of crosses carried out for each generation of the introgression programme for *Ae. speltoides* into wheat |
|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number of seeds sown | Wheat × *Ae. speltoides* | *F₁* | *BC₁* | *BC₂* | *BC₃* | *BC₅* | Total |
| Number of seeds that germinated (%) | NA | 20 | 22 | 400 | 187 | 173 | 802 |
| Number of plants that set seed (%) | NA | 3 (15) | 22 (100) | 292 (73) | 130 (70) | 135 (78) | 582 |
| Number of seed/total number of crosses (average number of seed set per crossed ear) | NA | 3 (100) | 22 (100) | 261 (89) | 124 (95) | 117 (87) | 527 |
| Number of crosses producing seed (%) | 127/26 (4.9) | 22/35 (0.6) | 509/357 (1.4) | 5743/2180 (2.6) | 3204/876 (3.7) | 348/416 (0.8) | 9963/3890 |
| Number of self seed produced | 127/26 (4.9) | 22/35 (0.6) | 509/357 (1.4) | 5743/2180 (2.6) | 3204/876 (3.7) | 348/416 (0.8) | 9963/3890 |
gene rearrangements are indicated where single markers cross-map to non-collinear positions on wheat chromosomes. The only major disruption in synteny between the two species is that *Ae. speltoides* does not carry the 4/5/7 translocation observed for chromosomes 4A, 5A and 7B of wheat (Liu et al., 1992; Naranjo et al., 1987). These data demonstrate that there is a close syntenic relationship between the A, B and D genomes of wheat and *Ae. speltoides*.

### Preferential transmission

The data obtained from SNP analysis was used to determine the transmission frequency of chromosome segments from each of the seven *Ae. speltoides* linkage groups through the female gametes to the BC₁, BC₂, BC₃, BC₄ and BC₅ generations. Chromosome segments from linkage group 2 were observed in all 536 backcross progeny observed (Table 4). The smallest segment carried only three SNP markers, AX94631566, AX95123223 and AX94601746, located near one of the terminal ends of the 2S linkage group (Fig. 2). These three SNP markers were found to be present in all the backcross individuals analysed. In order to determine whether the 2S chromosome was also preferentially transmitted through the male gametes, 41 BC₁ and BC₂ plants were allowed to self-fertilize and their progenies were analysed using GISH. The BC₁/BC₂ plants selected each carried a single copy of a 2S chromosome segment. Thus, these plants would produce two types of male and female gametes during gametogenesis: those that carried 2S and those that did not. If the 2S chromosome segment was preferentially transmitted through both the male and female gametes, only those that carried the 2S chromosome would be viable and as a result all the progeny observed in the BC₁/BC₂ self-populations would be homozygous for the 2S chromosome segment. Alternatively, if the 2S chromosome segment was not preferentially transmitted through the male or the female gametes, then the progeny would segregate for the presence of either one or two 2S chromosome segments. All progeny observed carried two copies of the 2S chromosome segment (Fig. 5), thus demonstrating that the 2S chromosome segment was preferentially transmitted through both the male and female gametes.

The frequency of transmission of chromosome segments from other linkage groups in each of the backcross progenies was much lower than that observed for linkage group 2. Of the remaining linkage groups, segments from linkage group 5 of *Ae. speltoides* had the highest frequency of transmission in all the backcross progenies observed (Table 4).

### DISCUSSION

*Aegilops speltoides* is a potentially important source of genetic variation for a range of agronomically important traits (Jia et al., 1996; Mago et al., 2009; Klindworth et al., 2012; Deek and Distelfeld, 2014). In the past, it has been difficult to detect and characterize wheat/wild relative introgressions due to the absence of high-throughput, genome-wide marker technologies. In this paper we have utilized an Affymetrix SNP array (King et al., 2017) complemented by a specific crossing strategy and the use of an automated, high-throughput microscope system for GISH image capture.

In this work we generated *F₁* interspecific hybrids between wheat and *Aegilops speltoides* and backcrossed these to wheat (Fig. 1). Hybrids were haploid for the A, B and D genomes of

### Table 3. Number of introgressed segments from *Ae. speltoides* present in BC₁ and BC₂ plants as detected by SNP genotyping and GISH. The *Ae. speltoides* chromosomes have been assigned to linkage groups via the comparative analysis of the SNPs with wheat

| Plant accession number | Number of segments | *Ae. speltoides* linkage group of segments |
|------------------------|-------------------|------------------------------------------|
|                        | Genotyping        |                                         |
| BC₁/BC₂                |                   |                                          |
| 45A                    | 3                 | 2, 5, 6                                  |
| 45C                    | 1                 | 1                                        |
| 45D                    | 3                 | 2, 5, 6                                  |
| 1A                     | 3                 | 2, 4, 5                                  |
| 1B                     | 1                 | 2                                        |
| 1C                     | 1                 | 2                                        |
| 1D                     | 2                 | 2, 5                                     |
| 2                      | 2                 | 2, 5                                     |
| 3                      | 1                 | 1                                        |
| 4A                     | 1                 | 1 + small end                            |
| 5A                     | 2                 | 1, 2                                     |
| 6A                     | 1                 | 1                                        |
| 6B                     | 2                 | 1, 2                                     |
| 6C                     | 1                 | 1                                        |
| 7A                     | 2                 | 2, 5                                     |
| 7C                     | 1                 | 1                                        |
| 8A                     | 1                 | 1                                        |
| 8B                     | 1                 | 1                                        |
| 8C                     | 1                 | 1                                        |
| 8D                     | 1                 | 1                                        |

### Table 4. Transmission frequencies of the seven linkage groups of *Ae. speltoides* in the backcross populations (BC₁ to BC₅) analysed by SNP genotyping

| Number of plants genotyped | BC₁ | BC₂ | BC₃ | BC₄ | BC₅ | Total |
|----------------------------|-----|-----|-----|-----|-----|-------|
| Linkage group 1 (%)        | 18  | 124 | 36  | 13  | 3   | 194   |
| Linkage group 2 (%)        | 22  | 252 | 123 | 117 | 22  | 536   |
| Linkage group 3 (%)        | 18  | 135 | 27  | 20  | 0   | 200   |
| Linkage group 4 (%)        | 15  | 89  | 23  | 7   | 0   | 134   |
| Linkage group 5 (%)        | 19  | 162 | 54  | 32  | 6   | 273   |
| Linkage group 6 (%)        | 17  | 115 | 31  | 25  | 0   | 188   |
| Linkage group 7 (%)        | 13  | 80  | 15  | 9   | 0   | 117   |
wheat and the S genome of *Ae. speltoides*. Thus, in the absence of homologous chromosomes the only recombination occurring at meiosis was between homoeologous chromosomes. This would normally be prevented by the presence of *Ph1*. However, the presence of the pairing promoters *Su1-Ph1* and *Su2-Ph1* located on chromosomes 3S and 7S of *Ae. speltoides* (Chen and Dvorak, 1984; Dvorak et al., 2006) enabled homoeologous recombination to occur.

Since the interspecific *F*₁ hybrids generated were haploid for the A, B, D and S genomes, their fertility was predicted to be low and this was found to be the case (Table 1). However, their fertility of 29% was higher than that previously observed in those between wheat and *Amblyopyrum muticum*, which was 16.2% (King et al., 2017). The low fertility of the *F*₁ hybrids resulted in the generation of only 22 BC₁ seeds that grew to maturity and set seed. Hence, if recombination did not occur in later generations, i.e. in the gametes of the BC₁, BC₂, BC₃ and BC₄ generations, then the total number of introgressions that could be generated was limited to the 22 female *F*₁ gametes giving rise to these 22 BC₁ plants. However, the level of interspecific recombination detected by the genetic mapping was such that it was possible to assemble the seven linkage groups of *Ae. speltoides*. Using the genetic linkage map of *Ae. speltoides*,

**Fig. 4.** Synteny of *Ae. speltoides* (genetic position in cM) with hexaploid wheat (physical position in Mb) [visualized using Circos v. 0.67 (Krzywinski et al., 2009)].

**Fig. 5.** GISH of a complete one-cell metaphase spread of a genotype produced by self-fertilization (BC₁F₃-6B) with labelled genomic *Ae. speltoides* DNA as probe, showing a homozygous pair of *Ae. speltoides* linkage group 2 introgressed chromosomes (green).
we estimated that 294 wheat/Ae. speltoides introgressions were generated spanning the entire Ae. speltoides genome. We were able to characterize these introgressions and track them through the backcross generations (Fig. 3). However, while using the genetic map to characterize the introgressions, it was important to treat the cM distances with caution, as the maps were not produced using proper mapping families. Tracking the introgressions through the different backcross generations via the SNP data showed that the majority of introgressions generated had occurred due to recombination in the F₁ gametes and were therefore present in the BC₁ plants.

Validation of introgressions identified by SNP analysis was carried out by GISH analysis. In most cases the number of wheat/Ae. speltoides introgressions detected by the SNP analysis corresponded to the number of introgressions detected by the GISH analysis (Table 3 and Fig. 3). The one exception (BC₅ -4A) can be explained in two possible ways. The first possibility is that linkage group 2S of Ae. speltoides has recombined with a wheat chromosome. Although the telomere is on a separate chromosome, the SNP markers would have detected the two introgressions as a complete chromosome. The second possibility is that the genetic linkage map of Ae. speltoides is not complete and therefore the smaller introgression has not been detected at all and the large segment has appeared complete in the SNP analysis.

Multicolour GISH analysis was carried out to verify which of the wheat genomes were involved in recombination with Ae. speltoides (Fig. 6). The majority of recombination events were shown to have occurred between Ae. speltoides and the B genome of wheat (91 %). Recombination had also occurred between Ae. speltoides and the D genome (18 %) but we found no evidence of recombination between Ae. speltoides and the wheat A genome. As the B genome progenitor of wheat is thought to be Ae. speltoides or a close relative (Sarkar and Stebbins, 1956; Riley et al., 1958; Dvorak and Zhang, 1990; Maestra and Naranjo, 1998), the higher level of recombination between Ae. speltoides and the B genome was expected. The low level of recombination observed with the D genome of wheat would also suggest that Ae. speltoides is more closely related to the D genome than to the A genome. This situation

![Fig. 6. Genomic in situ hybridization showing recombination between Ae. speltoides and the B and D genomes of wheat.](https://academic.oup.com/aob/article-abstract/121/2/229/4689502)
closely mirrors that observed between wheat and *Amblyopyrum muticum*, where most of the recombination occurred between the chromosomes of the wild relative and the B and D genomes of wheat (King et al., 2017). In addition, although the genetic location of the Phl pairing suppressors located on *An. muticum* are not currently known, the fact that both *Ae. speltoides* and *Am. muticum* carry suppressors indicates that the two species may have had a common ancestry.

We are currently unable to use the SNP markers to identify which of the chromosomes of wheat have recombined with the *Ae. speltoides* chromosomes. We are currently developing wheat chromosome-specific Kompetitive Allele Specific PCR (KASP) markers from the SNP markers on the 35K array. These markers will allow the analysis of large numbers of individuals, firstly to tag individual introgressions and track them through the generations (both via backcrossing and selfing) and secondly to identify which wheat chromosome(s) is (are) involved in each introgression once homozygous introgressions have been generated. Although considerably more labour-intensive and technically demanding, the latter could also be achieved using fluorescence in situ hybridization (FISH) with repetitive DNA sequences combined with GISH. The difficulty of inducing wheat/wild relative introgressions and (2) the fact that both *Ae. speltoides* introgressions have the potential to play a critical role in the development of superior wheat varieties in the future.

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