2 Cytogenetics, a Science Linking Genomics and Breeding: The *Brassica* Model

Anne-Marie Chèvre, Annaliese S. Mason, Olivier Coriton, Laurie Grandont, Eric Jenczewski and Martin A. Lysak

Abstract

Earlier, classical cytogenetics played a key role in taxonomic studies through identification of chromosome number and morphology. Similarly, the first identifications of polyploid species, and the analysis of relationships between different species from interspecific hybrids, were based on the observation of chromosome pairing during metaphase I of meiosis. Cytogenetics subsequently got a boost with the development of mapping and of next-generation sequencing technologies, enabling the development of modern molecular cytogenetics. In this chapter, we present the major impacts of molecular cytogenetics: shedding new light on genome organization and evolution as well as regulation of meiosis in the economically important genus *Brassica* and the tribe Brassicaceae. First, we present how comparative chromosome painting (CCP) using pools of *Arabidopsis thaliana* BAC clones is used to establish genome organization in diploid and polyploid species in conjunction with genotyping and sequencing data. This method complements phylogenetic analyses in establishment of the common ancestral genome and in the description of the three differentially fractionated *Brassica* ancestral subgenomes. Secondly, intergenomic relationships can be determined by BAC-fluorescent in situ hybridization (BAC-FISH) and genomic in situ hybridization (GISH); these techniques allow identification of the different genomes and chromosomes to quantify homologous and non-homologous pairing in haploids and hybrids, identifying structural rearrangements within allopolyploid species and between genomes in interspecific hybrids. Thirdly, meiosis and meiotic recombination in *Brassica napus* and its close relatives can be studied using antibodies developed against *Arabidopsis* proteins. From all these data, we show how molecular cytogenetics is essential for our understanding of genetics and genomics in the genus *Brassica* and how cytogenetics will undoubtedly play a significant role in the times to come.

A.-M. Chèvre (✉) · O. Coriton
INRA, UMR1349 IGEPP, BP35327,
35653 Le Rheu, France
e-mail: anne-marie.chevre@inra.fr

A. S. Mason
Department of Plant Breeding,
IFZ Research Centre for Biosystems,
Land Use and Nutrition, Justus-Liebig University,
Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

L. Grandont · E. Jenczewski
Institut Jean-Pierre Bourgin, INRA, AgroParisTech,
CNRS, Université Paris-Saclay, RD10, 78026
Versailles Cedex, France

M. A. Lysak
Central European Institute of Technology (CEITEC),
Masaryk University, 625 00 Brno, Czech Republic

S. Liu et al. (eds.), *The Brassica napus Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-319-43694-4_2

2.1 Introduction

Cytogenetics refers to the study of genetics at the cellular level, and most particularly to chromosome observations at mitosis and/or meiosis. Despite being an old method, it is still commonly used in high-quality scientific studies. Cytogenetics can deduce chromosome number, genome structure, and relationships between genomes in natural or artificial interspecific hybrids. Data generated by cytogenetics approaches have been widely used in taxonomic studies and to explore genetic diversity in genera, species, and populations and in breeding programs. The recent advent of next-generation sequencing (NGS) technologies has given a fresh boost to cytogenetics, allowing the development of molecular cytogenetics and providing new insights into bioinformatically obtained questions and information related to genome organization, evolution, and regulation.

The Brassicaceae tribe is one of the 49 tribes in the Brassicaceae family (Al-Shehbaz 2012). The first cytogenetic analyses in this tribe described 220 species in 46 genera, with 37 species belonging to the Brassica genus (Gomez-Campo 1980; Al-Shehbaz 2012 for review). These studies revealed chromosome numbers ranging from $n = 7$ to 75 in this tribe, thus including species of various ploidy levels. Within the *Brassica* genus, which is the main focus of this chapter, establishment of the karyotypes of the different species revealed that several chromosomes shared the same morphology in different species, probably due to their common origin (Prakash et al. 2009 for review). The genome structure of the diploid *Brassica* species (with chromosome numbers ranging from 7 to 12) was first analyzed from karyotypes and meiotic behavior in metaphase I of pollen mother cells. The comparison of autosyndetic pairing (non-homologous chromosome pairing between different chromosomes of the same genome) in haploids with the rate of chromosome pairing in interspecific hybrids allowed the first establishment of intra- and intergenomic relationships. These cytogenetic analyses revealed that each genome carried duplications. From chromosome morphology and chromosome pairing data, these studies postulated that genomes of Brassicaceae tribe species were derived from a common ancestor with six or seven chromosomes, with subsequent duplication (Mizushima 1980 for review).

The origin of the allopolyploid species was first depicted in the famous U triangle figure (U 1935). Confirming results from Morinaga (1934), U showed that *Brassica napus* (AACC, $2n = 38$), *B. juncea* (AABB, $2n = 36$), and *B. carinata* (BBCC, $2n = 34$) originated from natural interspecific hybridization events between *B. rapa* (AA, $2n = 20$), *Brassica oleracea* (CC, $2n = 18$), and *B. nigra* (BB, $2n = 16$). All three allotetraploid species show relatively strict disomic inheritance, indicating preferential pairing between homologous chromosomes with the formation of bivalents in metaphase I. However, occasional multivalents were also observed, suggesting that homeologous (non-homologous) pairing between chromosomes of related genomes can also generate exchanges between the two genomes in each of the allotetraploids (Prakash and Hinata 1980; Prakash et al. 2009 for review). These data were confirmed by the establishment of genetic maps with molecular markers (Parkin 2011 for review), and allelic segregation distortion revealed that homeologous exchanges did indeed generate reciprocal (Lombard and Delourme 2001; Osborn et al. 2003; Piquemal et al. 2005) and non-reciprocal (Udall et al. 2005) translocations in different *B. napus* varieties. Comparison of the published *B. rapa* (Wang et al. 2011) and *B. oleracea* (Liu et al. 2014; Parkin et al. 2014) genome sequences to the *B. napus* "Darmor" reference sequence revealed numerous small translocations and other rearrangements between the A and C genomes in the established allopolyploid *B. napus* genome (Chalhoub et al. 2014) relative to the diploid genomes, as already suggested by earlier work (Cheung et al. 2009). Structural variations such as translocations, deletions, duplications, and inversions are not purely of academic interest. Increasing evidence suggests that structural variation may play an important role in genome evolution (Chester et al. 2012; Edwards et al. 2013), gene expression regulation (Wang et al.

2012), and even in crop phenotypes (Zou et al. 2011; Schiessl et al. 2014). These homeologous rearrangements fundamentally result from ancestrally shared homeology between chromosomes from different subgenomes and are mediated by genetic control of chromosome pairing.

Variation for genetic control of chromosome pairing was later described in *B. napus*. Two main meiotic behaviors (high or low frequency of chromosome pairing) were detected in AC haploids of different *B. napus* varieties (Renard and Dosba 1980; Attia and Röbbelen 1986; Cifuentes et al. 2010). After the production of F_1 hybrids from varieties with contrasting meiotic behavior, meiotic analyses of large segregating AC haploid populations combined with genetic mapping allowed identification of QTL for genetic control of homeologous recombination. A major QTL, *PrBn* (for pairing regulator in *B. napus*) was identified, plus minor QTL and epistatic interactions (Jenczewski et al. 2003; Liu et al. 2006). This control was subsequently determined to mainly affect the frequency of crossovers between homeologous chromosomes (Nicolas et al. 2009; 2012).

Interspecific hybrids have frequently been produced in order to introduce agronomic traits from one species to another (Prakash et al. 2009 for review). Different strategies have been developed in *Brassica*: trait introgression via interspecific hybrids can be achieved by either direct crossing between diploid species, crosses between tetraploid species and parental diploid species, or by crosses between tetraploid species (Prakash et al. 2009 for review). Crosses between diploids produce interspecific hybrids (allohaploids or digenomic haploids) that are generally sterile (the few viable gametes produced are generally unreduced); colchicine doubling is classically used to produce new synthetic allopolyploids from these lines. Crosses between tetraploids and progenitor diploids generate hybrids with a diploid genome plus a haploid one; for example, *B. napus* crossed with *B. rapa* produces AAC hybrids, with a majority of cells containing 10 AA bivalents and 9 C univalents at metaphase I (Leflon et al. 2006). These plants can be fully fertile and also show boosted homologous recombination in the A genome (Leflon et al. 2010). Crosses between diploids and tetraploids that do not share a genome are also possible: Such trigenomic ABC hybrids can be used either as bridge species, subsequently backcrossed to introduce new variability in crops, or can be induced by colchicine doubling to generate allohexaploids, which may have potential as a new crop species (Chen et al. 2011). Finally, crosses between pairs of allotetraploid species can also be used with subsequent backcrossing for allotetraploid crop improvement and also increase the chance that homeologous recombination will occur between divergent genomes. Three genome hybrids with one genome at the diploid stage (e.g., AABC, BBAC, and CCAB) generated by crosses between the allotetraploids revealed more bivalents between remaining haploid genomes than the corresponding dihaploid (AC, BC, and AB) hybrids (Nagpal et al. 1996; Mason et al. 2010).

All these data highlight the role of classical cytogenetics in increasing our knowledge of genome structure and evolution, as well as how regulation of chromosome pairing between genomes can be manipulated to introduce new genetic variability into crops. However, in the last two decades, the development of new molecular cytogenetic techniques, including genomic in situ hybridization (GISH), fluorescent in situ hybridization (FISH), and immunolocalization of crossover proteins has opened new avenues of research. In this chapter, we will present the major impacts of molecular cytogenetics: shedding new light on the phylogenetic relationships between Brassica species, genome organization and evolution as well as regulation of meiosis in the agronomically important genus *Brassica*.

2.2 New Insights from Molecular Cytogenetics in Genome Organization and Evolution

Recently published genome assemblies of *B. napus* (Chalhoub et al. 2014) and its parental genomes (Wang et al. 2011; Liu et al. 2014) along with the available paleogenomic hypotheses (Lysak et al. 2006; Schranz et al. 2006; Mandáková and Lysak 2008) have permitted reconstruction of the origin and later evolution of *Brassica* genomes.

Even the first generations of *Brassica* researchers, working only with classical cytogenetics techniques, realized that *Brassica* species with diploid-like chromosome numbers (n = 7–12) were probably "balanced secondary polyploids," characterized by intra-genomic chromosomal homeologies (e.g., Catcheside 1934; Röbbelen 1960). However, it took almost 40 years until a genome triplication theory gained more solid ground due to results from comparative genetic mapping (Lagercrantz and Lydiate 1996; Lagercrantz 1998), and the ancestral hexaploid nature of the diploid *Brassica* and Brassicaceae genomes was eventually confirmed by cross-species (*Arabidopsis thaliana, B. napus*) restriction fragment length polymorphism (RFLP) mapping (Parkin et al. 2005) and, of course, comparative cytogenetic analysis (Lysak et al. 2005, 2007; Ziolkowski et al. 2006). Pools of chromosome-specific BACs from *A. thaliana* (Arabidopsis henceforth) were applied to paint large homeologous chromosome regions on pachytene chromosomes in *Brassica* and Brassicaceae species (Lysak et al. 2005, 2007; Ziolkowski et al. 2006). The technique has become known as comparative chromosome painting (CCP, Lysak et al. 2006). Howell et al. (2005) used *Arabidopsis* BACs as individual in situ probes to analyze a region on *B. oleracea* chromosome O6 which, from genetic mapping, was thought to be composed of two homeologous copies of a ~5 Mb region on the bottom arm of *A. thaliana* chromosome At1. Eleven *Arabidopsis* and three *B. oleracea* BAC clones were applied separately to pachytene spreads with a *B. oleracea* chromosome O6 BAC as a marker. The two copies were shown to be adjacent with the proximal one inverted relative to the homeologous region in *Arabidopsis*. However, this approach was designed specifically to investigate the *Brassica* region, and because only the signals on chromosome O6 could be unequivocally identified, signals seen elsewhere were not analyzed and the presence of further copies of the *Arabidopsis* region was not investigated (E. Howell, pers. comm.). Lysak et al. (2005) analyzed a ~8.7 Mb BAC contig from *Arabidopsis* chromosome At4 (genomic block U in Schranz et al. 2006) by CCP in 21 crucifer species traditionally classified as members of the tribe Brassicaceae or being closely related. Despite the contrasting chromosome numbers ($2n$ = 14–38), the analyzed segment was found as three copies (in 13 species) or as six copies in four species of Brassicaceae (including the six *Brassica* species of U's triangle). The homeologous chromosome segments resembled the *Arabidopsis*-like structure or were modified by paracentric inversions and translocations. To confirm the initial findings, CCP with BAC contigs covering the majority of the longer arm of *Arabidopsis* chromosome At3 (block F in Schranz et al. 2006) was carried out in ten species traditionally treated as members of the Brassicaceae. Three homeologous copies of the contig were identified per haploid chromosome complement in Brassicaceae species with $2n$ = 14, 18, 20, 32, and 36. In high polyploid species ($n \geq 30$; n = 30, 34, and 60), six or 12 copies of the analyzed block have been revealed. Congruent data have been published by Ziolkowski et al. (2006). These authors analyzed BAC contigs from *Arabidopsis* chromosomes At1, At2, and At3 (~8.3 Mb in total) in *B. oleracea*. Except for a short contig from At1, all *Arabidopsis* probes were found to be triplicated in the karyotype of *B. oleracea* (n = 9). The largest analyzed segment (~5.4 Mb) from the bottom arm of At3 was found to occur in three homeologous copies on three different *B. oleracea* chromosomes (O4, O6, and O8).

All the studies reviewed here suggested that single-copy *Arabidopsis* chromosome segments have usually three or six homeologous

counterparts within Brassicaceae and *Brassica* genomes. The presence of three copies in "diploid" species with $n = 7–12$ and of six or twelve copies in neopolyploid Brassicaceae species was most parsimoniously explained by descent from a mesohexaploid ancestor. The cytogenetic comparative studies and "the triplication theory" gained further support from comparative genetic mapping. Parkin et al. (2005) mapped over 1000 *B. napus* RFLP markers to *Arabidopsis* to estimate the level of genome colinearity shared by the two species. At least, 21 so-called conserved genomic units (analogous to genomic blocks sensu Schranz et al. 2006) were identified in the *Arabidopsis* genome, making up almost 90% of the *B. napus* genetic map. Conserved segments were present between four and seven times within the *B. napus* genome, with 86% of conserved units found in at least six copies. Again, these findings strongly supported the idea that "diploid" *Brassica* species are descendants of a hexaploid ancestor. In agreement with cytogenetic data, Parkin et al. also showed that "diploid" *Brassica* genomes underwent chromosome reshuffling following the *Arabidopsis–Brassica* split. Some rearrangements were shared by the A and C genomes of *B. napus* and, thus, most likely predated the divergence of *B. rapa* and *B. oleracea*. The authors conclude that "genome triplication followed by a small number of insertions/deletions/translocations would provide the simplest explanation for the present structure of the *Brassica* diploid genome" (Parkin et al. 2005). Indeed, the triplicated nature of *Brassica* genomes has been unambiguously confirmed by whole-genome and transcriptome sequencing in *B. oleracea*, *B. rapa*, *B. juncea*, and *B. napus* (Chalhoub et al. 2014; Liu et al. 2014; Paritosh et al. 2014; Parkin et al. 2014; Wang et al. 2011), and by reconstructing the origin and evolution of an ancestral mesohexaploid genome (Cheng et al. 2013, 2014). Moreover, the recently constructed sequence-based genome maps are to a larger extent congruent with pioneering cytogenetic analyses, as shown for the localization of block U in the *B. nigra* genome (Fig. 2.1).

For phylogenetic studies, Mandáková and Lysak (2008) showed that genomes in several tribes, including the Brassicaceae and falling into the so-called extended lineage II (Franzke et al. 2011), have descended from a common ancestral genome with seven linkage groups—the Proto-Calepineae Karyotype (PCK). The PCK genome is a younger derivative of the older Ancestral Crucifer Karyotype (ACK) with eight chromosomes, differentiated from it by a translocation-based chromosome fusion "that reduced the number of chromosomes from $n = 8$ to $n = 7$". In a younger clade of the extended lineage II, the structure of PCK was altered by a whole-arm reciprocal translocation to form the tPCK ancestral genome (t standing for translocation). When Mandáková and Lysak (2008) compared the A subgenome structure within the *B. napus* genome (Parkin et al. 2005) with the PCK (tPCK), two PCK-specific rearrangements were identified. Later on, whole-genome sequencing of *B. rapa* identified three differentially fractioned subgenomes, subsequently named MF1, MF2, and LF (Wang et al. 2011), which offered a new possibility to substantiate the hypothesis that the PCK is the ancestral genome of the genus *Brassica*. Cheng et al. (2013) showed that all three *B. rapa* subgenomes contain associations of genomic blocks (V/K/L/Wa/Q/X and O/P/W/R) diagnostic for both PCK and tPCK; moreover, associations D/V and M/E pointed to the younger tPCK genome. The authors thus concluded that the quasi-diploid genome of *B. rapa* and most likely genomes of all other Brassica species originated through re-diploidization of the hexaploid ancestor merging together three very similar diploid genomes, structurally resembling the seven chromosomes of tPCK. Considering the most parsimonious scenario for the origin of the *Brassica* mesohexaploid genome, three diploid tPCK-like genomes each with seven chromosomes were merged through hybridization/polyploidization to form a hexaploid genome with 21 chromosome pairs ($2n = 42$). Consequently, the 24 ancestral genomic blocks identified by Schranz et al. (2006) and comprising each

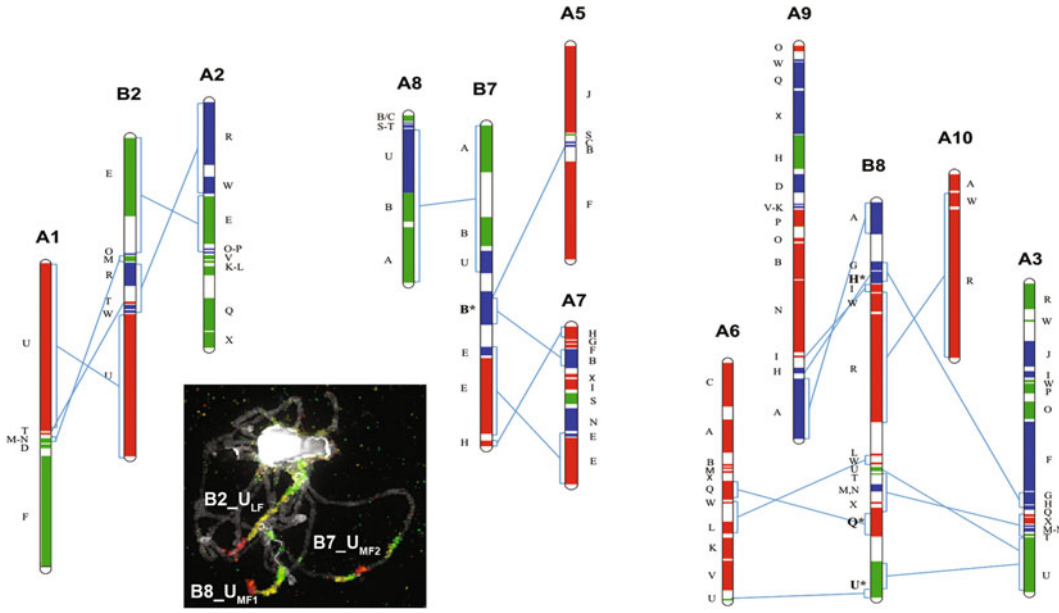

Fig. 2.1 Cross-specific localization of genomic block U on pachytene chromosomes of *Brassica nigra* and its position on chromosomes of *B. nigra* (B2, B7, and B8) and *B. rapa* (A1–A3, A5–A10). Block U was localized based on in situ hybridization of *A. thaliana* BAC contigs (labeled by yellow, red, and green fluorochrome, respectively) homeologous to this block in *B. nigra* (adopted from Lysak et al. 2005). The genomic block arrangement of three B genome chromosomes in *B. juncea* was based on single nucleotide polymorphism/intron polymorphism markers (adopted from Paritosh et al. 2014). Gene blocks on the B genome linkage groups (LGs) that show homeology to corresponding blocks on the A genome LGs are shown by the connecting lines. Genomic blocks assigned to the subgenomes LF (red), MF1 (green), and MF2 (blue) are color coded according to Cheng et al. (2013). Blocks showing variations in their fragmentation pattern in the B genome are shown in bold with an asterisk

of the three hybridizing genomes were multiplied to 72 blocks.

How was this hexaploid *Brassica* ancestor actually formed? Similar to the allohexaploid bread wheat (AABBDD, Marcussen et al. 2014), the origin of the *Brassica* hexaploid was probably via a two-step process of tetraploidization first, later followed by hybridization between this tetraploid and another diploid ancestor (Ziolkowski et al. 2006; Wang et al. 2011; Tang et al. 2012). This scenario is supported by the differential fractionation of the three (MF1, MF2, and LF) subgenomes in *B. rapa* (Wang et al. 2011; Tang et al. 2012; Cheng et al. 2013, 2014) and was also indicated by earlier cytogenetic studies (Lysak et al. 2005, 2007; Ziolkowski et al. 2006). The first to occur was an auto- or allotetraploid (XXXX or XXX^1X^1, $n = 14$) genome formed through a hybridization between two tPCK-like genomes (XX or X^1X^1, $n = 7$), followed by hybridization with a third tPCK-like genome (ZZ, $n = 7$). The second hybridization event was mediated either by the union of unreduced gametes or polyploidization of the primary triploid (XXZ or XX^1Z, $2n = 21$) to form the allohexaploid genome (XXXXZZ or XXX^1X^1ZZ, $n = 21$). This scenario is plausible considering the frequency with which intraspecific autotetraploid "chromosomal races" (sensu Müntzing 1936) and tetraploid species ($n = 14$) apparently originate(d) via autopolyploidy in tribes descending from PCK- to tPCK-like ancestral genome(s) (Mandáková and Lysak 2008; Mandáková et al. 2015). In the autotetraploid *Goldbachia laevigata* ($n = 14$, $2n = 28$), a genome derived from the PCK ancestor, the two duplicated chromosome sets are structurally identical apart from three chromosomes differentiated from

their homeologous partners by three pericentric inversions (Mandáková and Lysak 2008). These intra-genomic rearrangements illustrate how re-diploidization of the *Brassica* autotetraploid may have proceeded, to be followed by subsequent hybridization with another tPCK-like diploid genome.

2.3 Molecular Cytogenetics Provides New Insights into Intergenomic Relationships

A range of different molecular cytogenetics techniques exists to distinguish between genomes and individual chromosomes and to identify specific chromosome structural features. These molecular cytogenetic approaches can allow for quantification of homologous and non-homologous chromosome pairing in interspecific hybrids, investigation of genome structural variation such as duplications, deletions, and inversions, and can be used to characterize genomic introgressions and chromosome addition lines for crop improvement.

2.3.1 Using rDNA, GISH, and BAC-FISH Techniques to Distinguish Between Genomes and Individual Chromosomes

Initial molecular cytogenetic experiments on chromosome structure and identification were performed on ribosomal DNA loci, which are an effective cytogenetic marker for *Brassica* chromosomes but are difficult to map and organize after sequencing due to the large number of repeats at each locus. The rDNA units are composed of 500 to more than 40 000 genes copies per genome, arranged in tandem repeats on several loci (Long and Dawid 1980; Rogers and Bendich 1987). Recently, the sequence composition and gene content of the short arm of rye (*Secale cereale*) chromosome 1 (1RS) have been published: The short arm of rye 1R contains genes coding for 45S rRNA. The number of the 45S rDNA genes was estimated to be about 2000, amounting to about 3% of 1RS DNA. The 5S rDNA locus contained about 5000 copies of the 5S rDNA gene, constituting about 0.4% of 1RS DNA (Fluch et al. 2012). Shotgun 454 pyrosequencing of DNA was also obtained from flow-sorted 1RS. This novel approach permitted a detailed description of the gene space and the repetitive portion of this important chromosome arm. However, although NGS technologies offer powerful tools to generate suitable sequence reads, detection of different copies expected in these genomes is still challenging. FISH experiments with 45S and 5S rDNA probes enabled far more reliable identification of individual chromosomes (Maluszynska and Heslop-Harrison 1993; Snowdon et al. 1997; Fukui et al. 1998).

Combined FISH with 5S and 45S rDNA probes enables the discrimination of a number of chromosomes of diploid and tetraploid *Brassica* species of the "U triangle," allowing otherwise indistinguishable chromosomes to be identified. Twelve out of 20 chromosomes can be identified in diploid *B. rapa* (A genome) using a 45S rDNA probe. The strong FISH signal located on the A3 chromosomes likely reflects a large tandem repeat array of 45S rDNA sequences. The A3 locus has been shown to be the only one that carries transcriptionally active genes of the A genome nucleolar-organizing regions (NORs) (Hasterok and Maluszynska 2000). The second gene-rich locus is located on chromosome A1, proximal to the centromere. The remaining sites are located on cytogenetically undistinguishable A5, A6, and A9 chromosomes, collectively grouped as *Brassica* chromosomal type VIII (Hasterok et al. 2006). The 5S probe hybridizes to two major sites on the A10 and A1 chromosomes and to a minor site on chromosome A3 adjacent to the NOR. Based on the signal intensity, the small A10 metacentric chromosome contains the largest number of 5S genes. *B. oleracea* (C genome) has two pairs of chromosomes (C7 and C8) containing 45S loci. The active site is located on chromosome C8 according to Howell et al. (2002); it shows more decondensation, while loci on C7 are fully condensed. The 5S probe hybridizes to a unique

locus on chromosome C4. In natural *B. napus* allotetraploids, twelve 45S rDNA signals are observed. Four rDNA sites occur on C genome chromosomes (stained by GISH-like BAC-FISH specific for the C genome) implying that the C and A genomes carry four and eight sites, respectively. The decondensed signals are found on morphologically distinct A3 chromosomes (NORs), which are actively expressed (Chen and Pikaard 1997; Książczyk et al. 2011), while both C8 NORs are always condensed. Eight 5S rDNA sites are visible, with three loci in the A genome and one in the C genome (Fig. 2.2). The origin of the latter is evidenced by staining of chromosome C4 by GISH-like, with dispersed signals along the chromosome, and by the 5S probe which provides a highly condensed signal close to the centromere.

The first step to tie together cytogenetics and molecular genetics was to reconcile chromosome number and linkage group nomenclature. RFLP probes used for genetic map establishment were used either directly or to identify the corresponding BAC to design the different chromosomes; for example, the nomenclature of the different *B. oleracea* chromosomes was redefined by Howell et al. (2002), allowing the nine linkage groups of the *B. oleracea* genetic map to be assigned to the nine chromosomes of the karyotype derived from mitotic metaphase spreads of *B. oleracea* using BAC-FISH.

Use of GISH labeling of DNA from one parent can identify both genomes in interspecific hybrid or allopolyploid Brassica plants. This method is efficient for distinguishing chromosomes from the B genome (*B. nigra*) from A or C chromosomes, but the A and C genomes are too similar to be differentiated using GISH. Two strategies were developed to overcome this difficulty: the use of either rDNA as a blocking agent at the same time than *B. oleracea* DNA labeled (Howell et al. 2008) or a GISH-like method using *B. oleracea* BAC, BoB014O06 which selectively hybridizes to all C genome chromosomes in *B. napus* (Leflon et al. 2006; Nicolas et al. 2007) (Fig. 2.3).

Recently, a new technique for BAC-FISH analysis has been developed to allow identification of each chromosome in *B. napus* (Xiong and Pires 2011). This technique relies on a double

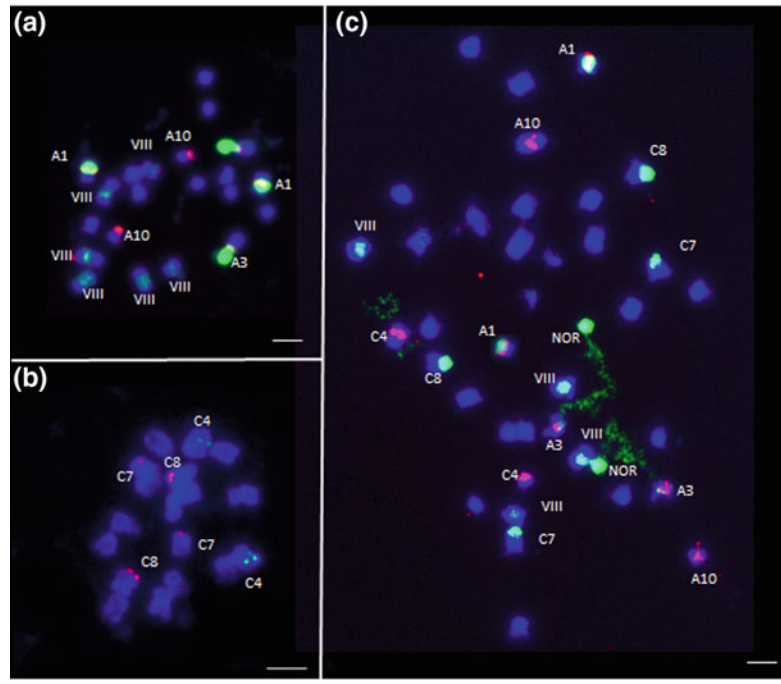

Fig. 2.2 FISH analyses of somatic metaphase chromosomes using 45S rDNA (green) and 5S rDNA (red) on *B. rapa* (**a**), on *B. oleracea* (**b**), and on *B. napus* (**c**). Bar = 5 µm

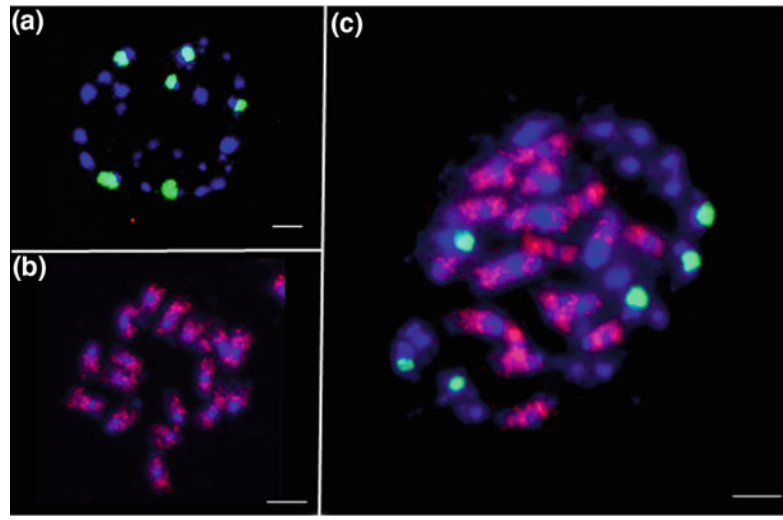

Fig. 2.3 FISH was carried out using BACs BoB014O06 probe (red) and 54B2 (green). FISH analyses of somatic metaphase chromosomes of *B. rapa* (**a**), *B. oleracea* (**b**), and *B. napus* (**c**). Chromosomes were counterstained with DAPI (blue). Bar = 5 μm

hybridization of the same slide, using a different set of three to four probes each time (45S, 5S, centBr1, centBr2, two BACs with multiple hybridization signals and a BAC that contained a C genome repeat to label the C genome chromosomes), and so is technically difficult. However, it was used successfully to identify chromosome rearrangements, duplications, and deletions in advanced resynthesized *B. napus* lines, showing selection for total number of homeologs (e.g., four copies of either A1 or C1, such as A1/C1/C1/C1 or A1/A1/A1/A1) in a "dosage balance" effect (Fig. 2.4) (Xiong et al. 2011; Grandont et al. 2014).

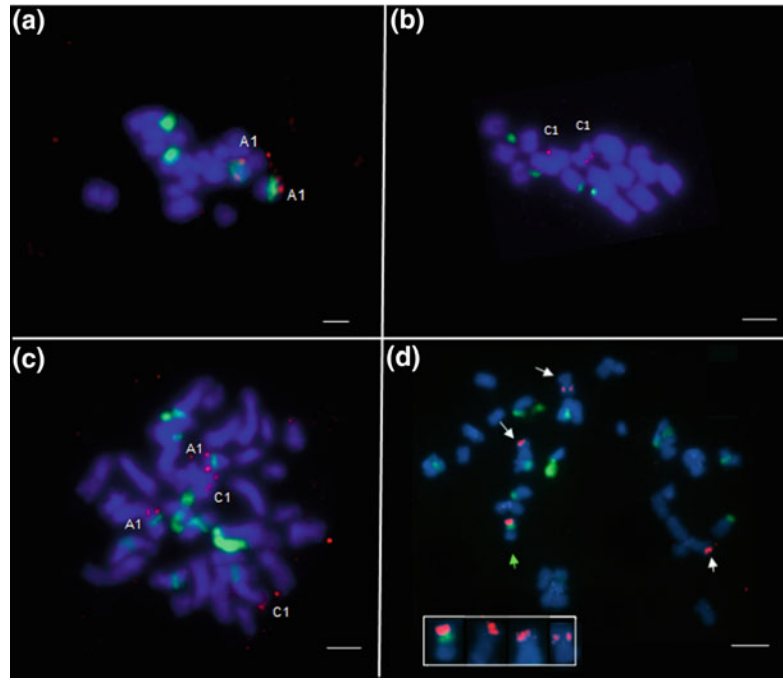

Fig. 2.4 FISH was carried out using BAC KBrB055A02 probe (A1/C1) (red) and 45S rDNA (green). FISH analyses of somatic metaphase chromosomes of *B. rapa* (**a**), *B. oleracea* (**b**), *B. napus* (DN) (**c**), and resynthesized *B. napus* line (**d**). Chromosomes were counterstained with DAPI (blue). Bar = 5 μm

2.3.2 Quantifying Homologous and Non-homologous Pairing Between Genomes in Haploids and Hybrids

Genomic sequence similarity is perhaps the primary factor in determining whether chromosomes will pair and recombine during meiosis I (Bozza and Pawlowski 2008), although genetic factors certainly play a role (Jenczewski and Alix 2004). In allopolyploid *B. napus*, mapping and sequencing data have revealed that the constitutive A and C genomes show highly conserved homeologous genomic regions along whole chromosomes, chromosome arms, and smaller genomic regions (Chalhoub et al. 2014; Parkin et al. 1995, 2003). Similar homeologous regions have been inferred between the less closely related B genome and the A and C genomes (Lagercrantz and Lydiate 1996; Navabi et al. 2013). This sequence homeology can lead to pairing and recombination between chromosomes belonging to related species during meiosis. Using FISH and GISH techniques as described above, the relative frequency of autosyndesis (pairing between chromosomes from the same genome) and of allosyndesis (pairing between the different genomes) can be determined in metaphase I of meiosis.

In haploid AC plants with one copy of each genome, it is possible to determine frequencies of both allosyndesis (AC) and autosyndesis (AA and CC). In these plants, autosyndetic bivalents are always observed to represent close to 20% of bivalents in metaphase I of meiosis; this result suggests that rearrangements between paralogous regions can occur (Nicolas et al. 2007, 2009). Similar analyses using genome labeling FISH and GISH in hybrids between *B. napus*, *B. carinata,* and *B. juncea* (genome compositions AABC, BBAC, and CCAB) also confirmed this result, as well as showing autosyndesis in the haploid B genome and AB, BC, and AC allosyndesis at metaphase I (Mason et al. 2010) (Fig. 2.5). Complementary analyses using BACs specific to particular homeologous chromosome pairs in *B. napus* haploids revealed that the rate of homeologous pairing depends on the pair of homeologs concerned (A1/C1, A3/C3, A10/C9, or A7/C6) and on the *B. napus* variety (Grandont et al. 2014). In AC hybrids produced from crosses between *B. rapa* and *B. oleracea*, the frequency of homeologous pairing is even higher than in *B. napus* haploids (Cifuentes et al. 2010; Szadkowski et al. 2011). This high rate of homeologous exchange was also observed in synthetic *B. napus* obtained by crosses between *B. rapa* and *B. oleracea*. GISH-like methods showed that A and C chromosomes frequently paired during the first meiosis in resynthesized AACC S0 plants, suggesting that the first meiosis acts as a genome blender (Szadkowski et al. 2010). These results were also confirmed by analyzing the dynamics of rDNA loci rearrangements in advanced generations of synthetic *B. napus* (Książczyk et al. 2011). However, when one genome is diploid and the other is haploid, such as in AAC hybrids, GISH-like methods show that the nine C chromosomes generally remain as univalents, with only low frequencies of homeologous pairing observed between the A and C genomes (Leflon et al. 2006). On the contrary, homeologous pairing is promoted between two haploid genomes when a third genome is at the diploid stage in the same plant: These data were confirmed by GISH and GISH-like analysis in AABC, BBAC, and CCBA hybrids by Mason et al. (2010). All of these molecular cytogenetic studies not only contribute to our understanding of meiosis and genomic relationships, but provide useful information for breeders targeting genomic introgressions or promoting non-homologous chromosome exchange for crop improvement.

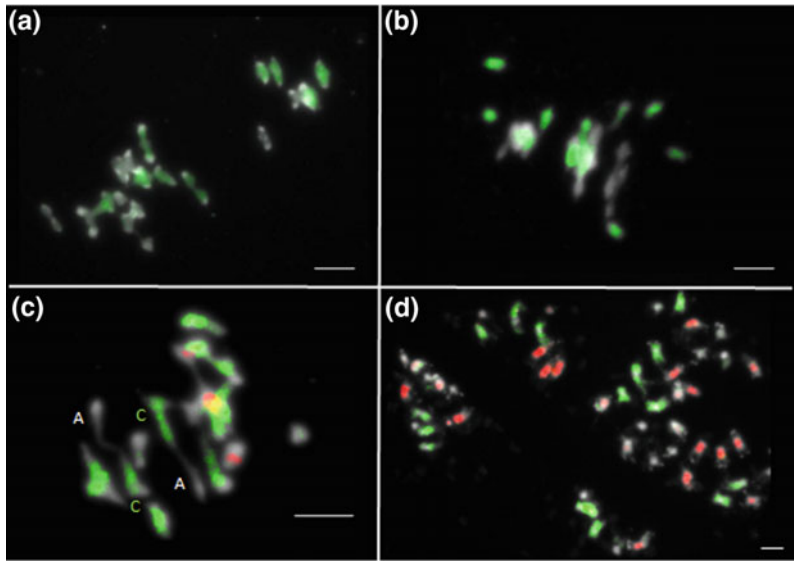

Fig. 2.5 **a–c** FISH analysis of metaphase I PMCs: FISH was carried out using BAC BoB014O06 which identifies all the C chromosomes (green). Nine C bivalent chromosomes and ten unmarked A bivalents (in blue DAPI) on *B. napus* (**a**) and nine C univalent chromosomes on AAC hybrid (**b**). Detection of autosyndesis (AA pairing)/ allosyndesis (AC pairing) at metaphase I using BAC BoB014O06 (green) and 54B2 specific of 3 A chromosomes (red) on pollen mother cells at MI from haploid Darmor-bzh (**c**); **d** FISH using genome labels for chromosomes in second-generation progeny derived from a near-allohexaploid *Brassica* (2*n* = AABBCC) plant [(*B. napus* × *B. carinata*) × *B. juncea*]: A genome chromosomes are blue (DAPI, background stain), B genome chromosomes are labeled red, and C genome chromosomes are labeled green. Bar = 5 μm

2.3.3 Identifying Structural Variation and Homeologous Exchanges Between Genomes

It is now clearly established that homeologous pairing can generate different structural rearrangements in natural allotetraploid species (Schubert and Lysak 2011) and that these rearrangements are difficult to detect only by mapping and sequencing strategies. Specifically, identification of reciprocal translocations and inversions between genotypes is extremely challenging using short-read sequencing technologies (Talkowski et al. 2011). This hinges on the fact that mapping of sequence reads to a reference genome assembly is the preferred method of comparing between genotypes or cultivars within a species. However, with this approach sequence inversions and reciprocal translocations will be almost invisible: Sequences from the target genotype will still map to the reference genome, but in incorrect locations determined by the reference genome rather than by the genome of the target genotype. Genetic mapping can assist with this by analysis of marker distortion, but requires the availability of linkage mapping populations for every genotype of interest. Despite the amount of work and inference involved, this method has been efficient in detecting translocations in several *B. napus* varieties (Lombard and Delourme 2001; Osborn et al. 2003; Piquemal et al. 2005). Similarly in hybrids carrying A, B, and C genomes, structural rearrangements between genomes are large enough to be detected by GISH using *B. nigra* labeled DNA to identify the B genome and GISH using the *B. oleracea* BAC BoB014O06 hybridized specifically to all C genome chromosomes (Howell et al. 2002). In near-allohexaploid lines

containing the A, B, and C genomes, this combined labeling technique effectively identified AC, AB, and BC recombinant chromosomes (Mason et al. 2014). It is important to also mention that this molecular cytogenetics technique is the best and perhaps only method available to detect rearrangements at the heterozygous stage (Fig. 2.4).

Loss of genomic regions resulting from non-reciprocal translocations (duplication/deletion events) can be detected in next-generation sequencing by lack of sequences from the target genotype mapping to the reference genome over the deletion region. However, this approach requires high sequence coverage to accurately identify copy number variants: Enough sequencing depth must be generated that absence of mapped reads (or presence of twice as many mapped reads in the case of duplications) in a chromosomal region can be definitively identified as a deletion (or duplication) event, rather than just natural variation in sequence coverage (Alkan et al. 2011). Both SNP array data and fluorescently labeled microsatellite marker data have also been used to assess duplication/deletion events in *Brassica* hybrids, although detection of duplications using relative allele fluorescence is challenging (Mason et al. 2011, 2015). Translocations can be directly physically detected at the homozygous and heterozygous stages by combining GISH and BAC-FISH, if the genomic regions are large and if a BAC is available specific to the translocated regions. Inversions, which are the most challenging of all to identify using sequencing data, can be readily confirmed by the use of several chromosome-specific BAC probes to hybridize to the chromosome of interest, as was done to integrate genetic and physical maps in *B. oleracea* (Howell et al. 2002). In future, technologies currently under development (such as optical mapping) may allow easier identification of structural rearrangements such as non-reciprocal translocations and inversions. Currently, the most direct and unambiguous method to assess these variants is still molecular cytogenetics.

2.3.4 Characterizing Chromosome Addition Lines and Genomic Introgressions for Crop Improvement

So-called chromosome addition lines provide a valuable resource for breeding and genetics analyses. These lines consist of a core genome, usually diploid, with the addition of a single chromosome from another genome, present in either one copy (monosomic addition line) or two copies (disomic addition line). The phenotypic characterization of these chromosome addition lines can allow localization of the trait of interest to particular chromosomes, as has been previously demonstrated for the yellow-seededness trait in *Brassica* AA + C addition lines by Heneen et al. (2012). However, the generation of these lines can be technically challenging. To produce lines with, for example, a complete diploid A genome with single additional C genome chromosomes in *Brassica*, usually a hybrid with $2n$ = AAC, will first be produced from crosses between *B. napus* (or resynthesized *B. napus* from the cross *B. rapa* × *B. oleracea*). This hybrid will then be backcrossed to *B. rapa* in order to eliminate all but one C genome chromosome. However, this approach offers plentiful opportunities for non-homologous recombination to occur, as well as being technically challenging. The use of FISH and GISH techniques to identify which chromosomes are present and in how many copies and to check for large-scale rearrangements such as translocations between the diploid genome and the addition chromosome/s is invaluable. Several studies have reported such analyses (e.g., Snowdon et al. 1997; Chèvre et al. 2007; Heneen et al. 2012; Mason et al. 2014), and BACs specific to different chromosomes allowed their identification in metaphase I (e.g., Suay et al. 2014).

The movement of traits from wild relatives or related species into crops first requires the production of a hybrid between the wild relative and the crop species. This hybrid must then allow

recombination between chromosomes from the crop genome and the donor species to occur to produce recombinant chromosomes. These recombinant chromosomes must then be successfully transferred in a backcross to crop to become an integral, stable part of the crop genome to establish the genomic transfer of the trait loci. Depending on the degree of genomic divergence between genomes (as well as genetic factors), a greater or fewer number of recombination events may occur in the hybrid. Currently, only cytogenetic analyses allow assessment of the chromosome number and genomic structure of subsequent introgressed plants. If only very few recombination events occur, then subsequent backcrossing may fail to recover any introgression events in the *B. napus* background. In this case, whole chromosomes may be retained if selection for the phenotype of interest is carried out, but these are generally less desirable as additional chromosomes or chromosome pairs from alien genomes can be frequently lost or selected against (Heneen et al. 2012). If a large number of recombination events take place, then many recombinant chromosomes may be transmitted in backcross generations. This can also cause problems, as many of these recombinants may carry undesirable allelic variants or have a negative effect on crop phenotype.

The larger the population size, the greater the chance of finding an individual with a single recombinant chromosome carrying desirable genomic introgressions for the phenotypic locus or loci of interest. However, the screening method is also critical: If individual lines of interest can be identified early and accurately, chances of success and cost-effectiveness and efficiency are greatly increased. For most species crossed to Brassica crop genomes, GISH is an efficient technique to determine the number and location of independent introgression events, as well as the size of the introgression in some cases (Snowdon et al. 1997; Fredua-Agyeman et al. 2014). In future, the development of BAC-FISH probes specific to introgression regions may offer a solution to detect smaller introgressions.

2.4 New Insights into Meiosis

Cytology and cytogenetics are central to all meiotic studies. Meiosis consists of two rounds of cell division during which chromosome number is halved (from diploid to haploid) and gametes are generated. In most organisms, accurate separation of homologous chromosomes during the first division requires that they first be connected to one another by crossovers (COs), which are one of the products of meiotic recombination. Meiotic COs are the reciprocal exchange of genetic material between chromosomes; they are formed during prophase I between all pairs of homologous chromosomes and start to become visible as chiasmata from diakinesis (they are more clearly resolved at metaphase I). Non-reciprocal recombination events, the so-called non-crossovers (NCOs), can also be recovered genetically but are not amenable to cytological analysis.

Despite its small chromosomes, *A. thaliana* has become a powerful model system for the analysis of meiosis and characterization of meiotic mutants (Mercier et al. 2015 for review). This has paved the way to meiotic studies in *Brassica*. Most notably, the high degree of primary sequence similarity between *A. thaliana* and *B. napus* has recently made possible the use of the antibodies developed against *Arabidopsis* proteins to study meiosis and meiotic recombination in *B. napus* and its close relatives. The first of these studies aimed to decipher the meiotic behavior of *Brassica* allotetraploid (AACC) and allotriploid (AAC) hybrids (Leflon et al. 2006). Polyclonal *Arabidopsis* antibodies that recognize the meiotic proteins ASY1, which associates with chromosome axis, and ZYP1, which is involved in the synaptonemal complex (SC) formation, were successfully used to demonstrate that some chromosomes were completely synapsed at pachytene (intimately associated by a fully formed SC along their length) while other remained unsynapsed even at a latter meiotic stage (i.e., diplotene). These unsynapsed chromosomes likely correspond to the C genome

chromosomes that remained as univalents (chromosomes that failed to form COs) at metaphase I, while the synapsed bivalents most probably correspond to pairs of homologous A chromosomes between which COs are formed. Actually, COs get a boost in the AAC hybrids compared to AA diploids (Leflon et al. 2010). Immunolocalization of *Arabidopsis* MLH 1 antibodies, which specifically mark the main fraction of COs (also called class I CO or CO I; Chelysheva et al. 2010), indicated that these crossovers undergo a 1.7-fold increase during male meiosis in the AAC hybrids compared to diploid controls. An even higher increase was detected in female meiosis by comparing genetic map distances (Leflon et al. 2010), suggesting either a sex-specific effect or a greater boost of the otherwise *minority* type of COs (which are not marked by MLH1). Although this has yet to be resolved, this study confirms the importance of combining both genetic and cytological approaches to study such an intricate biological process as meiotic recombination.

Finally, a wider range of cytological and cytogenetical tools was recently used to investigate the formation, progression, and completion of several key hallmarks of meiosis in *B. napus* allotetraploids (AACC) and allohaploids (AC, 19 chromosomes) (Grandont et al. 2014). This study has provided a thorough comparative description and analysis of sister chromatid cohesion, chromosome axes, the synaptonemal complex, and meiotic recombination in two representative *B. napus* accessions (Cifuentes et al. 2010). Analyses of surface-spread prophase I nuclei with electron microscopy have demonstrated a precocious and efficient sorting of homologous versus homeologous chromosomes during early prophase I in the two *B. napus* varieties that otherwise show a genotypic difference in the progression of homologous recombination. Most notably, the spatial-temporal localization of HEI10, an essential protein that can be used to follow the progressive channeling of recombination intermediates into the class I CO pathway (see Chelysheva et al. 2012 for details), was shown to vary from one genotype to another and to correlate with the two main meiotic behavior described at the haploid stage (see introduction). Moreover, the detailed comparison of meiosis in allohaploid and allotetraploid plants showed that the mechanism(s) promoting efficient chromosome sorting in allotetraploids is adjusted to promote crossover formation between homeologs in allohaploids. This suggests that, in contrast to other polyploid species, the threshold for committing a pair of chromosomes to form a CO is not fixed once and for all in *B. napus*, but depends on the operating chromosomes (Grandont et al. 2014). This probably remains an error-prone process, and a few COs can be expected to occasionally form between homeologs and generate homeologous exchanges in the allotetraploid plants (see Sect. 2.3.3). FISH analyses were also carried out to characterize CO formation between individual chromosomes during meiosis in allohaploid *B. napus*; these analyses indicated that both chromosome- and genotype-specific effects change the odds of forming a CO between a given pair of homeologous regions in these plants.

Interestingly, the cytological survey of meiosis in *B. napus* has tentatively pointed toward some of the genomic features of the *B. napus* genome. First, observation of genotype-specific bivalents in the allohaploids has led to the assumption that several chromosome(s) may carry the products of homeologous exchanges (HEs) (Grandont et al. 2014); the presence of numerous HEs was confirmed when assembling the *B. napus* genome sequence (see Sect. 2.3.3; Chalhoub et al. 2014). Likewise, the limited extent to which synaptic multivalents persisted to pachytene in *B. napus* allotetraploids has led to suppose that most interhomeolog recombination intermediates abort early and are redirected into intersister or non-crossover pathways (Grandont et al. 2014). Evidence for very short non-reciprocal exchanges between homeologous sequences, which possibly originated from meiotic non-crossovers, was recently obtained by Chalhoub et al. (2014). More work is needed to

confirm that all these HEs, whether they are large or small, have originated during or affect the meiotic behavior of *B. napus*.

2.5 Conclusions

Although cytogenetics as a technique significantly predates molecular genetics, let alone genome sequencing, this methodology and subsequent advances to molecular cytogenetic techniques provide extremely valuable information on physical organization of genomes that complements both mapping and sequencing data. Cytogenetics can provide new information on the genomic structure of ancient and recent polyploid species and shed light on evolutionary processes of genomic rearrangements, genome regulation, and genome structure variation. Cytogenetics can also be used to detect homeologous exchanges between constitutive genomes in interspecific hybrids or addition lines and to track chromosome introgressions from one genome to another. Comparative cytogenetics can be used for phylogenetic and taxonomic analysis, complementary to sequencing-based approaches, and can show evolutionary changes in functional features such as rDNA loci. Cytogenetics is of course essential for our basic understanding of meiosis, and progressively more sensitive molecular biology techniques are allowing visualization of this important biological process in *Brassica*. Use of interspecific hybridization coupled with cytogenetic analysis allows a deep insight into the occurrence and control of homologous and non-homologous chromosome pairing. Cytogenetics techniques are also currently the primary means of validating chromosome rearrangements such as duplications, deletions, and inversions, acting in a complementary fashion to confirm the sequence-based analysis. From the past to the future, cytogenetics has contributed a great deal to our understanding of genetics and genomics in the Brassica genus, and undoubtedly, cytogenetics will play a significant role in the times to come.

Acknowledgements M. A. L. was supported by a research grant from the Czech Science Foundation (P501/12/G090). ASM is supported by an Emmy Noether grant from the Deutsche Forschungsgemeinschaft (MA 6473/1-1).

References

Alkan C, Coe BP, Eichler EE (2011) Applications of next-generation sequencing genome structural variation discovery and genotyping. Nat Rev Genet 12:363–375

Al-Shehbaz IA (2012) A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon 61(5):931–954

Attia T, Röbbelen G (1986) Meiotic pairing in haploids and amphihaploids of spontaneous versus synthetic origin in rape, *Brassica napus* l. Can J Genet Cytol 28:330–334

Bozza CG, Pawlowski WP (2008) The cytogenetics of homologous chromosome pairing in meiosis in plants. Cytogenet Genome Res 120:313–319

Catcheside DG (1934) The chromosomal relationships in the Swede and turnip groups of *Brassica*. Ann Bot 48:601–633

Chalhoub B, Denoeud F, Liu SY, Parkin IAP, Tang HB, Wang XY, Chiquet J, Belcram H, Tong CB, Samans B, Correa M, Da Silva C, Just J, Falentin C, Koh CS, Le Clainche I, Bernard M, Bento P, Noel B, Labadie K, Alberti A, Charles M, Arnaud D, Guo H, Daviaud C, Alamery S, Jabbari K, Zhao MX, Edger PP, Chelaifa H, Tack D, Lassalle G, Mestiri I, Schnel N, Le Paslier MC, Fan GY, Renault V, Bayer PE, Golicz AA, Manoli S, Lee TH, Thi VHD, Chalabi S, Hu Q, Fan CC, Tollenaere R, Lu YH, Battail C, Shen JX, Sidebottom CHD, Wang XF, Canaguier A, Chauveau A, Berard A, Deniot G, Guan M, Liu ZS, Sun FM, Lim YP, Lyons E, Town CD, Bancroft I, Wang XW, Meng JL, Ma JX, Pires JC, King GJ, Brunel D, Delourme R, Renard M, Aury JM, Adams KL, Batley J, Snowdon RJ, Tost J, Edwards D, Zhou YM, Hua W, Sharpe AG, Paterson AH, Guan CY, Wincker P (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950–953

Chelysheva L, Grandont L, Vrielynck N, le Guin S, Mercier R, Grelon M (2010) An easy protocol for studying chromatin and recombination protein dynamics during *Arabidopsis thaliana* meiosis: immunodetection of cohesins, histones and MLH1. Cytogenet Genome Res 129(1–3):143–153

Chelysheva L, Vezon D, Chambon A, Gendrot G, Pereira L, Lemhemdi A, Vrielynck N, Le Guin S, Novatchkova M, Grelon M (2012) The *Arabidopsis* HEI10 is a new ZMM protein related to Zip3. PLoS Genet 8(7):e1002799

Chen ZJ, Pikaard CS (1997) Transcriptional analysis of nucleolar dominance in polyploid plants: biased

expression/silencing of progenitor rRNA genes is developmentally regulated in *Brassica*. Proc Natl Acad Sci U S A 94:3442–3447

Chen S, Nelson MN, Chèvre AM, Jenczewski E, Li Z, Mason AS, Meng J, Plummer JA, Pradhan A, Siddique KH (2011) Trigenomic bridges for *Brassica* improvement. Crit Rev Plant Sci 30(6):524–547

Cheng F, Mandáková T, Wu J, Xie Q, Lysak MA, Wang X (2013) Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. Plant Cell 25(5):1541–1554

Cheng F, Wu J, Wang X (2014) Genome triplication drove the diversification of *Brassica* plants. Hortic Res 1:14024. https://doi.org/10.1038/hortres.2014.24

Chester M, Gallagher JP, Symonds VV, da Silva AVC, Mavrodiev EV, Leitch AR, Soltis PS, Soltis DE (2012) Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). Proc Natl Acad Sci USA 109:1176–1181

Cheung F, Trick M, Drou N, Lim YP, Park J-Y, Kwon SJ, Kim JA, Scott R, Pires JC, Paterson AH, Town C, Bancroft I (2009) Comparative analysis between homoeologous genome segments of *Brassica napus* and its progenitor species reveals extensive sequence-level divergence. Plant Cell 21:1912–1928

Chèvre AM, Adamczyk K, Eber F, Huteau V, Coriton O, Letanneur JC, Laredo C, Jenczewski E, Monod H (2007) Modelling gene flow between oilseed rape and wild radish. 1. Evolution of chromosome structure. Theor Appl Genet 114:209–221

Cifuentes M, Eber F, Lucas MO, Lode M, Chèvre AM, Jenczewski E (2010) Repeated polyploidy drove different levels of crossover suppression between homoeologous chromosomes in *Brassica napus* allohaploids. Plant Cell 22(7):2265–2276

Edwards D, Batley J, Snowdon RJ (2013) Accessing complex crop genomes with next-generation sequencing. Theor Appl Genet 126:1–11

Fluch S, Kopecky D, Burg K, Šimková H, Taudien S, Petzold A, Kubaláková M, Platzer M, Berenyi M, Krainer S, Doležel J, Lelley T (2012) Sequence composition and gene content of the short arm of Rye (*Secale cereale*) chromosome 1. PLoS ONE 7(2):e30784

Franzke A, Lysak MA, Al-Shehbaz IA, Koch MA, Mummenhoff K (2011) Cabbage family affairs: the evolutionary history of Brassicaceae. Trends Plant Sci 16:108–116

Fredua-Agyeman R, Coriton O, Huteau V, Parkin IAP, Chevre AM, Rahman H (2014) Molecular cytogenetic identification of B genome chromosomes linked to blackleg disease resistance in *Brassica napus* × *B. carinata* interspecific hybrids. Theor Appl Genet 127(6):1305–1318

Fukui K, Nakayama S, Ohmido N, Yoshiaki H, Yamabe M (1998) Quantitative karyotyping of three diploid *Brassica* species by imaging methods and localization of 45s rDNA loci on the identified chromosomes. Theor Appl Genet 96(3–4):325–330

Gomez-Campo C (1980) Morphology and morphotaxonomy of the tribe Brassiceae. In: Tsunoda S, Hinata K, Gomez-Campo C (eds) *Brassica* crops and wild allies: biology and breeding. Japan Scientific Society Press, Tokyo, pp 3–31

Grandont L, Cuñado N, Coriton O, Huteau V, Eber F, Chèvre AM, Grelon M, Chelysheva L, Jenczewski E (2014) Homoeologous chromosome sorting and progression of meiotic recombination in *Brassica napus*: ploidy does matter! Plant Cell 26(4):1448–1463

Hasterok R, Maluszynska J (2000) Nucleolar dominance does not occur in root tip cells of allotetraploid *Brassica* species. Genome 43:574–579

Hasterok R, Wolny E, Hosiawa M, Kowalczyk M, Kulak-Ksiazczyk S, Ksiazczyk T, Heneen WK, Maluszynska J (2006) Comparative analysis of rDNA distribution in chromosomes of various species of Brassicaceae. Ann Bot 97:205–216

Heneen WK, Geleta M, Brismar K, Xiong ZY, Pires JC, Hasterok R, Stoute AI, Scott RJ, King GJ, Kurup S (2012) Seed colour loci, homoeology and linkage groups of the C genome chromosomes revealed in *Brassica rapa-B. oleracea* monosomic alien addition lines. Ann Bot 109:1227–1242

Howell EC, Barker G, Jones G, Kearsey MJ, King G, Kop EP, Ryder CD, Teakle GR, Vicente JG, Armstrong SJ (2002) Integration of the cytogenetic and genetic linkage maps of *Brassica oleracea*. Genetics 161:1225–1234

Howell EC, Armstrong SJ, Barker GC, Jones GH, King GJ, Ryder CD, Kearsey MJ (2005) Physical organization of the major duplication on *Brassica oleracea* chromosome O6 revealed through fluorescence in situ hybridization with *Arabidopsis* and *Brassica* BAC probes. Genome 48(6):1093–1103

Howell EC, Kearsey MJ, Jones GH, King GJ, Armstrong SJ (2008) A and C genome distinction and chromosome identification in *Brassica napus* by sequential fluorescence in situ hybridization and genomic in situ hybridization. Genetics 180(4): 1849–1857

Jenczewski E, Alix K (2004) From diploids to allopolyploids: the emergence of efficient pairing control genes in plants. Crit Rev Plant Sci 23:21–45

Jenczewski E, Eber F, Grimaud A, Huet S, Lucas MO, Monod H, Chèvre AM (2003) *Prbn*, a major gene controlling homoeologous pairing in oilseed rape (*Brassica napus*) haploids. Genetics 164:645–653

Książczyk T, Kovarik A, Eber F, Huteau V, Khaitova L, Tesarikova Z, Coriton O, Chèvre AM (2011) Epigenetic silencing precedes rDNA loci rearrangements during the stabilization of a polyploid species *Brassica napus*. Chromosoma 120:557–571

Lagercrantz U (1998) Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics 150:1217–1228

Lagercrantz U, Lydiate D (1996) Comparative genome mapping in *Brassica*. Genetics 144:1903–1910

Leflon M, Eber F, Letanneur JC, Chelysheva L, Coriton O, Huteau V, Ryder CD, Barker G, Jenczewski E, Chèvre AM (2006) Pairing and recombination at meiosis of *Brassica rapa* (AA) × *Brassica napus* (AACC) hybrids. Theor Appl Genet 113(8):1467–1480

Leflon M, Grandont L, Eber F, Huteau V, Coriton O, Chelysheva L, Jenczewski E, Chèvre AM (2010) Crossovers get a boost in *Brassica* allotriploid and allotetraploid hybrids. Plant Cell 22(7):2253–2264

Liu Z, Adamczyk K, Manzanares-Dauleux M, Eber F, Lucas MO, Delourme R, Chevre AM, Jenczewski E (2006) Mapping PrBn and other quantitative trait loci responsible for the control of homeologous chromosome pairing in oilseed rape (*Brassica napus* L.) haploids. Genetics 174:1583–1596

Liu S, Liu Y, Yang X, Tong C, Edwards D, Parkin IA, Zhao M, Ma J, Yu J, Huang S, Wang X, Wang J, Lu K, Fang Z, Bancroft I, Yang TJ, Hu Q, Wang X, Yue Z, Li H, Yang L, Wu J, Zhou Q, Wang W, King GJ, Pires JC, Lu C, Wu Z, Perumal S, Wang Z, Guo H, Pan S, Yang L, Min J, Zhang D, Jin D, Li W, Belcram H, Tu J, Guan M, Qi C, Du D, Li J, Jiang L, Batley J, Sharpe AG, Park BS, Ruperao P, Cheng F, Waminal NE, Huang Y, Dong C, Wang L, Li J, Hu Z, Zhuang M, Huang Y, Huang J, Shi J, Mei D, Liu J, Lee TH, Wang J, Jin H, Li Z, Li X, Zhang J, Xiao L, Zhou Y, Liu Z, Liu X, Qin R, Tang X, Liu W, Wang Y, Zhang Y, Lee J, Kim HH, Denoeud F, Xu X, Liang X, Hua W, Wang X, Wang J, Chalhoub B, Paterson AH (2014) The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. Nat Commun 5:3930

Lombard V, Delourme R (2001) A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. Theor Appl Genet 103:491–507

Long EO, Dawid IB (1980) Repeated genes in eukaryotes. Annu Rev Biochem 49:727–764

Lysak MA, Koch MA, Pecinka A, Schubert I (2005) Chromosome triplication found across the tribe Brassiceae. Genome Res 15:516–525

Lysak MA, Berr A, Pecinka A, Schmidt R, McBreen K, Schubert I (2006) Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. Proc Natl Acad Sci U S A 103:5224–5229

Lysak MA, Cheung K, Kitschke M, Bures P (2007) Ancestral chromosomal blocks are triplicated in Brassiceae species with varying chromosome number and genome size. Plant Physiol 145:402–410

Maluszynska J, Heslop-Harrison JS (1993) Physical mapping of rDNA loci in *Brassica* species. Genome 36:774–781

Mandáková T, Lysak MA (2008) Chromosomal phylogeny and karyotype evolution in x = 7 crucifer species (Brassicaceae). Plant Cell 20:2559–2570

Mandáková T, Singh V, Krämer U, Lysak MA (2015) Genome structure of the heavy metal hyperaccumulator *Noccaea caerulescens* and its stability on metalliferous and nonmetalliferous soils. Plant Physiol 169:674–689

Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifer M; International Wheat Genome Sequencing Consortium, Jakobsen KS, Wulff BB, Steuernagel B, Mayer KF, Olsen OA (2014) Ancient hybridizations among the ancestral genomes of bread wheat. Science 18;345(6194):1250092

Mason AS, Huteau V, Eber F, Coriton O, Yan G, Nelson MN, Cowling WA, Chèvre AM (2010) Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB *Brassica* interspecific hybrids. Chromosome Res 18:655–666

Mason AS, Nelson MN, Castello MC, Yan G, Cowling WA (2011) Genotypic effects on the frequency of homoeologous and homologous recombination in *Brassica napus* × *B. carinata* hybrids. Theor Appl Genet 122:543–553

Mason AS, Nelson MNN, Takahira J, Cowling WA, Moreira Alves G, Chaudhuri A, Chen N, Ragu ME, Dalton-Morgan J, Coriton O, Huteau V, Eber F, Chèvre AM, Batley J (2014) The fate of chromosomes and alleles in an allohexaploid *Brassica* population. Genetics 197:273–283

Mason AS, Takahira J, Atri C, Samans B, Hayward A, Cowling WA, Batley J, Nelson MN (2015) Microspore culture reveals complex meiotic behaviour in a trigenomic *Brassica* hybrid. BMC Plant Biol 15:173

Mercier R, Mézard C, Jenczewski E, Macaisne N, Grelon M (2015) The molecular biology of meiosis in plants. Annu Rev Plant Biol 66:297–327

Mizushima U (1980) Genome analysis in *Brassica* and allied Genera. In: Tsunoda S, Hinata K, Gómez-Campo C (eds) *Brassica* crops and wild allies: biology and breeding. Japan Scientific Society Press, Tokyo, pp 89–106

Morinaga T (1934) Interspecific hybridization in Brassica VI. The cytology of F1 hybrdis of *B. juncea* and *B. nigra*. Cytologia 6:62–67

Müntzing A (1936) The evolutionary significance of autopolyploidy. Hereditas 21:263–378

Nagaharu U (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap J Bot 7:389–452

Nagpal R, Raina SN, Sodhi YS, Mukhopadhyay A, Arumugam N, Pradhan AK, Pental D (1996) Transfer of *Brassica tournefartii* (TT) genes to allotetraploid oilseed *Brassica* species (*B. juncea* AABB, *B. napus* AACC, *B. carinata* BBCC): homoeologous pairing is more pronounced in the three-genome hybrids (TACC, TBAA, TCAA, TCBB) as compared to allodiploids (TA, TB, TC). Theor Appl Genet 92:566–571

Navabi ZK, Huebert T, Sharpe AG, O'Neill CM, Bancroft I, Parkin IAP (2013) Conserved microstructure of the *Brassica* B genome of *Brassica nigra* in relation to homologous regions of *Arabidopsis*

thaliana, *B. rapa* and *B. oleracea*. BMC Genom 14:250

Nicolas S, Le Mignon G, Eber F, Coriton O, Monod H, Clouet V, Huteau V, Lostanlen A, Delourme R, Chalhoub B, Ryder C, Chevre AM, Jenczewski E (2007) Homoeologous recombination plays a major role in chromosome rearrangements that occur during meiosis of *Brassica napus* haploids. Genetics 175:487–503

Nicolas S, Leflon M, Monot H, Eber F, Coriton O, Huteau V, Chèvre AM, Jenczewski E (2009) Genetic regulation of meiotic crossovers between related genomes in *Brassica napus* haploids and hybrids. Plant Cell 21:373–385

Nicolas S, Monot H, Eber F, Chèvre AM, Jenczewski E (2012) Non random distribution of extensive chromosome rearrangements in *Brassica napus* depends on genome organization. Plant J 70:691–703

Osborn TC, Butrulle DV, Sharpe AG, Pickering KJ, Parkin IA, Parker JS, Lydiate DJ (2003) Detection and effects of a homeologous reciprocal transposition in *Brassica napus*. Genetics 165:1569–1577

Paritosh K, Gupta V, Yadava SK, Singh P, Pradhan AK, Pental D (2014) RNA-seq based SNPs for mapping in *Brassica juncea* (AABB): synteny analysis between the two constituent genomes A (from *B. rapa*) and B (from *B. nigra*) shows highly divergent gene block arrangement and unique block fragmentation patterns. BMC Genom 15:396

Parkin I (2011) Chasing ghosts: comparative mapping in the Brassicaceae. In: RA Jorgensen (ed) Plant genetics and genomics: crops and models, vol 9, pp 153–170

Parkin IAP, Sharp AG, Keith DJ, Lydiate DJ (1995) Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome 38:1122–1131

Parkin IAP, Sharpe AG, Lydiate DJ (2003) Patterns of genome duplication within the *Brassica napus* genome. Genome 46:291–303

Parkin IA, Gulden SM, Sharpe AG, Lukens L, Trick M, Osborn TC, Lydiate DJ (2005) Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics 171: 765–781

Parkin IA, Koh C, Tang H, Robinson SJ, Kagale S, Clarke WE, Town CD, Nixon J, Krishnakumar V, Bidwell SL, Denoeud F, Belcram H, Links MG, Just J, Clarke C, Bender T, Huebert T, Mason AS, Pires JC, Barker G, Moore J, Walley PG, Manoli S, Batley J, Edwards D, Nelson MN, Wang X, Paterson AH, King G, Bancroft I, Chalhoub B, Sharpe AG (2014) Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. Genome Biol 15(6):R77

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet E, Perret D, Villeger M-J, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523

Prakash S, Hinata K (1980) Taxonomy, cytogenetics and origin of crop *Brassica*, a review. Opera Botanica 55:1–57

Prakash SR, Bhat SR, Quiros CF, Kirti PB, Chopra VL (2009) *Brassica* and its close allies: cytogenetics and evolution. Plant Breed Rev 31:21–187

Renard M, Dosba F (1980) Etude de l'haploidie chez le colza (*Brassica napus* L. var *oleifera* metzger). Annales de l'amélioration des Plantes 30:191–209

Röbbelen G (1960) Beiträge zur Analyse des *Brassica*-Genoms. Chromosoma 11:205–228

Rogers SO, Bendich AJ (1987) Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacers. Plant Mol Biol 9:509–520

Schiessl S, Samans B, Huttel B, Reinhard R, Snowdon RJ (2014) Capturing sequence variation among flowering-time regulatory gene homologs in the allopolyploid crop species *Brassica napus*. Front Plant Sci 5

Schranz ME, Lysak MA, Mitchell-Olds T (2006) The ABC's of comparative genomics in the Brassicaceae: building blocks of crucifer genomes. Trends Plant Sci 11:535–542

Schubert I, Lysak MA (2011) Interpretation of karyotype evolution should consider chromosome structural constraints. Trends Genet 27:207–216

Snowdon RJ, Kohler W, Friedt W, Kohler A (1997) Genomic in situ hybridization in *Brassica* amphidiploids and interspecific hybrids. Theor Appl Genet 95:1320–1324

Suay L, Zhang D, Eber F, Jouy H, Lodé M, Huteau V, Coriton O, Szadkowski E, Leflon M, Martin OC, Falque M, Jenczewski E, Paillard S, Chèvre AM (2014) Crossover rate between homologous chromosomes and interference are regulated by the addition of specific unpaired chromosomes in *Brassica*. New Phytol 201(2):645–656

Szadkowski E, Eber F, Huteau V, Lodé M, Huneau C, Belcram H, Coriton O, Manzanares-Dauleux MJ, Delourme R, King GJ, Chalhoub B, Jenczewski E, Chèvre AM (2010) The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol 186:102–112

Szadkowski E, Eber F, Huteau V, Lodé M, Coriton O, Jenczewski E, Chèvre AM (2011) Polyploid formation pathways have an impact on genetic rearrangements in resynthesized *Brassica napus*. New Phytol 191:554–894

Talkowski ME, Ernst C, Heilbut A, Chiang C, Hanscom C, Lindgren A, Kirby A, Liu ST, Muddukrishna B, Ohsumi TK, Shen YP, Borowsky M, Daly MJ, Morton CC, Gusella JF (2011) Next-generation sequencing strategies enable routine detection of balanced chromosome rearrangements for clinical diagnostics and genetic research. Am J Hum Genet 88:469–481

Tang H, Woodhouse MR, Cheng F, Schnable JC, Pedersen BS, Conant G, Wang X, Freeling M, Pires JC

(2012) Altered patterns of fractionation and exon deletions in *Brassica rapa* support a two-step model of paleohexaploidy. Genetics 190:1563–1574

Udall JA, Quijada PA, Osborn TC (2005) Detection of chromosomal rearrangements derived from homologous recombination in four mapping populations of *Brassica napus* L. Genetics 169:967–979

Wang X, Wang H, Wang J, Sun R, Wu J, Liu S, Bai Y, Mun JH, Bancroft I, Cheng F, Huang S, Li X, Hua W, Wang J, Wang X, Freeling M, Pires JC, Paterson AH, Chalhoub B, Wang B, Hayward A, Sharpe AG, Park BS, Weisshaar B, Liu B, Li B, Liu B, Tong C, Song C, Duran C, Peng C, Geng C, Koh C, Lin C, Edwards D, Mu D, Shen D, Soumpourou E, Li F, Fraser F, Conant G, Lassalle G, King GJ, Bonnema G, Tang H, Wang H, Belcram H, Zhou H, Hirakawa H, Abe H, Guo H, Wang H, Jin H, Parkin IA, Batley J, Kim JS, Just J, Li J, Xu J, Deng J, Kim JA, Li J, Yu J, Meng J, Wang J, Min J, Poulain J, Wang J, Hatakeyama K, Wu K, Wang L, Fang L, Trick M, Links MG, Zhao M, Jin M, Ramchiary N, Drou N, Berkman PJ, Cai Q, Huang Q, Li R, Tabata S, Cheng S, Zhang S, Zhang S, Huang S, Sato S, Sun S, Kwon SJ, Choi SR, Lee TH, Fan W, Zhao X, Tan X, Xu X, Wang Y, Qiu Y, Yin Y, Li Y, Du Y, Liao Y, Lim Y, Narusaka Y, Wang Y, Wang Z, Li Z, Wang Z, Xiong Z, Zhang Z; *Brassica rapa* Genome Sequencing Project Consortium (2011) The genome of the mesopolyploid crop species *Brassica rapa*. Nat Genet 43(10):1035–1039

Wang YP, Wang XY, Paterson AH (2012) Genome and gene duplications and gene expression divergence: a view from plants. Ann NY Acad Sci 1256:1–14

Xiong Z, Pires JC (2011) Karyotype and identification of all homoeologous chromosomes of allopolyploid *Brassica napus* and its diploid progenitors. Genetics 187:37–49

Xiong ZY, Gaeta RT, Pires JC (2011) Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. Proc Natl Acad Sci USA 108:7908–7913

Ziolkowski PA, Kaczmarek M, Babula D, Sadowski J (2006) Genome evolution in *Arabidopsis/Brassica*: conservation and divergence of ancient rearranged segments and their breakpoints. Plant J 47:63–74

Zou J, Fu DH, Gong HH, Qian W, Xia W, Pires JC, Li RY, Long Y, Mason AS, Yang TJ, Lim YP, Park BS, Meng JL (2011) *De novo* genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Plant J 68:212–224