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Abstract	Alien introgression breeding is an attractive approach to recover genetic variation that was lost during wheat domestication and breeding. New alleles and genes may be introduced from wild relatives from the tribe Triticeae, which exhibit large genetic variation and many potentially useful traits. Although a range of wheat–alien introgression lines has been developed, apart from the 1BL.1RS translocation, only a few commercial wheat cultivars benefitted from alien introgression. This is a consequence of poor knowledge of genome structure of wild donors, limited ability to control chromosome behavior during meiosis in interspecific hybrids and introgression lines, difficulties in eliminating unwanted chromatin transferred simultaneously with genes of interest, as well as a lack of tools permitting large-scale production and characterization of introgression lines. Recent advances in molecular and flow cytogenetics and genomics are bound to change the situation. New insights into the meiotic recombination raise the hopes for the ability to control its frequency and distribution. The progress in comparative genome analysis provides clues about the genome collinearity between wild species and wheat, making it possible to assess chances for chromosome recombination and predict its outcomes. Genomics tools enable massive and high-resolution screening of hybrids and their progenies and characterize their genomes, including the development of markers linked to traits of interest. Until recently, little attention has been paid to the function of introgressed genes and their interaction with the host genome. However, also this has been changing and all these achievements make the breeding of improved wheat cultivars using wild germplasm a realistic goal.

Keywords (separated by " - ")	Wheat breeding - Alien gene function - Chromosome flow sorting - Chromosome genomics - Epigenetic modifications - Genome sequencing - Interspecific hybridization - Molecular cytogenetics - Molecular markers

AUTHOR QUERIES

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Chapter 13
Genomics of Wild Relatives and Alien Introgressions

[AU1]

Elodie Rey, István Molnár, and Jaroslav Doležel

13.1 Introduction

As one of the most important staple food crops, bread wheat (*Triticum aestivum*, L.) continues to play a major role in ensuring global food security. The growing human population is estimated to reach nine billion by 2050, and in order to meet the expected demand, the annual yield increase of wheat should reach 2 %. This is a great challenge, as climate change and land degradation act against this endeavor. Apart from improved agronomic practice and reduction of postharvest losses, the key elements will be new varieties with increased resistance to diseases and pests, adverse environmental conditions, and with improved yield.

According to the most widely accepted scenario, bread wheat ($2n=6x=42$, BBAADD genome) arose about 8000 years ago when a cultivated form of tetraploid *Triticum turgidum* ($2n=4x=28$, BBAA genome) migrated to south of the Caspian Sea and in the area of Fertile Crescent crossed with a wild diploid grass *Aegilops tauschii* Coss. ($2n=2x=14$, DD genome). The union of unreduced gametes, or somatic chromosome doubling in the hybrid (Feuillet et al. 2008), resulted in a new allohexaploid species. The genetic diversity of bread wheat was restricted at the onset of its origin by the limited diversity of parental populations and was eroded subsequently during domestication and thousands years of cultivation and breeding.

One option to recover the useful variation that was lost and to acquire new and valuable genes and alleles is to utilize wild relatives of wheat, which were not

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subjected to human selection, and thus represent a rich source of diversity. The tribe Triticeae comprises wild annual and perennial species related to wheat, facilitating the production of interspecific hybrids. The efforts to use this approach date back 140 years, and the first experiments at the end of nineteenth century and beginning of twentieth century involved hybridization between wheat and rye (Wilson 1876), wheat and barley (Farrer 1904), and between wheat and *Aegilops* (Kihara 1937). However, larger-scale production of interspecific hybrids was delayed until the introduction of colchicine treatment in 1930s (Blakeslee 1937), allowing the production of fertile amphiploids by doubling chromosome number in otherwise sterile hybrids. Among other, this provided a way to develop triticale as a new cereal crop (Meurant 1982). With the advances in hybridization techniques (Kruse 1973) and establishment of in vitro embryo rescue methodology (Murashige and Skoog 1962), wide hybridization became more accessible, and the experiments involved a larger group of wild and cultivated wheat relatives (Mujeeb-Kazi 1995).

An extensively used approach to utilize wild germplasm in wheat breeding has been the production of synthetic hexaploid wheat by hybridizing tetraploid durum wheat (*T. turgidum* ssp. *durum* (Desf.) Husn.) ($2n=4x=28$; BBAA genome) with *Ae. tauschii*. Both synthetic hexaploid and bread wheat have the same genomic constitution and therefore can be readily hybridized to transfer novel alleles and genes from different accessions of the D-genome progenitor. This strategy has been employed at CIMMYT where more than 1000 synthetic wheats were created (del Blanco et al. 2001; Warburton et al. 2006; van Ginkel and Ogbonnaya 2008; Li et al. 2014).

Genetic diversity suitable for wheat improvement is not limited to *Ae. tauschii*, and over the years, a range of interspecific hybrids, chromosome addition and translocation lines were obtained between perennial and annual Triticeae species and bread wheat (Mujeeb-Kazi 1995; Friebe et al. 1996; Schneider et al. 2008; Molnár-Láng et al. 2014). Probably the best example of a successful wheat–alien introgression has been the spontaneous 1BL.1RS chromosome translocation (Mujeeb-Kazi 1995). It was estimated that between 1991 and 1995, 45 % of 505 commercial cultivars of bread wheat in 17 countries carried 1BL.1RS translocation, which confers increased grain yield by providing race-specific disease resistance to major rust diseases (including *Lr29/Yr26* leaf and yellow rust resistance genes), improved adaptation and stress tolerance, superior aerial biomass, and higher kernel weight (Rabinovich 1998; Feuillet et al. 2008; Zarco-Hernandez et al. 2005). However, too few other alien introgressions into wheat made their way to agricultural practice.

This chapter reviews the progress in characterizing nuclear genomes of wild relatives of wheat and wheat–alien introgression lines at chromosomal and DNA levels, and the potential of these approaches to support wheat–alien introgression breeding. After introducing the diversity of wild relatives of wheat and the difficulties of the introgression breeding, methods of cytogenetics and genomics are outlined and examples of their uses are given. The need for better understanding the mechanisms controlling chromosome behavior and for better knowledge of genome structure of wild relatives is explained. The last part of the chapter is devoted to the interaction of the introgressed chromatin with the host wheat genome. This research area has been poorly developed so far, and the lack of information may hamper the attempts to develop improved cultivars of wheat with alien introgressions.

13.2 Wild Relatives of Wheat and Difficulties with Alien Introgression

The tribe Triticeae comprises a group of species belonging to the Poaceae grass family commonly named Gramineae. In addition to economically important bread wheat (*T. aestivum* L.), durum wheat (*T. turgidum* ssp. *durum*), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.), the tribe comprises over 500 wild and cultivated species of genera *Aegilops*, *Agropyron*, *Ambylopyrum*, *Anthosachne*, *Campeiostachys*, *Dasypyrum*, *Elymus*, *Hordeum*, *Leymus*, *Lophopyrum*, *Psathyrostachys*, *Pseudoroegneria*, *Secale*, *Thinopyrum*, and *Triticum*.

The Triticeae species exhibit a large diversity in terms of geographical distribution, environmental requirements, and agronomically interesting traits. The latter include increased yield (Reynolds et al. 2001), resistance to pests and diseases (Friebe et al. 1996), early maturity (Koba et al. 1997), drought tolerance (Fatih 1983; Molnár et al. 2004; Dulai et al. 2014), salt tolerance (Fatih 1983; Dulai et al. 2010; Darkó et al. 2015), micronutrient content and efficiency (Schlegel et al. 1998, Farkas et al. 2014), lodging resistance (Chen et al. 2012), heat tolerance (Pradhan and Prasad 2015), high dietary fibre content (Cseh et al. 2011), and high protein content (Pace et al. 2001). Donors for these traits have been identified and some of the traits have been transferred to wheat (Gill et al. 2011). Some of the genes responsible for the traits have been tagged, and a few of them were even cloned (Feuillet et al. 2008; Hajjar and Hodgkin 2007; Jiang et al. 1993). However, the degree of genetic and genomic characterization of wild Triticeae species is highly variable and uneven.

Although the potential of wild relatives for wheat improvement has been recognized since a long time, the available genetic diversity remains largely underexploited. In order to utilize its full potential, it is important to understand genome organization in wild wheat relatives, increase the number of genome-specific molecular tools and identify loci underlying traits of interest (Hajjar and Hodgkin 2007). The poor knowledge on genome structure of Triticeae species and the lack of high resolution genetic maps hampers identification of genes underlying important traits, identification of unwanted sequences and their elimination using suitable large-scale screening platforms.

Elimination of unwanted alleles may be challenging due to low level of recombination between chromosomes of wild relatives and wheat. Two principal approaches have been developed to overcome this hindrance. The first is based on decreasing the effect of *Ph1* locus by the use of wheat genotypes ph1b or Ph^{I} (Riley and Chapman 1958; Griffiths et al. 2006), which promotes recombination between homoeologous wheat and alien chromosomes. The second approach involves induction of donor chromosome breakage by ionizing irradiation, or gametocidal chromosomes (Jiang et al. 1993) to stimulate insertion of alien chromosome fragments into wheat chromosomes.

Evolutionary chromosome rearrangements broke down the collinearity between the homoeologous wheat and alien chromosomes (Devos and Gale 1993). As a consequence, genes on alien chromosome segments may not compensate for the loss of wheat genes. This may negatively affect agricultural performance of the wheat–alien introgression lines and represents another obstacle in using wheat–alien translocations

in breeding. Little is known about different levels of interaction between the host genome and the alien chromatin, which may lead to unexpected and even undesirable effects. Insertion of alien chromosome segment may interfere with functionality of the host genome at genomic, epigenomic, transcriptomic and proteomic levels, and may explain the failure of some introgressed genes to function in the host background, although their sequences remained intact after the introgression.

13.3 Tools to Support Alien Introgression in Wheat

13.3.1 Cytogenetics Techniques

The development of alien chromosome addition and translocation lines and their characterization greatly profits from the ability to identify chromosomes involved. Originally, the repertoire of selection methods was limited to cytological techniques that visualize mitotic and meiotic chromosomes. When Sears (1956) transferred leaf rust resistance from Ae. umbellulata to wheat, cytological characterization of the wheat—Ae. umbellulata addition line was limited to microscopic observation of mitotic chromosomes in root tips, and the translocation event was identified based on the leaf rust-resistance phenotype (Sears 1956). The advent of chromosome banding techniques such as Giemsa C-banding (Gill and Kimber 1974), permitted description of genomic constitution in interspecific hybrids, identification of alien chromosomes and characterization of translocations at subchromosomal level. C-banding was particularly effective in characterizing wheat–rye translocations because of diagnostic terminal bands of rye chromosomes (Lukaszewski and Gustafson 1983; Lapitan et al. 1984; Friebe and Larter 1988). However, it has been less useful if chromosomal segments of interest lacked diagnostic bands.

Introduction of techniques for in situ hybridization further stimulated the development and characterization of alien introgression lines. Following the pioneering work of Rayburn and Gill (1985), fluorescence in situ hybridization (FISH) was developed in wheat (Yamamoto and Mukai 1989). The potential of FISH to identify chromosomes and their segments depends on the availability of suitable probes. The most popular probes included the pAs1 repeat (Rayburn and Gill 1985; Nagaki et al. 1995), which permits identification of D-genome chromosomes, the rye subtelomeric repeat pSc119.2 (Bedbrook et al. 1980), which is useful to identify B-genome chromosomes, and pTa71 DNA clone (Gerlach and Bedbrook 1979), which identifies nucleolus organizing regions on satellite chromosomes. FISH with these probes discriminates the whole set of D- and B-genome chromosomes and, depending on the quality of hybridization, partially or completely the A-genome chromosomes of bread wheat. The same set of DNA probes has been applied to examine genetic diversity and construct karyotypes of wild species in Aegilops (Badaeva et al. 1996a, 1996b), Agropyron (Linc et al. 2012), and Hordeum (de Bustos et al. 1996; Szakács et al. 2013;), and to identify their chromosomes introgressed into wheat (Molnár et al. 2009; Sepsi et al. 2008; Nagy et al. 2002, Molnár-Láng et al. 2012) (see Figs. 13.1 and 13.2)

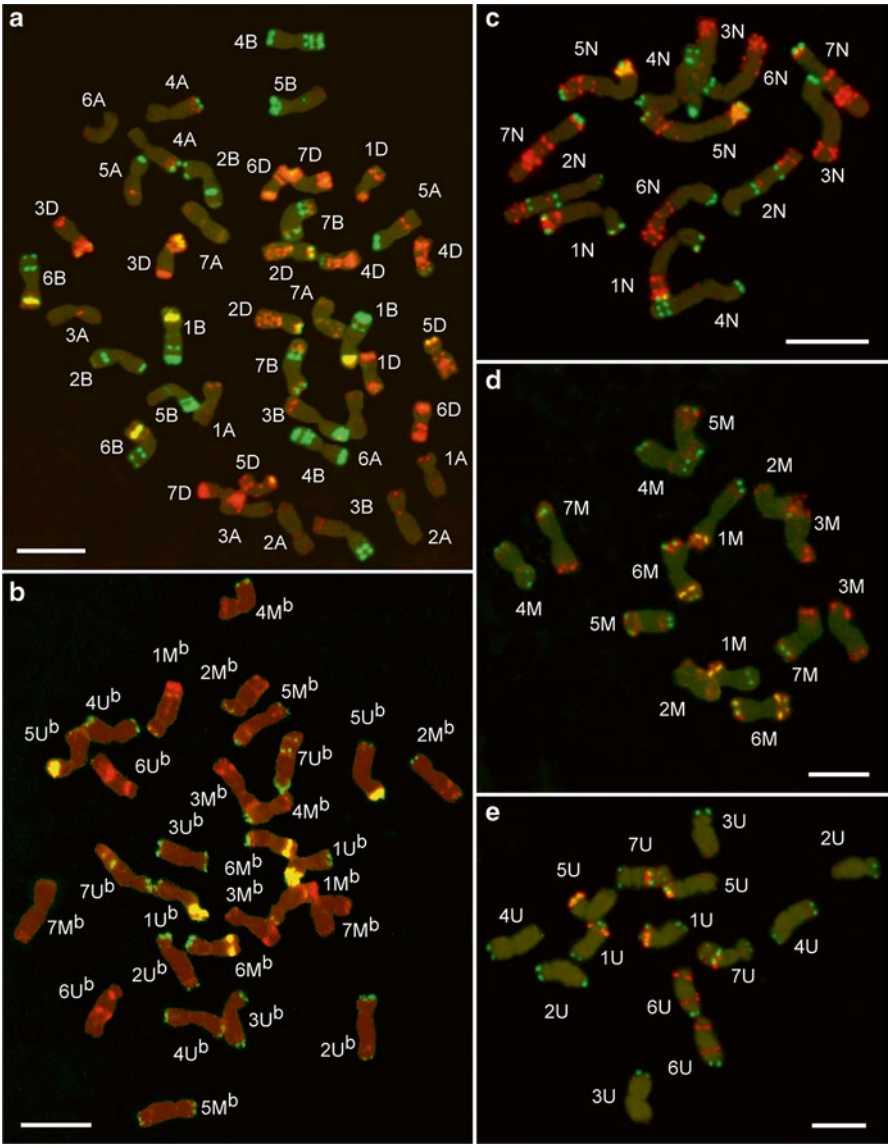

Fig. 13.1 Molecular cytogenetic identification of mitotic metaphase chromosomes in (**a**) T. aestivum cv. Chinese Spring (2n = 6x = 42; BBAADD); (**b**) Ae. biuncialis MvGB382 (2n = 4x = 28; $U^bU^bM^bM^b$); (**c**) Ae. uniaristata JIC2120001 (2n = 2x = 14; NN); (**d**) Ae. comosa MvGB1039 (2n = 2x = 14; MM); and (**e**) Ae. umbellulata AE740/03 (2n = 2x = 14; UU). Fluorescence in situ hybridization (FISH) was done using repetitive DNA probes for Afa family repeat (red), pSc119.2 repeat (green) and pTa71 repeat (yellow) and allowed identification of all chromosomes in the karyotypes. Scale bar = 10 μm

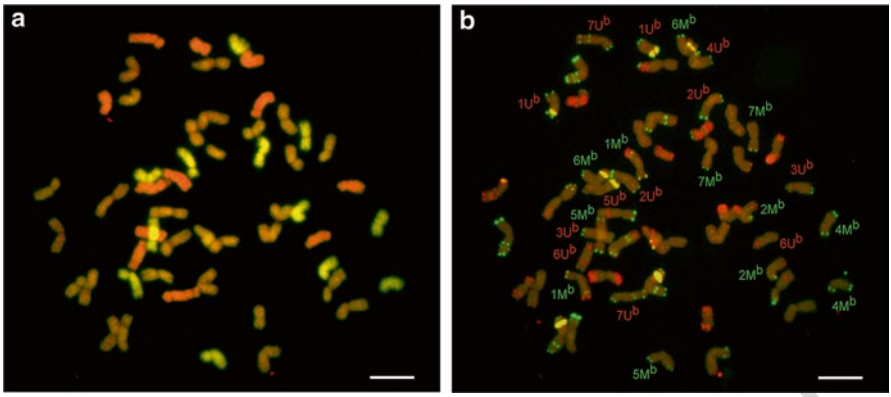

Fig. 13.2 Multicolor genomic in situ hybridization (mcGISH) using U- and M-genomic probes (**a**) and FISH with probes for DNA repeats (**b**) on mitotic metaphase chromosomes of a partial meristem root tip cell of wheat-Ae. biuncialis amphiploid plant. (**a**) McGISH allows discrimination of U^b genome (red color), M^b genome (green color), and wheat (brown color) chromosomes. (**b**) FISH with probes for pSc119.2 repeat (green color), Afa family repeat (red color), and pTa71 repeat (yellow color) enables identification of all alien chromosomes in the wheat background. Scale bar = 10 μm

Characteristic FISH labeling patterns of HvT01 tandem repeat (Schubert et al. 1998), and the Triticeae-specific AT-rich tandem repeat pHvMWG2315 (Busch et al. 1995), permitted identification of all chromosomes in barley. In wheat genetic background, barley chromosomes could be discriminated with various combinations of repetitive DNA probes (Szakács and Molnár-Láng 2007). In rye, FISH with the 120-bp repeat family pSc119.2 together with pTa71 or AAC repeats identifies the whole chromosome complement (McIntyre et al. 1990; Szakács and Molnár-Láng 2008). In order to enrich chromosomes with diagnostic landmarks, microsatellite trinucleotide repeats (GAA, AAC, ACG) were found useful in wheat, barley, and rye (Cuadrado et al. 2008) as well as in Aegilops (Molnár et al. 2011a) and Dasypyrum (Grosso et al. 2012).

Inserts from DNA libraries cloned in a BAC (Bacterial Artificial Chromosome) vector were also tested to identify new repetitive sequences (both dispersed and tandem types), and to develop locus-specific cytogenetic markers (Zhang et al. 2004a). FISH with BAC clones (BAC FISH) was shown useful to discriminate the three subgenomes in hexaploid wheat (Zhang et al. 2004b), and for physical mapping of a powdery mildew-resistance gene (Yang et al. 2013). Unfortunately, BAC FISH suffers from the presence of dispersed repetitive DNA sequences in BAC clones, which often prevent localization of BAC clones to single loci. A possible solution is to use short single-copy probes free of repeats (Karafiátová et al. 2013).

Danilova et al. (2014) used wheat cDNAs as probes for FISH to develop cytogenetic markers specific for single-copy genic loci in wheat. They localized several cDNA markers on each of the 14 homoeologous chromosome arms and studied chromosome structure and homoeology in wild Triticeae species. The work revealed

1U-6U chromosome translocation in Ae. umbellulata, showed collinearity between the chromosome A of Ae. caudata and group-1 wheat chromosomes, and between chromosome arm 7S#3L of Thinopyrum intermedium and the long arm of the group-7 wheat chromosomes. A limitation inherent to performing FISH on condensed mitotic and meiotic chromosomes is the low spatial resolution. This can be improved by performing FISH on stretched mitotic chromosomes (Valárik et al. 2004), on extended DNA fibers (Fiber-FISH) (Jackson et al. 1998; Ersfeld 2004), and on hyper-expanded chromosomes obtained by flow cytometry (Endo et al. 2014).

Genomic in situ hybridization (GISH) uses genomic DNA as a probe (Schwarzacher et al. 1989) and permits determination of genomic constitution of allopolyploid Triticeae, and to detect alien chromatin introgressed into wheat. Combined with chromosome banding and/or FISH, the method allows location and identification of wheat–alien translocation breakpoints (Friebe et al. 1992, 1993; Jiang et al. 1993; Molnár-Láng et al. 2000, 2005; Liu et al. 2010; Kruppa et al. 2013). While cytogenetic methods are irreplaceable to verify genomic constitution in interspecific hybrids, the limited sensitivity and spatial resolution, and especially their laborious and time consuming nature seriously limit their suitability for large scale selection of wheat–alien introgressions. High-resolution and high-throughput methods are needed to increase the screening capacity and to identify micro-introgressions and chromosome breakpoints. These include the use of DNA markers and, more recently, DNA sequencing.

13.3.2 Molecular Markers

Morphological, isozyme, and seed storage protein markers were the first markers used in wheat–alien introgression breeding to identify and characterize alien chromosome addition lines (Guadagnuolo et al. 2001; Hart et al. 1980; Tang and Hart 1975). Because of their limited number, they were not suitable to reveal chromosomal rearrangements.

The restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD) (Williams et al. 1990), and amplified fragment length polymorphism (AFLP) (Vos et al. 1995), were the first DNA markers used to characterize wheat–alien introgression lines (Fedak 1999), since they do not require prior sequence information. They were used in a number of studies to identify chromosome/chromosome-arm addition and substitution lines (Devos and Gale 1993; King et al. 1993; Hernández et al. 1996; Qi et al. 1996; Peil et al. 1998; Wang et al. 1995; Francki et al. 1997; Qi et al. 1997). Despite their temporal popularity, the markers suffered from some drawbacks. Their application was time-consuming, often labor-intensive and expensive, and they were not appropriate for high-throughput genotyping. Moreover, the low level of polymorphism revealed by RAPD markers, and low transferability/conversion of AFLP markers into STS markers, prevented the extensive use of these markers in wheat breeding (Gupta et al. 1999).

RFLPs became the molecular markers of choice for some time due to their codominance and locus specificity (Qi et al. 2007). Wheat RFLPs were used to develop high-resolution genetic and physical maps (Qi et al. 2004; Qi et al. 2003), characterize homoeology of alien chromosomes, and reveal their rearrangements relative to wheat (Devos et al. 1993; Devos and Gale 1993; Zhang et al. 1998; McArthur et al. 2012). RFLP markers identified cryptic alien introgressions where cytogenetic techniques failed (Yingshan et al. 2004), such as the T5DL.5DS-5MgS wheat-Ae. geniculata translocation conferring resistance to leaf rust and stripe rust (Kuraparthy et al. 2007). With the advances in molecular biology, informative but cumbersome to use RFLP markers were converted to PCR-based markers such as the sequence-tagged site (STS) markers, which were more suitable for tagging interesting genes (Cenci et al. 1999; Seyfarth et al. 1999; Langridge et al. 2001).

Transposable elements, randomly distributed in nuclear genomes have also been used as molecular markers (Queen et al. 2003; Nagy and Lelley 2003). The sequence-specific amplified polymorphism (S-SAP) technology (Waugh et al. 1997) amplifies regions representing flanking genomic sequences of individual retrotransposons. The advantages of S-SAP for studying genetic diversity are higher amount of accessible polymorphism (Waugh et al. 1997), the markers are more evenly distributed throughout the genome (Nagy and Lelley 2003), and the estimated genetic distances are more consistent with physical mapping (Ellis et al. 1998). Nagy et al. (2006) used the short interspersed nuclear element (SINE) Au identified in Ae. umbellulata (Yasui et al. 2001) to develop S-SAP markers specific for U- and M-genome chromosomes of Aegilops (Nagy et al. 2006).

Simple Sequence Repeat (SSR) markers (Tautz 1989), or microsatellite markers, were the next generation of molecular markers employed in wheat–alien introgression breeding. Efficient development of SSRs requires genomic sequence information, and thus they were developed concomitantly with expressed sequence tags (ESTs), cDNA and BAC libraries. A list of genomic resources currently available for Triticeae is given in Table 13.1.

Together with cDNA libraries and draft genome sequences of barley, bread wheat, Ae. tauschii and T. urartu (Table 13.2), ESTs are currently the most abundant type of sequence information available for not less than 25 species from 15 Triticeae genera. The release of 16,000 EST loci mapped to chromosome deletion bins (Qi et al. 2004) provided excellent resource for development of markers from specific chromosome regions and helped designing locus-specific markers. Because of the genic and thus conserved nature of ESTs, EST-derived SSR markers are transferable between Triticeae species (Gupta et al. 2008). As ESTs and cDNA resources are much less abundant in other Triticeae, e.g., Elymus, Aegilops and Leymus, numerous studies profited from the high transferability of wheat EST-derived SSR markers across distantly related species for comparative mapping, trait-marker associations and to carry out evolutionary studies to establish the phylogenetic relationships among the wild relatives of wheat and between them and bread wheat (Adonina et al. 2005; Jing et al. 2007; Kroupin et al. 2012).

The conserved orthologous set (COS) markers allowed identification of orthologous regions between wild species and wheat in order to facilitate alien gene-transfer

t1.1 **Table 13.1** Genomic resources available for Triticeae species

t1.2 t1.3 t1.4 Genus (no. of taxonomy entries in NCBI)	Bio Project[a]	Number of genes	Number of ESTs	BAC libraries	cDNA clones	Probe[b]	Map data[c]	SRA[d]	GSS[e]	Genome[f]
t1.5 Aegilops (42)	35	1172	4546	8	2303	787	4	161	5172	1
t1.6 Agropyron (16)	0	4	17	–	–	0	–	1	0	1
t1.7 Amblyopyrum (3)	–	–	–	–	–	–	–	–	–	–
t1.8 Anthosachne (10)	–	–	–	–	–	–	–	–	–	–
t1.9 Australopyrum (6)	–	–	–	–	–	–	–	–	–	–
t1.10 Avena (35)	11	28	79,657	–	–	11,542	24	–	3063	–
t1.11 Campeiostachys (11)	–	–	–	–	–	–	–	–	–	–
t1.12 Connorochloa (2)	–	–	–	–	–	–	–	–	–	–
t1.13 Critesion (4)	–	–	–	–	–	–	–	–	–	–
t1.14 Crithopsis (2)	–	–	–	–	–	–	–	–	–	–
t1.15 Dasypyrum (3)	–	–	–	–	–	–	–	–	14	–
t1.16 Douglasdeweya (3)	–	–	–	–	–	–	–	–	–	–
t1.17 Elymus (116)	1	–	45,580	–	–	–	–	–	–	1
t1.18 Eremopyrum (5)	-	–	–	–	–	–	–	–	–	–
t1.19 Festucopsis (3)	–	–	–	–	–	–	–	–	–	–
t1.20 Haynaldia (2)	–	–	10	–	–	–	–	–	13	–
t1.21 Henrardia (5)	–	–	–	–	–	–	–	–	–	–
t1.22 Heteranthelium (4)	–	–	–	–	–	–	–	–	–	–
t1.23 Hordelymus (2)	–	–	–	–	–	–	–	–	–	–
t1.24 Hordeum (62)	148	717	840,120	2	89,452	11,196	76	1894	574,028	4
t1.25 Hystrix (5)	–	147	–	–	–	–	–	50	–	–
t1.26 Kengyilia (22)	–	–	–	–	–	–	–	2	–	–
t1.27 Leymus (50)	4	–	30,749	–	–	1853	3	6	13	–
t1.28 Lophopyrum (5)	2	–	2	–	–	56	–	1	–	–

(continued)

Table 13.1 (continued)

Genus (no. of taxonomy entries in NCBI)	Bio Project[a]	Number of genes	Number of ESTs	BAC libraries	cDNA clones	Probe[b]	Map data[c]	SRA[d]	GSS[e]	Genome[f]
Pascopyrum (2)	–	–	–	–	–	–	–	–	–	–
Peridictyon (2)	–	–	1	–	–	–	–	–	–	–
Psammopyrum (2)	–	–	–	–	–	–	–	–	–	–
Psathyrostachys (16)	1	–	–	–	–	–	–	1	44	–
Pseudoroegneria (9)	–	–	–	–	–	–	–	–	–	–
Secale (16)	21	113	15,903	2	6617	1091	12	36	2956	–
Stenostachys (4)	–	–	–	–	–	–	–	–	–	–
Taeniatherum (6)	–	–	2	–	–	–	–	–	–	–
Thinopyrum (12)	4	–	2385	–	–	–	–	3	7	–
Triticum (84)	239	3170	1,358,421	16	10,527	21,164	69	2558	72,374	4
× Aegilotriticum(14)	1	–	–	–	–	–	–	–	–	–
× Triticosecale (10)	3	–	11	–	–	–	–	–	8	–
× Tritordeum (6)	–	–	4	–	–	57	–	–	11	–

The information in this table was collected from NCBI taxonomy (http://www.ncbi.nlm.nih.gov/taxonomy) and GrainGene (http://wheat.pw.usda.gov/GG3/) databases in May 2015. Triticeae genera comprising cultivated species are underlined

[a]Projects initiated in the fields of genomics, functional genomics and genetic studies (NCBI)

[b]Public registry of nucleic acid reagents designed for use in a wide variety of biomedical research applications (NCBI)

[c]Genetic and physical maps available for Triticeae (GrainGene database)

[d]Sequence Read Archive (NCBI) stores sequencing data

[e]Genome Survey Sequences (NCBI) is a collection of unannotated short single-read primarily genomic sequences from GenBank including random survey sequences, clone-end sequences and exon-trapped sequences

[f]Genome (NCBI) reference whole genomes sequencing information, both completely sequenced organisms and those for which sequencing is in progress

t2.1 **Table 13.2** Whole genome sequencing projects in cereals

t2.2 t2.3 Species/cultivar	Genome size (1C)	Sequence description	Consortium/team
t2.4 t2.5 t2.6 Oryza sativa ssp. Japonica	400–430 Mbp	Pseudomolecule	International Rice Genome Sequencing Project (2005)
t2.7 Zea maize cv. B73	2.4 Gbp	Pseudomolecule	Schnable et al. (2009)
t2.8 t2.9 Sorghum bicolor cv Moench	750 Mbp	Whole-genome draft assembly	Paterson et al. (2009)
t2.10 t2.11 t2.12 Brachypodium distachyon inbread line Bd21	~355 Mbp	Pseudomolecule	The International Brachypodium Initiative (2010)
t2.13 t2.14 t2.15 Hordeum vulgare cv Morex	~5.3 Gbp	Whole-genome draft assembly	The International Barley Genome Sequencing Consortium (2012)
t2.16 t2.17 t2.18 Aegilops tauschii ssp. strangulata accession AL8/78	4.02 Gbp	Whole-genome draft assembly	Luo et al. (2013)
t2.19 t2.20 Triticum urartu accession G1812	4.94 Gbp	Whole-genome draft assembly	Ling et al. (2013)
t2.21 t2.22 t2.23 t2.24 t2.25 t2.26 t2.27 Triticum aestivum cv Chinese spring (CS) 3B chromosome of Triticum aestivum cv CS	~16 Gbp ~16 Gbp (~1 Gbp)	5× whole-genome draft assembly Chromosome-based draft assemblies of each 21 chromosomes Reference sequence assembly of chromosome 3B	Brenchley et al. (2012) IWGSC (2014) Choulet et al. (2014)

through a better characterization of the potentially recombining regions (Molnár et al. 2013). As the COS markers are PCR based and span exon–intron junctions, they are conserved enough to be transferrable across genera, while the intron sequences provide relatively high polymorphism that allows variants of genes to be discriminated (e.g., between species). Although these markers present interesting tools to support alien-wheat gene transfer, they remain underexploited in this area.

13.3.3 High-Throughput Genotyping

Diversity Arrays Technology (DArT) markers were initially developed as microarray hybridization-based sequence-independent marker system, and allowed screening thousands of polymorphic loci in a single assay at low cost per data point (Jaccoud et al. 2001). Among other, DArT markers were used to develop high-density genetic map of wheat × wild emmer (Peleg et al. 2008). A new version of DArT marker technology (DArT-seq) is based on next-generation sequencing where the polymorphisms are genotyped by sequencing. Because of their advantages,

DArT has been employed extensively in genetic mapping, genotyping, and diversity assessment in wheat (Cabral et al. 2014; Jighly et al. 2015; Bentley et al. 2014; Yu et al. 2014; Colasuonno et al. 2013; Iehisa et al. 2014), and more recently in its wild and cultivated relatives (Montilla-Bascón et al. 2015; Kalih et al. 2015; Castillo et al. 2014; Bolibok-Brągoszewska et al. 2014; Alheit et al. 2014; Yabe et al. 2014; Cabral et al. 2014; Jing et al. 2009).

The advent of the next generation sequencing technologies changed the paradigm of wheat genetics and genomic and led to the development of Single Nucleotide Polymorphism (SNP) markers. Various platforms have been developed for wheat genotyping such as the 9K and 90K Illumina iSelect platforms with 9000 and 90,000 SNP markers, respectively (Cavanagh et al. 2013; Wang et al. 2014), the Illumina infinium platform (up to 1,000,000 SNP markers), as well as the Axiom 820K and 35K arrays (with up to 820,000 and 35,000 features) (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/axiom_download.php). These platforms provide tools to obtain detailed information on germplasm diversity and characterize allelic variation. However, low representation of wild wheat relatives in the SNP design process may limit the utility of the platforms in wheat alien introgression breeding (Wulff and Moscou 2014). Consequently, a few studies made use of SNP molecular markers to support alien gene transfer in wheat (Tiwari et al. 2014) and very few SNPs derived from wild species are available.

Due to the low cost per data point and ease of development, Kompetitive Allele Specific PCR (KASP) SNP markers (He et al. 2014), a genotyping technology based on allele-specific oligo extension and fluorescence resonance energy transfer for signal generation, are becoming popular and are used in large-scale projects (Petersen et al. 2015). KASP markers can genotype SNP polymorphism, deletions and insertions variations, and have been used in screening wheat–alien hybrids and their back-crossed derivatives to detect recombinants and isolate desired introgressions (King et al. 2013). In order to promote the use of KASP markers, it is important to generate new genomic sequences from wild relatives of wheat.

13.3.4 Genome Sequencing

13.3.4.1 Whole Genome Approaches

Despite the importance of Triticeae species for the humankind (Feuillet et al. 2008), attempts to sequence their genomes were delayed due to the size and complexity. The nuclear genome of bread wheat comprises three structurally similar (homoelogous) subgenomes A, B, and D and with the size of about 17 Gb/1C, it is 40 times bigger than rice (0.43 Gb) and 126 times bigger than Arabidopsis thaliana (0.135 Gb). As the other Triticeae genomes, it is highly redundant and composed mostly from various classes of repetitive DNA sequences (IWGSC 2014).

High throughput of the next generation sequencing technologies makes it possible to sequence even the biggest genomes. However, the problem is to assemble and

order the short reads thus obtained (IWGSC 2014). Due to large genome complexity and sequence redundancy, high-quality reference genome assemblies are not yet available for any of the Triticeae species. To date, only draft genome sequences are available for barley (The International Barley Genome Sequencing Consortium 2012), T. urartu (Ling et al. 2013)—a progenitor of the A genome of bread wheat, Ae. tauschii (Luo et al. 2013)—a D genome progenitor of bread wheat, as well as the whole genome shotgun assembly of hexaploid bread wheat (Brenchley et al. 2012) (see Table 13.2).

Due to their nature, draft sequence assemblies are only partial representations of the genomes, often accounting for less than 50 % of their estimated size. A significant part of expressed genes may be absent, which may compromise efforts with gene discovery and cloning, while the fragmentation of genome sequence and large numbers of unanchored contigs hamper comparative genome analyses.

Despite their preliminary nature, draft genome sequences provided useful insights into the Triticeae genome organization, evolution, and function. They were useful to develop protein-coding gene models, analyze genome organization, assess recombination rates along chromosomes, and characterize synteny and collinearity with other species (Ling et al. 2013; Luo et al. 2013; The International Barley Genome Sequencing Consortium 2012). They served as templates to characterize agronomically important genes and develop genome-specific molecular markers for plant breeding (Ling et al. 2013). The utility and extensive use of whole genome sequences from the main Triticeae crops confirm the need for such resources in wild wheat relatives. Although it may not be possible to sequence genomes of all wild species employed in wheat alien introgression breeding, efforts should be made to obtain as much information on their genomes as possible in order to understand better the genome relationships among Triticeae.

13.3.4.2 Reduced-Complexity Sequencing

One approach to facilitate sequencing and assembly of the huge Triticeae genomes is to reduce sample complexity prior to sequencing. Various strategies have been developed to achieve this, and can be classified into two groups: (1) Transcriptome sequencing and sequence capture approaches, which sequence only certain parts of genomes, and (2) the chromosome-centric approaches, which reduce the complexity in a lossless way by dissecting genomes to small parts (chromosomes and chromosome arms) that are sequenced and assembled separately.

Sequencing conserved genic portions of genomes enables development of cross-species transferable tools, and facilitates functional understanding of important traits. Haseneyer et al. (2011) sequenced transcriptome in five winter rye inbred-lines and identified over 5000 SNPs between the transcriptomes that were subsequently used for genotyping 54 inbred lines using SNP genotyping array. This analysis does not require prior knowledge of genome sequence and allows large-scale molecular marker development for high-throughput genotyping. A recent analysis of Agropyron cristatum transcriptome permitted identification of 6172

unigenes specific to A. cristatum, including many stress-resistant genes and alleles potentially useful in wheat improvement (Zhang et al. 2015).

Another option to reduce sequencing efforts are sequence-capture approaches, which are used to enrich samples for sequences of interest before carrying out NGS. They are based on hybridization of target sequences to bait probes in solution, or on solid support. This approach usually necessitates preliminary sequence information. However, since it allows high level of mismatches, it permits capturing diverged sequences. Known sequences from more characterized species such as wheat, barley, Brachypodium, and rice can be employed to discover uncharacterized sequences from related species and varieties. Accordingly, Jupe et al. (2013) developed an exome capture for nucleotide-binding leucine-rich repeat (NB-LRR) domain for the so-called Resistance gene enrichment Sequencing (RenSeq) in potato. Their work resulted in discovery of 317 previously unannotated NB-LRRs and the method could aid in discovery of new resistance genes in wild relatives of wheat (Wulff and Moscou 2014).

Alternative approach to reduce complexity of large and polyploid genomes is to isolate chromosomes by flow cytometric sorting and sequence them individually (Fig. 13.3). This strategy is called chromosome genomics (Dolezel et al. 2007, 2014) and has been adopted by the IWGSC for the bread wheat genome sequencing (IWGSC 2014). The method, originally developed in Vicia faba (Doležel et al. 1992), relies on cell cycle synchronization of meristem root tip cells of young seedlings and their accumulation at mitotic metaphase. After mild formaldehyde fixation, intact chromosomes are released into a buffer solution by mechanical homogenization of root tips. Chromosome samples are stained by a DNA fluorochrome DAPI and classified at rates of several thousand per second according to their relative DNA content using flow cytometry. Chromosomes that differ in DNA content from other chromosomes form distinct peaks on histograms of DNA content (flow karyotypes). Such chromosomes, can be sorted individually at rates of about 20 s^{-1}, and several hundred thousand chromosomes of the same type can be collected in 1 day.

In a majority of species, chromosomes have similar DNA content and cannot be discriminated after DAPI staining alone. The most frequent approach to overcome this difficulty has been the use of cytogenetic stocks in which the size of one or more chromosomes has been changed so that the chromosome of interest can be discriminated and sorted. The stocks included chromosome translocations (Kubaláková et al. 2002), deletions (Kubaláková et al. 2005), alien chromosome addition (Kubaláková et al. 2003) and alien chromosome arm additions (Suchánková et al. 2006). As such stocks are not available for many species, it is important that Giorgi et al. (2013) developed a protocol termed FISHIS, to fluorescently label repetitive DNA on chromosomes prior to flow cytometric analysis. This approach permits discrimination of chromosomes, which have the same or very similar relative DNA content (Fig. 13.3), and has been used successfully to sort chromosomes in Ae. umbellulata, Ae. comosa, Ae. speltoides, and Ae. markgrafii (Molnár ~~et al. in preparation~~). [AU2]

To date, chromosome flow-sorting has been reported in at least 29 plant species, including 15 Triticeae (Doležel et al. 2014; Table 13.3). High purity in the sorted

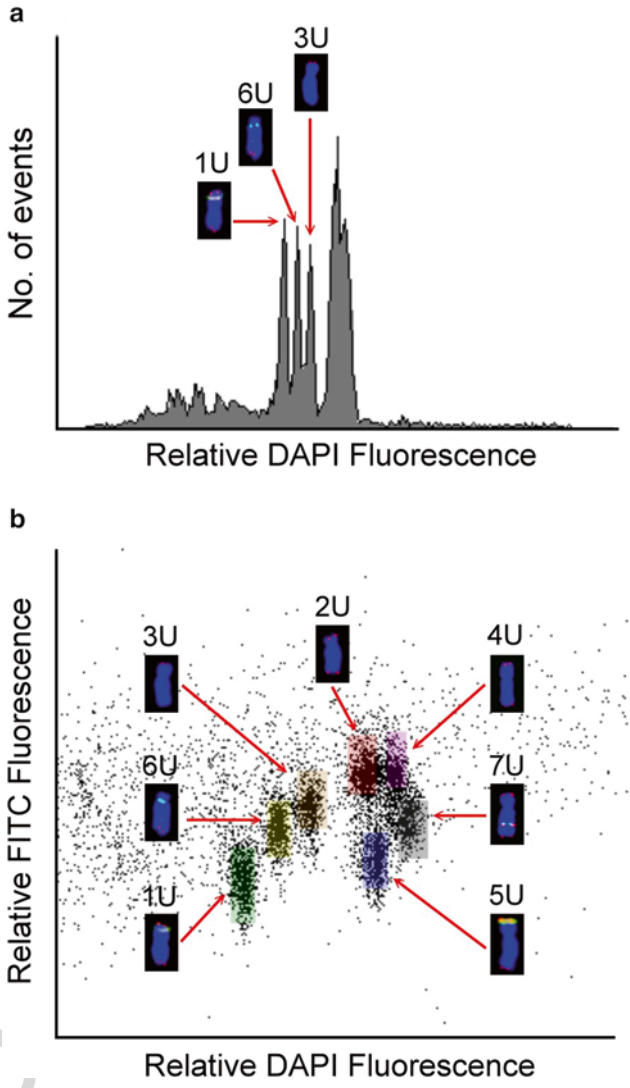

Fig. 13.3 Mono- (**a**) and biparametric (**b**) flow cytometric analysis and sorting of mitotic metaphase chromosomes from Ae. umbellulata (2n = 2x = 14; UU). (**a**) Monoparametric analysis of chromosomes stained by DAPI results in a histogram of relative fluorescence intensity (flow karyotype) in which three peaks representing chromosomes 1U, 6U and 3U are discriminated. The remaining four chromosomes form a composite peak and cannot be sorted individually. Biparametric analysis of chromosomes stained by DAPI and with GAA repeats labeled by FITC results in a bivariate flow karyotype on which all seven chromosomes (colored regions) can be discriminated and flow-sorted at a purity of 90–99 %

t3.1 **Table 13.3** List of Triticeae species in which flow cytometric chromosome sorting has been
t3.2 reported (adapted from Doležel et al. (2014))

t3.3 t3.4 Genus	Species	Common name	*n*	Reference[a]
t3.5 *Aegilops*	*biuncialis*	Goatgrass	14	Molnár et al. (2011b)
t3.6	*comosa*		7	Molnár et al. (2011b)
t3.7	*cylindrica*		14	Molnár et al. (2015)
t3.8 t3.9	*geniculata*		14	Molnár et al. (2011b); Tiwari et al. (2014)
t3.10	*markgrafii*		7	Molnár et al. (2015)
t3.11	*speltoides*		14	Molnár et al. (2014)
t3.12	*triuncialis*		14	Molnár et al. (2015)
t3.13	*umbellulata*		7	Molnár et al. (2011b)
t3.14 *Avena*	*sativa*	Oat	21	Li et al. (2001)
t3.15 t3.16 *Dasypyrum*	*villosum*	Mosquito Grass	7	Grosso et al. (2012); Giorgi et al. (2013)
t3.17 t3.18 t3.19 *Hordeum*	*vulgare*	Barley	7	Lysák et al. (1999); Lee et al. (2000); Suchánková et al. (2006); Mayer et al. (2009, 2011)
t3.20 t3.21 *Secale*	*cereale*	Rye	7	Kubaláková et al. (2003); Bartoš et al. (2008); Martis et al. (2013)
t3.22 t3.23 t3.24 t3.25 t3.26 t3.27 t3.28 t3.29 t3.30 *Triticum*	*aestivum*	Bread wheat	21	Wang et al. (1992); Schwarzacher et al. (1997); Lee et al. (1997); Gill et al. (1999); Vrána et al. (2000); Kubaláková et al. (2002); Giorgi et al. (2013); Hernandez et al. (2012); IWGSC (2014); Helguera et al. (2015); Tanaka et al. (2014); Sergeeva et al. (2014); Lucas et al. (2014); Berkman et al. (2011)
t3.31 t3.32	*durum*	Durum wheat	14	Kubaláková et al. (2005); Giorgi et al. (2013)
t3.33	*urartu*		7	Molnár et al. (2014)

t3.34 [a]Reports on chromosome sequencing are underlined

408 fractions and high molecular weight DNA of flow-sorted chromosomes makes
409 them ideal substrate for downstream applications such as PCR-based analysis,
410 development of markers, BAC-vector cloning and construction of optical maps
411 (for review see (Doležel et al. 2014)). Chromosomal DNA can be sequenced or
412 used for other applications either directly, if a sufficient number of chromosomes is
413 sorted, or after representative amplification (Šimková et al. 2008). It is now even
414 possible to sequence a single flow-sorted chromosome (Cápal et al. submitted). [AU3]
415 The latter is particularly important in cases when the chromosome of interest cannot
416 be discriminated from other chromosomes in karyotype, or if the focus is on the
417 analysis of structural chromosome heterozygosity and allele phasing.
418 For example, BAC-end sequences obtained using 1RS-specific BAC library
419 were used to develop Insertion Site-Based Polymorphism markers (ISBP) specific
420 for 1RS and to identify loci carrying microsatellites suitable for the development

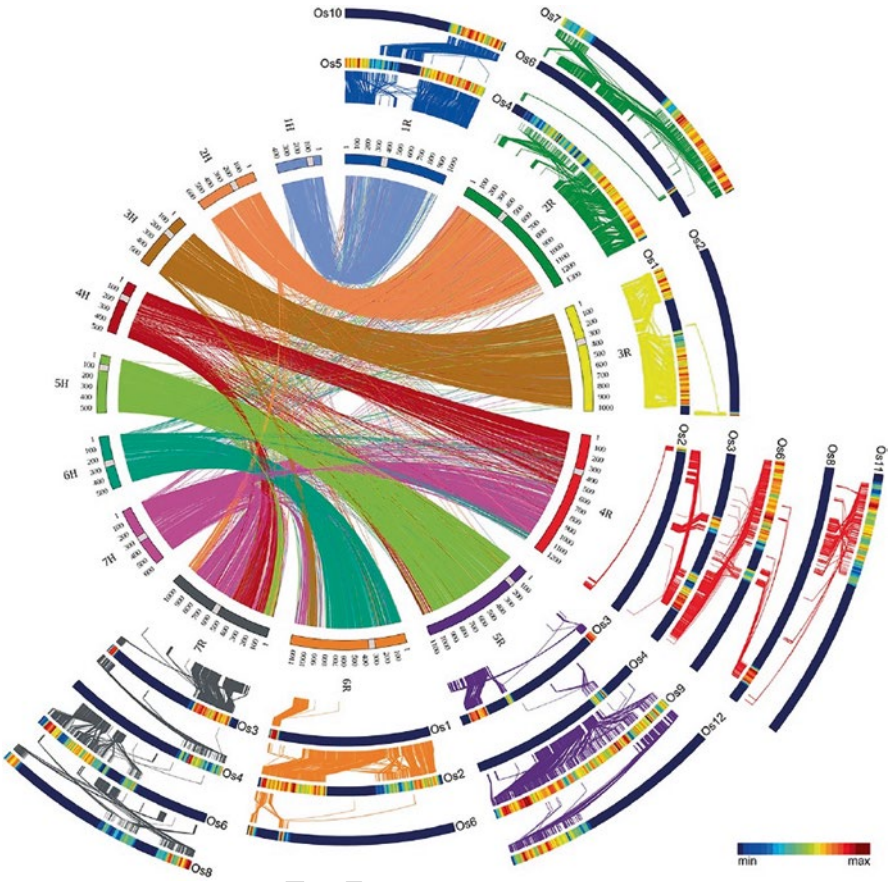

Fig. 13.4 Next-generation sequencing of flow-sorted rye chromosomes allowed characterization of synteny between rye, barley, and rice genomes. Collinearity of the rye and barley genomes is depicted by the inner circle of the diagram. Rye (1R–7R) and barley (1H–7H) chromosomes were scaled according to the rye genetic and barley physical map, respectively. Lines (colored according to barley chromosomes) within the inner circle connect putatively orthologous rye and barley genes. The outer partial circles of heat map colored bars illustrate the density of rice genes hit by the 454 chromosome sequencing reads of the corresponding rye chromosomes. Conserved syntenic blocks are highlighted by yellow-red-colored regions of the heat maps. Putatively orthologous genes between rye and rice are connected with lines (colored according to rye chromosomes) and centromere positions are highlighted by grey rectangles. Martis et al., Plant Cell 25: 3685–3698, 2013. www.plantcell.org Copyright American Society of Plant Biologists. Reproduced with permission

of 1RS-specific SSR markers (Bartoš et al. 2008). Next-generation sequencing flow-sorted chromosomes of rye enabled establishing linear gene order model comprising over 22 thousand genes, i.e. 72 % of the detected set of 31,000 rye genes. Chromosome sequencing together with transcript mapping and integration of conserved synteny information of Brachypodium, rice and sorghum enabled a genome-wide high-density comparative analysis of grass genome synteny (Fig. 13.4).

The chromosome genomics approach has been particularly fruitful in genomics of wheat. The chromosome-based draft sequence of bread wheat was obtained by sequencing flow-sorted chromosome arms (except of chromosome 3B), each of them representing only 1.3–3.3 % of the genome. Chromosome arms were sequenced with Illumina technology and the reads were assembled to contigs representing 10.2 Gb (61 %) of the genome with a L50 of repeat-masked assemblies ranging from 1.7 to 8.9 kb. A total of 133,090 loci homologous to related grass genes were classified as high-confidence gene calls. Out of them, 93.3 % were annotated on individual chromosome arm sequences, and 53.2 % were located on syntenic chromosomes compared to brachypodium, rice and sorghum. In total, 81 % raw reads and 76.6 % assembled sequences contained repeats, explaining the difficulty of assembling such genomes from short sequence reads. As demonstrated in chickpea, chromosome genomics can be coupled with whole genome next-generation sequencing to validate whole genome assemblies (Ruperao et al. 2014). This powerful combination could speed up production of good quality whole genome assemblies in wild wheat relatives.

Chromosome genomics was also shown useful to characterize chromosome segments of alien origin, develop markers from these regions, and support cloning alien genes of interest. In a pioneering study, Tiwari and coworkers sequenced DNA from flow-sorted short arm of chromosome $5M^g$ of Ae. geniculata to develop genome-specific SNP markers Tiwari et al. (2014). The markers allowed development of two SNP markers identifying introgression of a segment of $5M^g$ to wheat chromosome 5D carrying resistance to leaf rust (Lr57) and stripe rust (Yr40) (Fig. 13.5). In order to simplify the identification of alien chromatin introgressed into wheat, Abrouk (pers. comm.) developed a method based on comparative analysis. Briefly, using the linear gene order map of a recipient wheat chromosome (IWGSC 2014) and the sequence of flow-sorted chromosome carrying alien introgression, the density of orthologs is calculated along the wheat chromosome. The variation in density makes it possible to detect the alien segment. This approach has been validated recently in wheat T. aestivum cv. Tahti—T. militinae introgression line 8.1 (Jakobson et al. 2006, 2012), which carries a major QTL for powdery mildew resistance on the distal part of the chromosome 4AL (Abrouk pers. comm.)

13.4 Functional Aspects of Alien Gene Transfer

When introducing alien genes to wheat, the function of introgressed chromosomes or chromosome segments and their interaction with the host genome needs to be considered. It may occur at different levels and concern chromosome behavior during meiosis, changes in chromosomes structure and genome organization, as well as gene expression. Understanding the interaction between the host and alien genomes, the evolution of this relationship from the moment of F1 hybrid formation to a stabilized wheat–alien introgression line, and the way the final equilibrium impacts the performance of the introgression line may contribute to the success of alien gene transfer in wheat improvement.

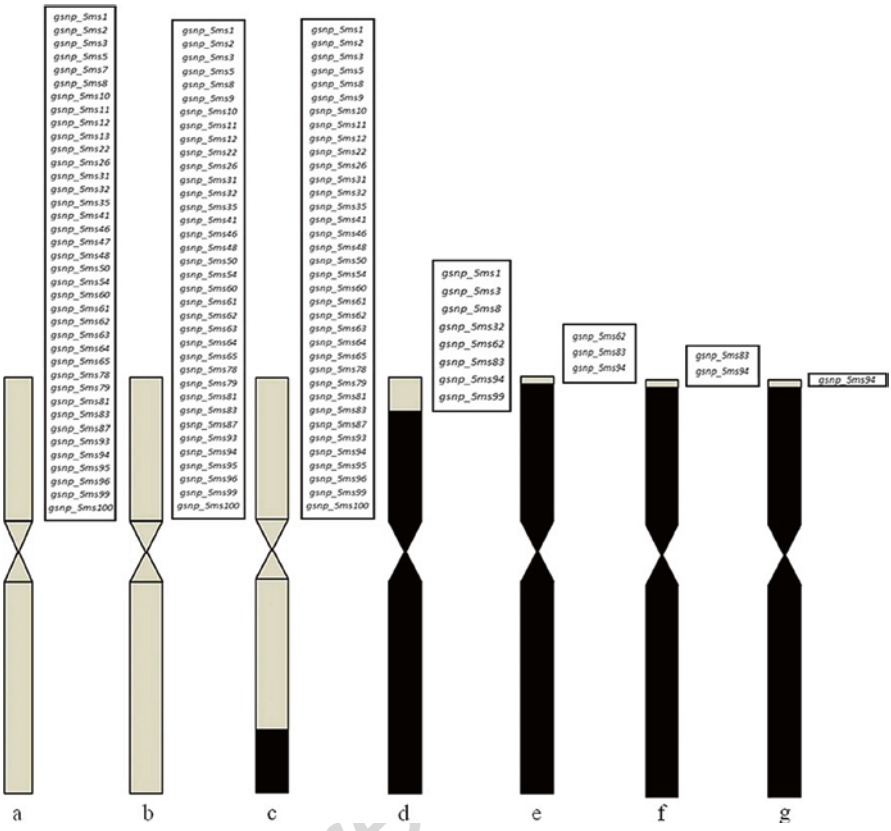

Fig. 13.5 Distribution of validated 5MgS-specific SNPs developed from flow-sorted ditelosomic 5Mg in different alien introgression-based addition, translocation, and released wheat lines. (a) disomic addition line TA7657, (b) disomic substitution line TA6675, (c) translocation line TA5599, (d) terminal translocation line TA5602, (e) TA5602 (with very small 5Mg segment), (f) SNPs validated in germplasm KS11WGGRC53-J and (g) SNP validated in germplasm KS11WGGRC53-O. Tiwari et al., BMC Genomics 15: 273, 2014. http://www.biomedcentral.com/bmcgenomics BioMed Central Ltd. Reproduced with permission

13.4.1 Interaction Between Host and Donor Genomes

Alien gene transfer involves hybridization and creation of interspecific hybrids, followed by genome duplication to establish fertile amphiploids. A consequence is a shock for both genomes, which may result in activation of mobile genetic elements, various structural changes and lead to changes in epigenetic status of chromatin and novel patterns of gene expression (Comai 2000).

Elimination of specific sequences is commonly reported as rapid genomic rearrangement accompanying allopolyploidization in wheat. The changes include elimination of noncoding and low-copy DNA sequences, and gain of novel fragments

(Feldman et al. 1997; Liu et al. 1998). Elimination of rye-specific fragments often representing transposable elements (TEs) and their derivatives was observed in allopolyploid triticales (Ma and Gustafson 2006, 2008; Bento et al. 2008). The analysis of a newly synthesized triticale (Bento et al. 2008; Han et al. 2003) revealed rapid changes in coding sequences upon the induction of allopolyploidy, but the changes did not extend to alterations discernible at cytological level. The molecular mechanisms underlying genome reorganization are not yet fully understood (Tayalé and Parisod 2013). 'Genomic stress' due to polyploidization may activate TEs and promote their proliferation and mobility. At the same time, massive elimination in a TE family-specific manner may be observed (Comai et al. 2003; Parisod and Senerchia 2012). It seems that the degree of TE sequence divergence between progenitors correlates with the degree of restructuring in polyploid TE fractions (Senerchia et al. 2014).

A general observation made in newly created polyploids and synthetic allotetraploids, including wheat, is a change in gene expression immediately after polyploidization (Kashkush et al. 2002; Levy and Feldman 2004). Both genetic and epigenetic mechanisms may alter gene expression (Lynch and Conery 2000; Lee and Chen 2001; Osborn et al. 2003; Soltis et al. 2004). The analysis of cytosine methylation in Aegilops–Triticum F1 hybrids and their derivative allotetraploids revealed 13 % of the loci with altered patterns of methylation affecting both repetitive DNA and low-copy DNA (Xiong et al. 1999; Shaked et al. 2001). In leaves of Arabidopsis autopolyploids and allotetraploids and their progenitors, Ng et al. (2012) could associate rapid changes in gene expression with quantitative proteomic changes, suggesting rapid changes in posttranscriptional regulation and translational modifications of proteins as a consequence of polyploidization.

Epigenomic rearrangements after allopolyploidization seem to be involved in the processes of uniparental chromosome elimination, a phenomenon observed frequently in interspecific hybrids between T. aestivum and H. bulbosum (Bennett et al. 1976), H. vulgare (Islam et al. 1981) and Zea mays (Laurie and Bennett 1986). The loss of centromere-specific histone H3 (CENH3) caused centromere inactivation and triggered mitosis-dependent uniparental chromosome elimination in unstable H. vulgare × H. bulbosum hybrids (Sanei et al. 2011). Bento et al. (2010), found that chromosome structural rearrangements were more drastic in wheat–rye disomic addition lines than in triticale, indicating that the lesser the amount of rye genome introgressed into wheat, the higher the likelihood of wheat chromosome breakage, chromosome elimination, and chromosome structural rearrangement, including sequence-specific elimination, translocations and TE movement (Fu et al. 2013).

13.4.2 Alien Gene Expression

Various studies indicate complex relationships between the alien and host genes (Pumphrey et al. 2009; Jeffrey Chen and Ni 2006; Bougas et al. 2013; Wu et al. 2015; Yoo et al. 2013; Wulff and Moscou 2014) and, as a result, in some cases

alien genes may not function as expected. For example, weaker effect in the wheat background as compared to the wild species was observed in studies involving resistance gene transfer (Wulff and Moscou 2014; Chen et al. 2005; Riley and Chapman 1958; Riley and Macer 1966). One explanation may be that the introgressed genes are involved in polygenic resistance together with other loci, which are not introgressed simultaneously. However, in some cases, resistance genes had no effect at all, as was the case of resistance to wheat leaf rust (Puccinia triticina Erikss.) introduced to wheat from rye (Riley and Macer 1966). It seems that the polyploid status of wheat itself may impact alien gene expression. When Kerber and Dyck (1973) transferred stem rust resistance from diploid einkorn wheat (T. monococcum L.) to tetraploid durum and hexaploid bread wheat, a progressive loss of the resistance with increasing ploidy from diploid to hexaploid was observed. Chen et al. (2005) described different levels of scab resistance in progenies that involved the same wheat-Leymus racemosus alien chromosome translocation, or the same alien chromosome addition, possibly related to other components of resistance in the genetic background.

Suppression of resistance due to negative interaction of homoeologous and non-homoeologous loci between genomes is another effect observed in hexaploid wheat, and the examples include a conserved gene on chromosome 7DL that suppresses stem rust resistance, and suppression of powdery mildew locus Pm8 by Pm3 locus (Kerber and Aung 1999; Wulff and Moscou 2014). The suppression of introgressed Pm8 resistance gene by its Pm3 host ortholog in some wheat–rye 1BL.1RS translocation lines was not due to gene loss, mutation or gene silencing (Hurni et al. 2014). A coexpression analysis of Pm8 and Pm3 genes in Nicotiana benthamiana leaves followed by co-immunoprecipitation analysis showed that the two proteins interact and form a heteromeric complex, which might result in inefficient or absent signal transmission for the defense reaction. Stirnweis et al. (2014) suggested that the frequently observed failure of resistance genes introduced from the secondary gene pool into polyploid crops could be the result of the expression of closely related NB-LRR-resistance genes or alleles in the host genome, leading to dominant-negative interactions through a posttranslational mechanism involving LRR domains. A recent study showed that genes with low similarity between rye sequences and their closest matches in the Triticum genome have a higher probability to be repressed or deleted in the allopolyploid genome (Khalil et al. 2015).

13.4.3 Spatial Genome Organization and Function

Little is known how alien chromosome(s) and/or translocated alien chromosome segments influence behavior and position of wheat chromosomes within the 3D space of interphase nucleus, how the position and behavior of alien chromosome differs from that in the nucleus of donor wild relative, and how changes in chromosome position influence gene expression of wheat and alien genes. Numerous studies in human and mouse indicate that chromosome territories are not

randomly positioned in the nucleus (Gibcus and Dekker 2013). Small and gene-rich chromosomes localize near the center of nucleus, whereas larger and less-gene-rich chromosomes are more frequently located near the nuclear periphery. In plants, however, 3D-nuclear genome organization has been studied only in a few cases and mostly in Arabidopsis (Schubert et al. 2014; Grob et al. 2014) and rice (Mukhopadhyay et al. 2013) with small genomes, whose interphase organization may differ from that of large genomes. The results obtained in rice (Mukhopadhyay et al. 2013) correlated transcriptional regulation with alteration in nucleosome positioning, histone modifications and gene looping, but not DNA methylation. A recent observation using 3D-FISH in wheat–rye chromosome arm introgression lines indicated that the rye alien chromosomes were positioned at the periphery of nuclei (Veronika Burešová, pers. comm.). These preliminary results are consistent with the general observation of negative regulation of the expression of the alien genes introgressed in wheat.

13.5 Concluding Remarks

During more than one century of wheat–alien introgression breeding, a significant progress has been made in developing strategies to produce hybrids of wheat with distant relatives, in devising chromosome engineering techniques to integrate alien-chromosome segments into wheat genome, in the improvement of cytogenetic techniques to identify and characterize introgressed chromatin, and in phenotypical characterization of new introgression lines. These advances led to development of a formidable panel of introgression lines of various types and from a number of wild wheat relatives, carrying important traits. Nevertheless, only a small number of commercially successful wheat cultivars benefitted from these advances, and the potential of alien introgression breeding remains underused.

In order to fully explore it and benefit from the extant genetic diversity of wild wheat relatives, implementation of improved and novel approaches and tools is needed. It is fortunate that new methods of cytogenetics, genomics and phenomics are becoming available for better and, in case of genomics and phenomics, high-throughput characterization of genetic diversity, and identification of donors of important traits. On the other hand, improvement of chromosome engineering methods and better knowledge of molecular mechanisms controlling meiotic recombination are needed to facilitate introgression of alien chromatin. This will require a better knowledge of genome structure of wild relatives to assess chances for chromosome recombination and predict its outcomes, in order to decide the best experimental approach to be applied.

The advances in DNA sequencing and DNA marker technologies make it possible to compare genomic organization of wheat and wild relatives, and judge the degree of collinearity. In order to cope with the huge and complex genomes of Triticeae, strategies have been developed to reduce genome complexity prior to sequencing and mapping, such as exome capture and chromosome genomics.

The advances in DNA sequencing technologies make it possible to develop powerful and high-throughput DNA marker technologies such as SNP, DArT and KASPAR, which are suitable for development of markers linked tightly to traits of interest, large-scale screening of progenies of wild hybrids and support production of lines with the introgressed genes of interest and minimum of unwanted chromatin.

Altogether these advances provide a toolbox to develop wheat lines enriched for gene(s) of interest with the smallest amount of undesired alien chromatin. At the same time, it is obvious that we are still at the beginning of what one day may become a routine transfer of alien genes to wheat by interspecific hybridization. In fact, there is another potential obstacle, which so far has received little attention, and that is the genome biology. Almost nothing is known on the behavior of introgressed chromosomes, chromosome segments and/or minute amounts of alien chromatin introgressed into the wheat genome. It is not clear how the wheat genome interacts with introgressed genes and how it influences their function. At the same time, it is important to understand if and how the alien DNA affects the function of the recipient wheat genome. There is an urgent need to clarify the interaction between the host and alien genomes to avoid failed attempts. Luckily, the recent advances in genomics, transcriptomics, epigenomics, proteomics, as well as in cytogenetics, and the analysis 3D organization of interphase nuclei in particular, are promising to deliver the much needed insights.

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