Chapter 1

Cytogenetics in the Study of Chromosomal Rearrangement during Wheat Evolution and Breeding

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Abstract

Cytogenetic methods such as chromosome banding and *in situ* hybridization remain relevant in the post-genomic era, especially for allopolyploid species where genome duplication in some cases makes it difficult to assess the reorganization of chromosomes during evolution. In this review, we give a brief description of cytogenetic methods for the analysis of homoeological chromosomes in cereals. Emphasis is placed on the development of methods for the study of polyploid wheat and its progenitors and on tandem repeats and retrotransposons as markers to evaluate chromosome reorganization throughout evolution and breeding. The most effective cytological probes used for the identification of chromosomes in wheat and Triticeae species by fluorescence and genomic *in situ* hybridization are described. Particular attention is paid to ribosomal genes used as markers in phylogenetic studies and for chromosome identification. Utility of these cytogenetic methods in the evaluation of breeding lines is demonstrated. A strategy for cytological analysis of wheat hybrids according to the degree of relationships between the species involved in crosses is also discussed.

Keywords: wheat, homoeological chromosomes, FISH, GISH, tandem repeats, retrotransposons, ribosomal genes

1. Introduction

The genus *Triticum* occupies a special position among cereals due to their different levels of ploidy and their adaptation to widely differing ecological and geographical regions of the world. The wealth of studies available on the origin of polyploid wheat provides the opportunity to comparatively analyze their genomes and those of putative donors, gaining insight into the reorganization of chromosomes in the process of evolution, domestication, and breeding.

Interest in chromosome organization and evolution in common wheat diploid progenitors and wild wheat is primarily because these species are a valuable source of new genes that were lost in the process of domestication.

The first allopolyploid of the Emmer wheat group is *Triticum dicoccoides* Koern. (2n = 28, genome BBAA), which arose as a result of hybridization of the diploid species *T. urartu* Thum. ex Gandil. and *Aegilops speltoides* Tausch (the most probable donor of the B-genome) [1]. Hexaploid wheat (*T. aestivum* L.) appeared about 7–10 thousand years ago as a result of a second round of hybridization between tetraploid wheat and the wild species *Ae. tauschii* Coss. (donor of the D-genome).

Wheat of Timopheevi group (tetraploids—*T. araraticum* Jakubz., *T. timopheevii* Zhuk., *T. militinae* Zhuk. et Migusch., and hexaploid *T. zhukovskyi* Menabde et Ericzjan) also came about through the hybridization of *T. urartu* and *Ae. speltoides*, but in another time scale. They have a genome designated as GGA^tA^t or GGA^tA^tDD, pointing at their partial homology with the genomes of wheat species in the Emmer group [2].

Diploid progenitors and species of the Timopheevi group are the source of new genes for the resistance to biotic and abiotic stresses. They are regularly involved in hybridization with common wheat, either directly or through synthetic polyploid forms. In this regard, knowledge on the structure and evolution of chromosomes of common wheat, its cultural and wild relatives is particularly relevant.

Genome sequencing methods actively displace cytogenetic analysis in current research. However, in working with hybrid material and introgressed lines of common wheat, we argue that the relevance of cytogenetic methods endures in the post-genomic era.

This review gives a brief description of the cytogenetic methods that remain relevant at this time and their use in the study of chromosomal rearrangement during wheat evolution and breeding.

2. Development of cytogenetic methods for studying chromosomes of polyploid wheat and their progenitors

2.1. Chromosome banding and *in situ* hybridization

Classical cytogenetic methods such as chromosome banding are currently relevant for wild species of plants as well as for polyploid species. Genome duplication within polyploids in some cases makes it difficult to assess the reorganization of chromosomes during evolution and hybridization.

The development of chromosome banding techniques allowed for the identification of the chromosomes not only in morphology but also in individual-specific patterns. Descriptions of the results of chromosome banding are based on the chromosomal region (band) and the intensity of staining, which differs from the neighboring regions. There are several methods of chromosome banding, namely, C-, N-, F-, Hy-, G-, Re-, and AgNOR-banding [3]. The most

common method of staining used in the analysis of cereal genomes is C-differential staining (C-banding), first demonstrated by Pardu and Gal [4]. This method identifies the regions of constitutive heterochromatin after denaturation of the chromosomes and subsequent processing by Giemsa reagent. The karyotypes of many cereal crops, including polyploid wheat and their wild relatives were characterized based on C-banding [3, 5–8]. The use of C-banding allows the study of chromosomal rearrangement during evolution and breeding. For example, Badaeva with co-authors [9] used C-banding to analyze 460 samples of polyploid wheat and 39 forms of triticale (x *Triticosecale* Wittmack) from 37 countries. Fifty-eight main types of chromosomal rearrangements were identified. The results obtained by the authors showed that chromosomes of the B genome are more often involved in chromosomal rearrangements than chromosomes of A and D genomes.

Thus, it is clear that studies like these are necessary for a better understanding of the laws of evolutionary processes in the plant world. C-banding is also currently used to characterize hybrid material and wheat cultivars, especially when other methods of analysis do not reveal chromosome polymorphism [10].

In addition to the differential staining, a specific pattern on chromosomes can be obtained by hybridization *in situ*. Hybridization *in situ* is a direct method of localizing DNA sequences on chromosomes. It is based on the ability of denatured DNA molecules to form duplexes with homologous DNA sequences of chromosomes on a slide. *In situ* hybridization was first performed on animal chromosomes [11] and later applied to plants chromosomes [12]. Over its 50-year history, this method has undergone significant changes aimed at increasing the sensitivity in the detection of labeled probes. This is primarily due to the development of simpler and more efficient DNA tagging systems and better visualization of the hybridization signal. Currently, fluorescence *in situ* hybridization (FISH) is used to study the distribution of individual DNA sequences on chromosomes. Genomic *in situ* hybridization (GISH) is commonly used to identify alien DNA or to study the genomic composition of wheat amphiploids and hybrids.

2.2. Repetitive DNA as a source of markers for chromosome painting

Most often, various repetitive DNA sequences are used as probes for FISH. This is not surprising since repeats are the largest and most rapidly evolving part of the genome. According to the latest sequencing data, repetitive DNA accounts for about 80% of the cereal genome [13, 14]. Groups of repeats with similar structure, formed by amplification from a common original sequence, are called families. Families of repeats differ in their structure, the size of the monomer (from one to several thousand nucleotide pairs), the number of copies, and the type of proliferation.

Transposable elements are the most common repeat elements and account for more than 90% of the entire fraction of cereal repetitive DNA. All families of transposable elements are united into two larger categories—classes, according the mechanism of transposition (retrotransposons and DNA transposable elements). The current detail classification of transposable elements was described by Wicker et al. [15]. Mostly, the transposable elements are dispersed on chromosomes.

There are families of repeats whose members are organized in tandem and assembled into one or more loci. Depending on the length of the repeating unit (monomer), tandem repeats are divided into microsatellites (monomer length 1–6 bp), minisatellites (from 10 to 60 bp), and satellites (average monomer length from 100 to 700 bp) [16].

In fact, each chromosome has an individual "pattern" of repeats, which can be used effectively for marking and identification of individual chromosomes, and the whole genome.

2.2.1. Tandem repeats as markers to study the reorganization of chromosomes in the process of evolution

This group of repetitive DNA sequences is well studied in plants, especially cereals, and is widely used as markers in genomic research and in identifying chromosomes. According to their distribution on chromosomes, the repetitive sequences can be classified as centromeric, subtelomeric or intercalary. In combination, they generate a diagnostic "pattern" on the chromosome. Tandem repeats, such as microsatellites and satellites, and genes of ribosomal RNA are most frequently used for marking the chromosomes of wheat and its relatives.

Microsatellites are repeats with motifs from 1 to 6 bp. In plant genomes, they are also referred to as simple sequence repeats (SSRs) [17]. Microsatellites are used extensively as PCR markers for mapping chromosomes of many plant species and for gene labeling in applied research. Microsatellites are also used as cytogenetic markers. There are a few studies in which the distribution of various microsatellites on *T. aestivum* chromosomes has been examined in detail using FISH [18, 19]. For example, the dinucleotide probes $(AT)_{10}$ and $(GC)_{10}$ recorded no signal on chromosomes. This confirms the earlier hypothesis that the wheat genome does not contain extended clusters of these microsatellites [20, 21]. A dispersed distribution on chromosomes was established for probes $(AC)_8$ and $(GCC)_5$. The large microsatellite blocks detected by the probes $(AGG)_5$, $(CAC)_5$, $(ACG)_5$, $(AAT)_5$, and $(CAG)_5$ were found mainly in the pericentromeric regions of the B genome. Strong intercalary signals were detected after hybridization with the probe $(ACT)_5$ on a number of chromosomes of A and B genomes. Molnar and co-authors [22] investigated the distribution of microsatellites (ACG)n and (GAA)n on the chromosomes of *Ae. biuncialis* Vis (2n = 4x = 28, $U^bU^bM^bM^b$) and *Ae. geniculata* Roth. (2n = 4x = 28, $U^gU^gM^gM^g$) and on the chromosomes of their diploid progenitors: *Ae. umbellulata* Zhuk (UU) and *Ae. comosa* Sm. In Sibth.& Sm. (MM). They concluded that the break points for intergenomic translocations are often localized in regions saturated with microsatellite repeats. Thus, a number of studies have demonstrated that probes based on microsatellites can be useful for the identification of chromosomes and for a better understanding of the principles of chromatin organization in cereals. An important methodologically significant result was obtained by Cuadrado and Jouve [23]. They found that labeled oligonucleotides with a repeating mono-, di-, tri-, or tetra-nucleotide motif have the unexpected ability to detect the corresponding SSR loci even on nondenatured chromosomes, which in some cases can greatly facilitate and accelerate cytological analysis.

The (GAA)n microsatellite is the most widely used marker for the identification of chromosomes. The first works on its localization in the genome of cereals were carried out at the end of the twentieth century [24]. The GAA microsatellite was used as a marker for identification

and sorting of polyploid wheat chromosomes [25, 26]. Phylogenetic studies using the GAA microsatellite were previously problematic due to the deficiency of hybridization signals on the A- and D-genomes and the presence of a number of major hybridization sites on the B-/G-genomes [19, 26].

In recent years, additional publications using GAA microsatellites for the identification of chromosomes of the A-genome of diploid wheat species and for phylogenetic analysis have appeared. Two works published in 2012 included data on the karyotypic analysis of single samples of *T. monococcum* L. and *T. urartu* using the oligonucleotide probe $(GAA)_9$ or GAA fragments obtained by PCR from genomic DNA of wheat [27, 28]. We performed a comparative analysis of the A-genome chromosomes in a diploid and polyploid wheat species consisting of two evolutionary lineages, Timopheevi and Emmer, using the pTm30 probe cloned from the *T. monococcum* genome and containing $(GAA)_{56}$ microsatellite sequences (**Figure 1**) [29].

Up to four pTm30 sites located on 1AS, 5AS, 2AS, and 4AL chromosomes have been revealed in the wild diploid species, although most accessions contained one to two (GAA)n sites (**Figure 1**). The (GAA)n loci on chromosomes 2AS, 4AL, and 5AL found in *T. dicoccoides* were retained in *T. durum* Desf. and *T. aestivum*. In species of the Timopheevi lineage, only one large (GAA)n site has been detected in the short arm of the $6A^t$ chromosome [29].

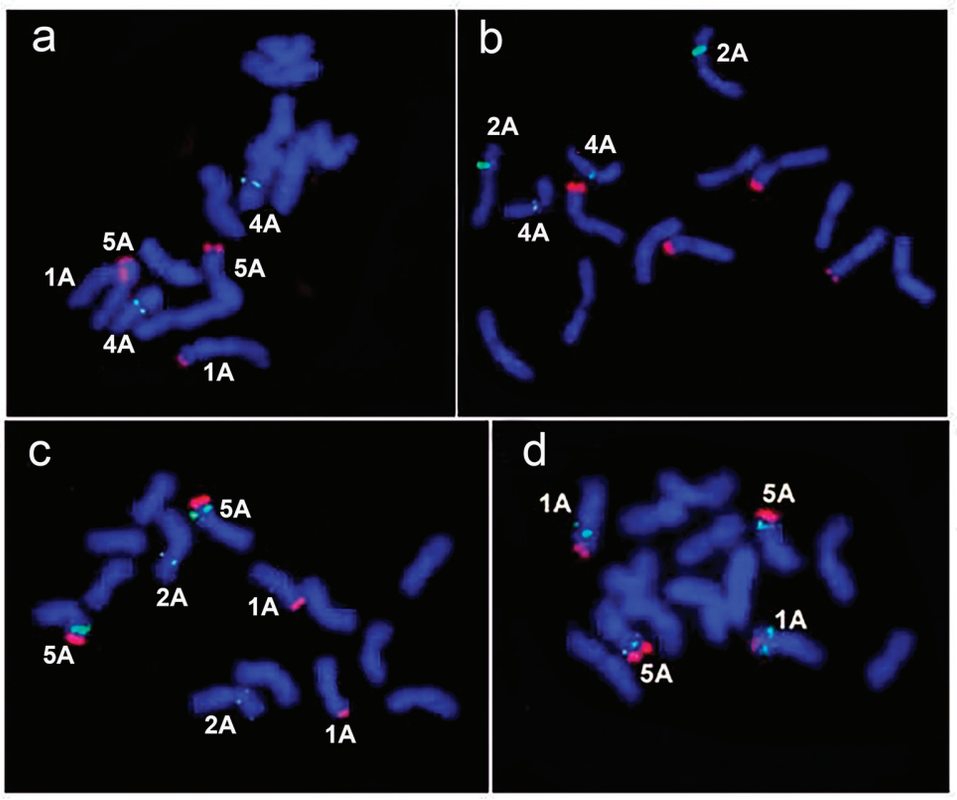

Figure 1. FISH with probes (GAA)n (green) and pTa71 (red) on the chromosomes of diploid *Triticum* species: (a) *T. monococcum* (acc. K-18140), (b) *T. boeoticum* Boiss (acc. K-25811), (c) *T. boeoticum* (acc. PI427328), and (d) *T. urartu* (acc. IG45298).

It was shown that changes in the distribution of (GAA)n sequences on the A-genome chromosomes of diploid and polyploid wheats are associated with chromosomal rearrangements/modifications involving mainly the NOR (nucleolus organizer region)-bearing chromosomes, throughout the evolution of wild and domesticated species.

Satellite DNA repeating units are longer than 100 bp. These sequences are characterized by a high copy level (10^4–10^6) and form clusters of repeats, the length of which is rather difficult to estimate by high-throughput sequencing of genomes due to the "ejection" of the main part of tandem repeats during this process. Earlier studies of cereal genomes using pulsed field gel electrophoresis made it possible to estimate the length of the tandem repeat regions in a cluster as 90–600 kb [30]. Satellite DNA can comprise up to 5% of the genome and is the cause of significant differences in the content of heterochromatin DNA blocks in closely related species. Due to the high copy numbers of satellite DNA in the chromosomal locus, they are well detected in the FISH assay.

We can distinguish the following families of satellite DNA, whose units (in the form of cloned DNA sequences or PCR fragments) have been successfully used for the analysis of the genome of wheat and Triticeae species, including the study of the reorganization of genomes during evolution:

1. A family of repeats pAs1/Afa/pHcKB6/dpTa1 [31, 32], localized predominantly in the subtelomeric and intercalary chromosome regions of *Ae. tauschii*, D-genome of *T. aestivum*, species of the genus *Hordeum*, *Elymus*, and several other species.
2. The family of repeats 120 bp/pSc119.2 [33], widely distributed in subtelomeric and intercalary regions of chromosomes in many species of the tribe Triticeae (**Figure 2**) and in the closely related tribe Avenae [34]. This family of repeats was first isolated from *Secale cereale* L. and described as one of the families of telomeric rye heterochromatin [35].
3. The family of repeats 350 bp/pSc200/pSc74 and pSc250, which are the main tandem repeats of telomeric heterochromatin in rye *Secale cereale* [36]. During evolution, these sequences were amplified in the genome of individual species of *Secale*, as well as in certain species of the genera *Agropyron* and *Dasypyrum* of the Triticeae tribe.
4. pAesKB52/pGC1R-1/Spelt52 are tandem repeats of subtelomeric regions of chromosomes *Ae. speltoides*, *Ae. longissima* Schweinf, & Musch L., and *Ae. sharonensis* Eig (**Figure 2**) [37].
5. Spelt1 is a genome-specific sequence associated with telomeric heterochromatin of *Ae. speltoides* (**Figure 2**). Sequences of this family have not yet been detected by hybridization methods in the genomes of other Triticeae species, with the exception of *T. monococcum* (weak hybridization signal) and polyploid species formed with the participation of *Ae. speltoides* [37].

The probes, pSc119.2 and pAs1, are most often used for intraspecific identification of Triticeae tribe chromosomes by the FISH method. Thus, simultaneous hybridization of two DNA probes (pSc119.2 and pAs1) makes it possible to identify 17 (out of 21) chromosomes of the genome of common wheat [34, 38].

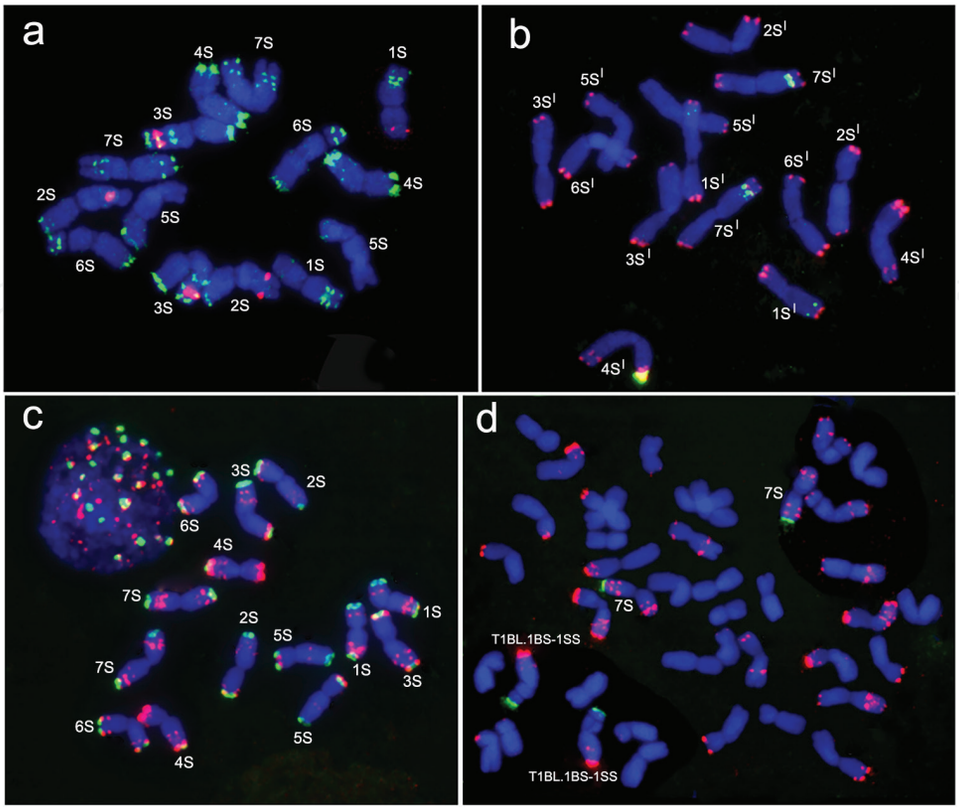

Figure 2. FISH to mitotic metaphase chromosomes. (a and c) *Ae. speltoides*, (b) *Ae. longissima*, and (d) hybrid line (*T. aestivum* × *Ae. speltoides*). Probe combinations used were (a) pSc119.2 (green) and Spelt52 (red), (b) pSc119.2 (red) and Spelt52 (green), and (c and d) pSc119.2 (red) and Spelt1 (green).

The combination of pSc119.2 and Spelt52 probes is effective for the study of all *Aegilops* species of the Sitopsis section (putative donors of the B/G genomes of polyploid wheats) (**Figure 2**). The probe combination pSc119.2 and Spelt1 is effective for only *Ae. speltoides* chromosome identification (**Figure 2**) [37]. The S-genomes of *Aegilops* species within the Sitopsis section are very similar to the common wheat B-genome. Accordingly, GISH with the DNA of these species is difficult on hybrid wheat lines. Therefore, we used FISH with the Spelt1 and Spelt52 probes to identify the *Aegilops* genetic material in the investigated lines (**Figure 2**). Simultaneous hybridization with probes pSc119.2 and pAs1 allows identification of wheat chromosomes.

The different level of homology within various families of tandem repeats depends on the rate of homogenization of repeats within the cluster, within each genome and species. The Spelt1-family is highly conserved, according to the sequencing of 10 Spelt1 sequences isolated from different accessions of *Ae. speltoides*, which shows a high level of homology (98%) [37]. It should be noted that more often a high level of interspecific polymorphism of satellite DNA families was demonstrated. This suggests that it may be possible to obtain efficient probes for the identification of chromosomes of the studied species by cloning DNA sequences of the repetitive families from these species.

Reports of the discovery of new families of highly repetitive DNA sequences are still emerging despite the existence of a large number of cereal tandem repeats already described. A previously unknown class of repeating DNA sequences named "Fat" was identified in the genome of common wheat [39]. Fat repeats are organized in clusters but with a dispersed distribution throughout the genome. The Fat-element content varies considerably across the genomes of different cereal species. The highest intensity of hybridization was found in the D-genome of wheat and *Aegilops* and in the S genome of *Agropyron*. This sequence was not found in oats or domesticated barley *Hordeum vulgare* L, but was present in minimal amounts in other species of the genus *Hordeum*. Based on this, it was concluded that the Fat-element first appeared in the evolution of cereals after the divergence of oats, during the separation of domestic barley from related grasses. The most intensive hybridization of Fat-repeats in the form of a large cluster of signals characterizes the chromosomes of the 4th homoeological group of wheat and *Aegilops*. This sequence is found only on the chromosomes of the D-genome of wheat and *Aegilops*, enabling it to be used as a FISH marker for identifying chromosomes and studying chromosome reorganization during evolution.

To discover new probes, 2000 plasmid wheat clones were examined by Komuro et al. [40]. Among them, 47 clones produced strong discrete signals on wheat chromosomes. Especially, valuable is combining pTa-535, pTa-713, and pTa-86 (pSc119 homolog) sequences, which allows to completely identify all 21 wheat chromosome pairs.

In addition, it seems promising to use oligonucleotides synthesized for various sites in the above-mentioned families of repeats identified in high-throughput sequencing, including sequencing data on individual chromosomes of wheat made for the identification of chromosomes. These probes have been shown to provide an easier, faster and more cost-effective method for the FISH analysis of wheat and hybrids [41, 42].

2.2.2. Ribosomal genes

An important and well-studied family of tandem repeats is the family of ribosomal RNA genes (rRNA). A detailed analysis of the monomers and cluster organization was carried out for these genes. Their localization on chromosomes in various species of cereals and possible mechanisms of evolutionary variability, including the processes of divergence and homogenization, were described by Hillis et al. [43]. There are two classes of rRNA-genes in the cereal genome: genes encoding 5S rRNA and 45S rRNA. 45S and 5S rDNA are located independently of each other, even in cases when they are both localized on one arm of a chromosome.

The 45S rDNA of cereals contains a coding region and a nontranscribed spacer sequence. The gene region includes three DNA sequences encoding 18S, 5.8S, and 26S rRNA, which are separated by internally transcribed spacer sequences. Polymorphism exists in the number of 45S rDNA loci in the Triticeae species genomes. The "major" loci of these genes are located on the short arms of homoeologous chromosome groups 1, 5, and 6. Nucleolus-forming regions are found on chromosomes 1A, 1B, 6B, and 5D of *T. aestivum* [44]. In addition, minor loci of 45S rDNA are also present, in which active RNA synthesis is not observed.

The genes encoding the 5S rRNA have the smallest repeating unit length among the ribosomal genes (320–500 bp). The repeating unit of 5S rDNA contains a 120 bp conserved coding

region and a variable nontranscribed spacer sequence. There are from 1000 to 4000 copies of 5S rRNA genes per haploid genome in cereals. Two subfamilies of 5S rDNA are distinguished in the Triticeae genomes, depending on the length of the spacer: 5SDna1 (200–345 bp) and 5SDna2 (350–380 bp). Hybridization of 5S-repeats on chromosomes of various *Triticeae* species showed that in most species, they are located in homoeological groups 1 and 5. An analysis of the chromosome distribution of 5SDna1 and 5SDna2 subfamilies showed that the short units of 5S rDNA have preferential localization on the chromosomes of homoeologous group 1, while the long units are located on group 5. It was shown that 5S rRNA genes with a monomer length of 290 bp are located on chromosomes 1B and 1D of common wheat, and genes with a monomer length of 410 bp are located on chromosomes 5B and 5D [45]. Further work on the isolation and sequencing of individual monomers led to division of the 5S rRNA genes into a larger number of subfamilies [46].

Analysis of chromosome 5B sequencing data, as well as individual BAC-clones containing 5S rDNA, showed that long and short types of subunits can be located on one chromosome, but they form separate clusters interrupted by the insertion of mobile elements [47].

The presence of conservative (coding) and polymorphic (noncoding) sequences in rDNA promoted their widespread use as molecular markers in phylogenetics. 5S and 45S rDNA are also widely used as cytogenetic markers for FISH due to their large copy number and localization in certain regions of chromosomes. A number of phylogenetic studies using individually cloned copies of 45S and 5S rDNA have been carried out for wheat and its relatives [48, 49]. An interesting fact is that among *Triticum* and *Aegilops*, two species (*T. timopheevii* and *Ae. speltoides*) lost the 5S rDNA locus on the chromosome of homoeologous group 1 (1G and 1S, correspondently) during evolution [50].

2.2.3. Transposable genetic elements as markers of genomic rearrangements

Another class of repetitive DNA, widely represented in the genome of plants, is transposable genetic elements (TEs), which are divided into two classes: class I elements (retrotransposons) and class II elements (DNA transposable elements).

At present, it seems likely that the diverse TEs, which have a mainly dispersed chromosomal localization, are the major contributors to the observed interspecies differentiation of chromosomes revealed by genomic *in situ* hybridization (GISH). GISH, a method based on the hybridization of labeled genomic DNA of one species to metaphase chromosomes of another species or hybrid, is widely used to assess the degree of genome homology. GISH serves as a unique approach to studying the formation of genomes of polyploid species and revealing the nature of their relationship, the analysis of introgression of alien genetic material, and the localization of break points in intergenomic translocations in remote hybrids [51, 52].

The development of BAC (bacterial artificial chromosome) libraries containing clones with very large inserts (>100 kb) of genomic DNA has opened up new possibilities for studying the reorganization of genomes by BAC *in situ* hybridization (BAC-FISH). The localization of BAC clones on chromosomes is mainly connected with families of TEs in their composition. Thus, carrying out BAC-FISH on wheat chromosomes showed a different BAC localization in the genome depending on which family of TEs or other repeats were present in them [53, 54].

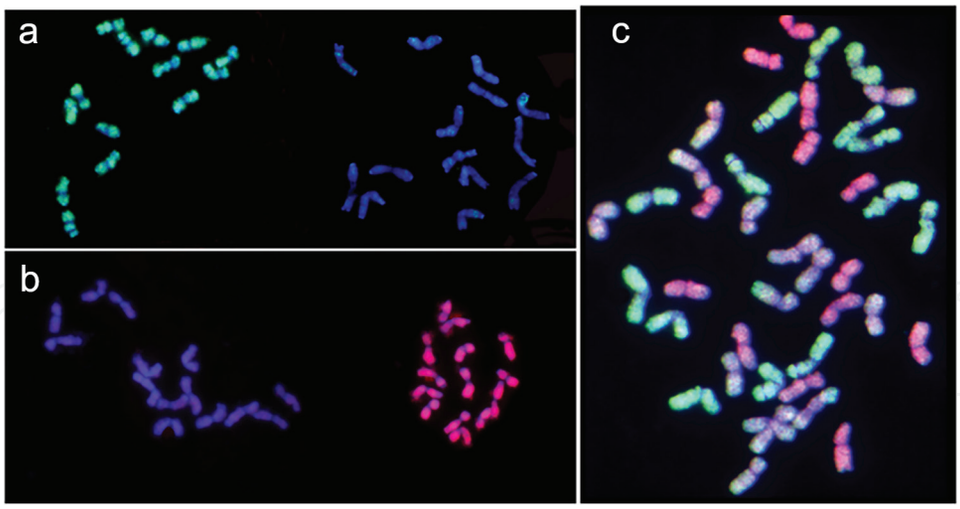

Figure 3. FISH to mitotic metaphase chromosomes of (a) *Ae. speltoides* and *T. urartu*, (b) *T. urartu* and *Ae. tauschii*, and (c) *T. aestivum*. With the probe combinations: (a) BAC clone 2383A24 (green), (b) BAC clone 112D20 (red), (c) BAC clone 2383A24 (green), and BAC clone 112D20 (red).

It is interesting to note the differential amplification of individual members of retrotransposon families belonging to the subclass Ty3-*gypsy*-retrotransposons in the genomes of diploid species, which retains genomic specificity in allopolyploid wheats (**Figure 3**) [55, 56].

Thus, FISH analysis of a BAC clone (BAC_2383A24) demonstrated its predominant localization to chromosomes of the B-genome of allopolyploid wheats and its putative diploid progenitor *Ae. speltoides* (**Figure 3**).

Analysis of the complete BAC_2383A24 nucleotide sequence revealed that three elements of the gypsy LTR retrotransposon family *Fatima* make up 47.2% of all the LTR retrotransposons in this BAC. Phylogenetic analysis, as well as FISH, showed that these *Fatima* elements are predominantly from the B genome of common wheat and its putative progenitor *Ae. speltoides* (**Figure 3**). Similar approaches, including hybridization with BAC clone 112D20, demonstrated that the *Lila* family of Ty3-*gypsy*-retrotransposons is predominantly from the D-genome and its progenitor *Ae. tauschii*. Multiple FISH with both clones allows the identification of all three subgenomes of hexaploid wheat (**Figure 3**).

Dating of the LTR retrotransposon insertion showed that TE proliferation mainly occurred in this diploid species before it entered into allopolyploidy [55, 57].

3. Chromosomal rearrangement during wheat breeding

Genetic erosion, caused by modern agricultural breeding practices, has led to the observed decrease in genetic variation in crops, including common wheat *T. aestivum*. Wheat relatives—wild and cultivated cereals—are used as sources of effective genes for resistance to biotic and abiotic stresses and to increase genetic diversity.

Introgression of genes from related species to wheat depends on the level of divergence between the species involved in the cross. Species belonging to the primary gene pool have homoeologous genomes. This group includes wild and cultivated forms of *T. turgidum* and species of donors A and D of the genomes of common wheat: *T. urartu*, *T. monococcum*, and *T. boeoticum*, as well as *Ae. tauschii*. The transfer of genes from these species can be carried out by crossing, homoeologous recombination, backcrossing and selection, as well as through the development of synthetic amphiploids.

The secondary gene pool includes polyploid species of wheat and *Aegilops* which have at least one homoeologous genome with *T. aestivum*. The transfer of genes from these species to common wheat by means of homoeologous recombination is also possible if recombination has taken place between the target homoeologous chromosomes. This group also includes hexaploid species with GGA^tA^tDD genome: *T. kiharae*, Dorof. et Migusch., *T. miguschovae*; tetraploid species with GGA^tA^t genome: *T. timopheevii*, *T. militinae*, and *T. araraticum*; and diploid species of *Aegilops* from the Sitopsis section, which are close to the B genome of *T. aestivum*. Cytogenetic analysis of hybrids from crosses of common wheat with *T. timopheevii* showed homoeologous introgression of G genome fragments to practically all chromosomes of both the B genome and the D genome of common wheat [58]. It should also be noted that the extent of introgressive regions varies among wheat lines [59]. Genetic material from *Ae. speltoides* (SS genome), the putative progenitor of the B and G genomes of polyploid wheat, was successfully transferred to all three genomes of common wheat, but especially, as expected, in the chromosome of the B-genome [60, 61].

Species that do not carry the genomes A, B, and D, and those related to the tertiary gene pool, are considered more distant relatives of wheat. The transfer of genes from these species is difficult since it cannot be accomplished by recombination and therefore requires the use of other strategies. Currently, there are standard methods that facilitate the transfer of genes from species that do not have related genomes with common wheat. Some are based on the methods of chromosome engineering, and others manipulate the genetic control of meiotic recombination or employ genetic engineering. The transfer of genetic material in this case occurs both in the partly homoeologous group of chromosomes and into other groups [62].

The strategy used in cytological analysis of hybrids depends first on the nature of the relationships between the species involved in crossing. In instances where the donor species belongs to the tertiary gene pool with respect to *T. aestivum*, GISH is first used, which allows the estimation of the size and localization of the alien translocation. GISH can be used successfully to identify translocations of rye, wheatgrass, and *Aegilops* species (with the exception of the Sitopsis group) in the wheat genome (**Figure 4**).

However, GISH does not answer the questions: which wheat chromosome is replaced by an alien chromosome or which alien chromosome took part in the translocation. In addition, if the genomes of the crossed species are evolutionarily close, that is, if donor species refer to primary and secondary gene pools, then GISH will also be difficult. A similar problem occurs, for example, in the analysis of hybrids from the crossing of hexaploid wheat with *Ae.*

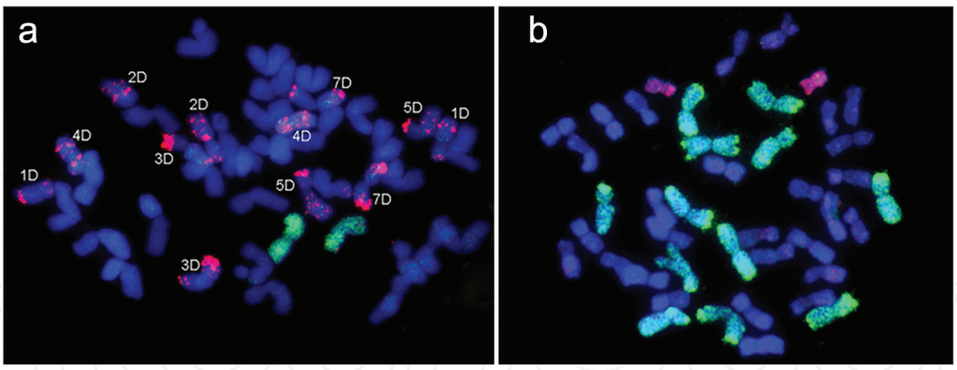

Figure 4. GISH with *Th. intermedium* DNA (green) in combination with pAs1 probes (red) of common wheat cultivar Tulaikovskaya 5 (a) and GISH with *S. cereale* DNA (green) and with *Ae. umbellulata* DNA (red) of the triticale line with introgression from *Ae. umbellulata* (b).

speltoides (B-genome putative donor) or with the species *Ae. tauschii* which is a donor of the D genome. In this case, species-specific markers are used to identify chromosomes in hybrids or introgressive wheat lines, such as, for example, Spelt1 for the *Ae. speltoides* genome (**Figure 2**).

It should be noted that when identifying the alien introgressions in lines/varieties developed by remote hybridization of cereals, best results are usually obtained by combining different methodological approaches and using different chromosomal markers.

4. Conclusion

The wheat allopolyploids have long attracted the attention of researchers, both from the perspective of studying the processes of genome reorganization during amphiploidization and to develop new wheat lines for breeding.

To accomplish these fundamental and applied tasks, various approaches are used. In recent years, SNP markers and various technologies for their identification have been actively involved, as well as reference genome data for wheat and related species. This allows us to obtain more detailed information about the organization and evolution of the wheat genome and the structure of gene families present in reference genomes. Despite continued progress in deciphering the complex wheat genome, a complete understanding of the reorganization of the wheat genome during evolution can only be obtained by combining molecular methods of analysis with cytogenetic ones. The latter makes it possible to identify rearrangements of homoeological chromosomes in the process of evolution and breeding.

The first translocations in wheat varieties were detected by cytogenetic methods. Later, the molecular markers developed for these translocations allowed the use of marker-assisted breeding for selection of the desired genotypes.

One of the most successful used in selection is the translocation of the short 1R chromosomal arm to 1A and 1B of the wheat chromosome during breeding. At present, more than 300 soft

wheat varieties carry the T1RS.1BL translocation [http://www.rye-gene-map.de/rye-introgression], which determines the resistance to phytopathogens and increased productivity. Interestingly, the presence of an intact wheatgrass chromosome in Russian wheat varieties was found to be significant for resistance to fungal diseases and the maintenance of grain quality over the last 30 years [10]. It should be noted that only a set of C-banding methods used in conjunction with *in situ* hybridization and assays with PLUG and SSR markers revealed that wheat chromosome 6D in the wheat cultivars was substituted by the *Thinopyrum intermedium* (Host) homoeologous chromosome, 6Ai.

It should be emphasized once again that, despite extensive development of molecular markers for genome analysis, including high-throughput genotyping, it is impossible to characterize the modern diversity within the genus *Triticum* without involving cytogenetic methods.

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Conflict of interest

The authors declare that they have no competing interests.

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