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Cytogenetics and genome analysis in *Brassica* crops

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Abstract

The genus *Brassica* contains a wide range of diploid and amphipolyploid species including some of the most important vegetable, condiment and oilseed crops worldwide. As members of the Brassicaceae family the brassicas are the closest crop relatives to the model plant *Arabidopsis thaliana*, and hence are major beneficiaries from the vast array of *Arabidopsis* molecular genetic and genomic tools and the increasingly good annotation to major *Brassica* crop genomes. In this review examples are shown from recent studies that demonstrate the potential for intergenome navigation from model to crop plant and for comparisons among genetic and cytogenetic maps between the model and crop species and among different crop brassicas. The use of interspecific and intergeneric hybridization for introgression of novel traits into *Brassica* genomes from the secondary and tertiary crucifer genepools is described. In this context the use of the *Brassica* triangle of three diploid species and their corresponding amphiploids as an excellent model system for studying the mechanisms and control of homeologous recombination and polyploidization is discussed from a crop breeding perspective.

Introduction

The chromosomal relationships among the A, B and C genomes of the diploid species *Brassica rapa* (genome AA, 2n = 20; turnip rape, turnip, Chinese cabbage), *B. nigra* (genome BB, 2n = 16; black mustard) and *B. oleracea* (genome CC, 2n = 18; cabbage, cauliflower, broccoli, kale, kohlrabi, brussel sprouts) and their natural spontaneous amphidiploids *B. carinata* (genome AABB, 2n = 34; Abyssinian or Ethiopian mustard), *B. napus* (genome AACC, 2n = 38; oilseed rape, swede) and *B. juncea* (genome BBCC, 2n = 36; Indian or brown mustard) were elucidated through interspecific crosses and meiotic analyses by the Asian cytogeneticists Morinaga and U in the early 20th century (Morinaga 1933, 1934, U N 1935). Because the *Brassica* amphidiploid species can be generated synthetically with the help of embryo rescue techniques, this complex of three diploid species and their corresponding polyploids (Figure 1) is today one of the most useful model systems for investigations of polyploidy in crop plants (e.g. Song *et al*. 1995, Lukens *et al*. 2006). Colchicine treatment can also be used to artificially synthesize autotetraploid brassicas, which can potentially be used to compare the corresponding effects of gene dosage, autoploidy, alloploidy and amphiploidy on gene regulation and expression.

Brassica–*Arabidopsis* comparative genomics: model-based breeding tools

Comparative genome analyses between *Brassica* and the model crucifer *Arabidopsis thaliana* have

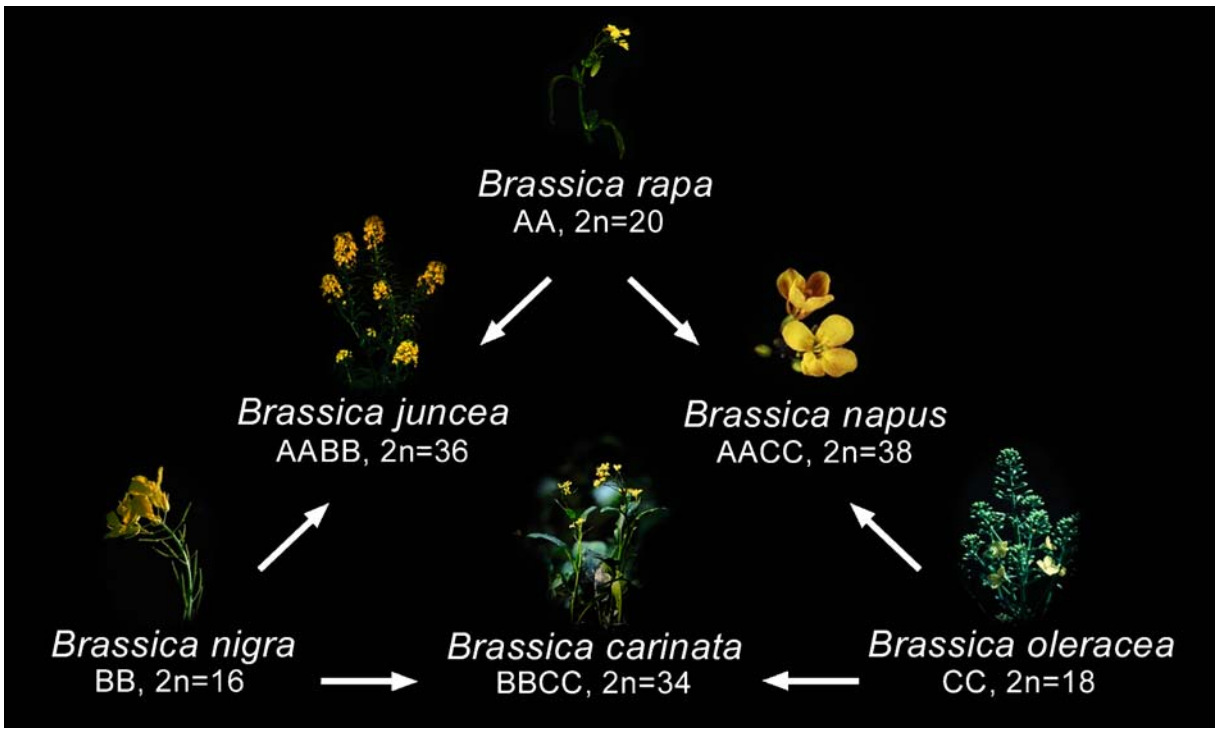

Figure 1. The *Brassica* triangle of species, as described by U N (1935), representing the A, B and C genomes and their respective amphidiploids that arose from spontaneous chromosome doubling via meiotic nondisjunction after interspecific hybridizations in regions of overlapping geographical distribution of the respective diploid progenitors.

revealed that *Brassica* chromosomes show a complex rearrangement in comparison to the *A. thaliana* genome (Lagercrantz & Lydiate 1996), presumably the result of numerous rounds of polyploidization. As a consequence the synteny and microsynteny relationships between the more or less triplicated diploid *Brassica* genomes (Schmidt *et al*. 2001, Lan *et al*. 2000, Lysak *et al*. 2005, Parkin *et al*. 2005, Ziolkowski *et al*. 2006) and the paleopolyploid genome of *Arabidopsis* are extremely complicated. Detailed comparative genomic information is therefore needed for directed utilization of genome data from *Arabidopsis* in *Brassica* molecular breeding.

The most intensively studied *Brassica* amphidiploid is the most economically important crop, oilseed rape (*B. napus* ssp. *napus*). The A and C genome chromosomes in *B. napus* have remained essentially unaltered after their amphipolyploidization (Olsson & Hagberg 1955, Attia *et al*. 1987, Sharpe *et al*. 1995, Snowdon *et al*. 2002). Hence it is possible to compare genetic maps, chromosomes and gene positions among the three species and to use the smaller diploid genomes to obtain genomic data that can be extrapolated to the amphidiploid. A large number of independent genetic maps among different *B. napus* crosses have been generated (reviewed by Snowdon *et al*. 2006) with a particular emphasis on investigation of quantitative traits, development of markers for marker-assisted breeding, and potential map-based cloning of genes involved in agronomically important traits. The ability to navigate between the *Arabidopsis* sequence and the major *Brassica* genomes is improving constantly as the genomic sequencing of *B. rapa* (Bancroft 2006, Yang *et al*. 2006, see also http://www.brassica.info) progresses and the annotation of the new sequence data to the *Arabidopsis* genome is updated. On the other hand, comparative genomics from *Arabidopsis* sequence information is a valuable asset for *in-silico* chromosome walking within the *B.* rapa sequencing project (Bancroft 2006, Lim *et al*. 2006). For example, evenly spaced seed BAC (bacterial artificial chromosomes) for the sequencing of *B. rapa* chromosome 1 were selected using mapped expressed sequence tags (EST). The physical positions and

ordering of these BAC on chromosome 1 were confirmed by fluorescence *in-situ* hybridization (FISH) to mitotic and meiotic chromosomes. Because the majority of the BAC show collinearity to the corresponding homoeologous chromosome region in *Arabidopsis* it is possible to validate the positions of the *B. rapa* sequencing clones by *in-silico* comparative physical mapping (Yang *et al*. 2006).

A detailed comparative genetic map between the chromosomes of *A. thaliana* and *B. napus* was generated by Parkin *et al*. (2005) by localization of orthologous sequences from mapped *B. napus* RFLP markers to the corresponding chromosome positions in *A. thaliana*. A total of 21 chromosomal blocks were identified in the genome of *A. thaliana* that could be duplicated and rearranged to reconstruct the basic chromosome structure of *B. napus*. Each block contained several closely linked homologous loci with more or less conserved order in both the *A. thaliana* and *B. napus* genomes. For each *B. napus* chromosome numerous blocks of conserved synteny were found between *B. napus* and *A. thaliana*, each presumably representing chromosomal segments that have been maintained since the divergence of *Arabidopsis* and *Brassica* from a common ancestor. According to Parkin *et al*. (2005) a minimum of 74 gross rearrangements (38 in the A genome and 36 in the C genome) appear to have occurred since the divergence of the brassicas from the *Arabidopsis* lineage some 14–24 million years ago (Koch *et al*. 2000). In similar studies Lukens *et al*. (2003) and Lan *et al*. (2000) compared the positions of sequenced loci with a known position on a *B. oleracea* genetic map to the physical positions of their putative orthologues in *A. thaliana*. By distinguishing orthologous from paralogous loci and establishing criteria to identify significant regions of collinearity between the genomes a total of 34 significant *A. thaliana* regions were found by Lukens *et al*. (2003) to be collinear with 28% of the *B. oleracea* genetic map. The large number of macrosynteny breakpoints coupled with the identification of extensive duplications in the *B. oleracea* genome also confirmed the extremely high degree of chromosomal rearrangement since divergence of the *Brassica* diploids from *A. thaliana*. Nevertheless it is potentially possible to utilize rough macrosyntenic data to navigate between particular regions of interest in the *Arabidopsis* genome and *Brassica* genetic maps. On the other hand evidence from microsyntenic studies of gene order in duplicated chromosome regions suggests that interspersed gene loss is prevalent in homoeologous *Brassica* chromosome regions (O'Neill & Bancroft 2000, Rana *et al*. 2004, Town *et al*. 2006).

The conservation of gross macrosynteny can prove extremely useful in the identification and characterization of *Brassica* genes involved in important quantitative traits based on comparative analysis with chromosome regions in *Arabidopsis* corresponding to QTL in the crop species. For example, considerable knowledge has been gained on the genetic control of vernalization-responsive flowering time in *B. rapa* and *B. napus* through QTL analysis and comparative mapping of genes associated with this trait in homoeologous *Brassica* and *Arabidopsis* genome regions. Osborn *et al*. (1997) found that a chromosome region containing a homoeologous QTL for flowering time in *B. rapa* and *B. napus*, respectively, was collinear with a region of *A. thaliana* chromosome 5 containing the flowering time genes *FLC*, *FY* and *CO*. A second QTL region showed fractured collinearity with several regions of the *Arabidopsis* genome, including the top of chromosome 4 where another flowering-time gene, *FRI*, is located. Detailed analysis enabled identification of *FLC* and *FRI* as the major candidate genes for regulation of flowering time in the *Brassica* species.

On a sequence level high similarity is found between the exons of putative orthologous genes in *Arabidopsis* and *Brassica* (Schmidt 2002), meaning that knowledge from *Arabidopsis* is highly relevant for gene isolation and characterization in *Brassica* crops. With an ever-growing resource of *Brassica* sequence data it is today becoming increasingly possible – despite the complex rearrangements among *Brassica* genomes – to annotate, align and compare chromosomal and genomic data between the crop brassicas and the model species and to use this new information for genomic studies in the comparatively large crop genomes. Navigation between *Brassica* and *Arabidopsis* physical maps using published genome annotation and synteny data uncovers an enormous wealth of tools for fine-mapping, synteny-based gene cloning and marker development for marker-assisted selection. For example, we have successfully used online SSR search engines to scan *Arabidopsis* chromosome regions flanking candidate genes of interest or major QTL positions. Many of the *Brassica* SSR primers we identified in this manner were found to amplify polymorphic markers at one or more homologous loci in oilseed rape. The

linkage of these markers to the trait of interest could often be confirmed by re-mapping to QTL regions or by allele-trait association analysis in genetically diverse material (unpublished results). If such markers are in linkage disequilibrium with the gene of interest, this strategy can be extremely useful for indirect mapping of candidate genes on *Brassica* chromosomes. Because SSR markers are codominant, this approach also has the potential to enable map localization of duplicated copies of a given candidate gene, for example to allow comparisons with major QTL positions.

Integration of karyotypes with genetic and physical maps

Development of classical cytogenetic resources for *Brassica* crop species is difficult due to the small size of the chromosomes and the lack of distinct karyological features that can be readily identified in metaphase preparations. This restricts the exact cytological characterization of *Brassica* addition, substitution and particularly introgression lines. However, *Brassica* interspecific addition and introgression lines can be generated with relative ease via interspecific hybridization and recurrent backcrossing using embryo rescue techniques in early generations. Characterization of addition lines with molecular markers has to a certain extent enabled the successful characterization of specific additions or introgressions for genome analysis or associations with particular traits of interest from the donor genome. Quiros *et al.* (1987) generated a set of monosomic addition plants and disomic addition lines by crossing and backcrossing *B. napus* to *B. rapa* (syn. *campestris*) and confirmed the genome-specificity of the additions using genome-specific molecular markers. The marker analyses revealed extensive gene duplications on individual addition chromosomes (McGrath *et al.* 1990) and the presence of deletions in some chromosomes (Hu & Quiros 1991), a phenomenon that may be a contributing factor to the extensive chromosomal rearrangement that has occurred during the evolution of the Brassicaceae. In a similar manner Chen *et al.* (1992, 1997a) generated addition lines containing monosomic addition chromosomes from the C-genome donor *Brassica alboglabra* in a *B. rapa* background by backcrossing a resynthesized *B. napus* containing the donor genome to its parental *B. rapa* line. One addition line was found to contain, on the same addition chromosome, independent genes for erucic acid biosynthesis, white flower colour and an isoenzyme of leucine aminopeptidase. Again, intergenomic recombination was frequently observed in the monosomic addition line and resulted in the introgression of one or two loci from the alien chromosome into the *B. rapa* genome. Another addition line was found to contain a gene controlling seed colour in a region where a homoeologous recombination or chromosomal deletion was detectable (Chen *et al.* 1997b). Disomic *B. napus* + *B. nigra* addition lines were described by Chèvre *et al.* (1991) and Struss *et al.* (1991), respectively. A number of other studies have reported individual addition or substitution lines between *B. napus* and related genera that have been generated in an effort to introgress specific traits into oilseed rape (e.g. Sjödin & Glimelius 1989, Skarzhinskaya *et al.* 1998, Snowdon *et al.* 2000, Voss *et al.* 2000, Peterka *et al.* 2004). In comparison to cereals, however, where chromosome additions, translocations and introgressions are well characterized and can be closely integrated with genetic maps, such detailed cytogenetic information is not yet available for *Brassica* materials and broad, well-defined cytological stocks are not available.

The use of FISH techniques offers new potential not only for more reliable chromosome identification in *Brassica*, but also in terms of potential information regarding the integration of genetic and physical maps, for ordering molecular markers and measuring physical genome distances, and for structural and functional chromosome analyses. Methods for the accurate *in-situ* localization of repetitive DNA sequences at chromosomal sub-arm level, particularly repetitive DNA sequences, have enabled a considerably more accurate identification of chromosomes and the elucidation of karyotypes for diploid and amphidiploid *Brassica* species (e.g. Figure 2, see also Armstrong *et al.* 1998, Fukui *et al.* 1998, Snowdon *et al.* 2002, Maluszynska & Hasterok 2005). FISH hybridization of BAC clones to *B. oleracea* (Howell *et al.* 2002) and *B. rapa* chromosomes (Jackson *et al.* 2000) represents a first step towards integration of physical and genetic maps with the karyograms of the diploid species and their amphidiploid hybrid *B. napus*. The first integration of a complete *Brassica* genetic map with the corresponding mitotic chromosome karyotype was achieved by Howell *et al.* (2002), who assigned all nine linkage groups of a *B. oleracea* genetic map to the nine mitotic metaphase chromosomes using FISH. The probes were

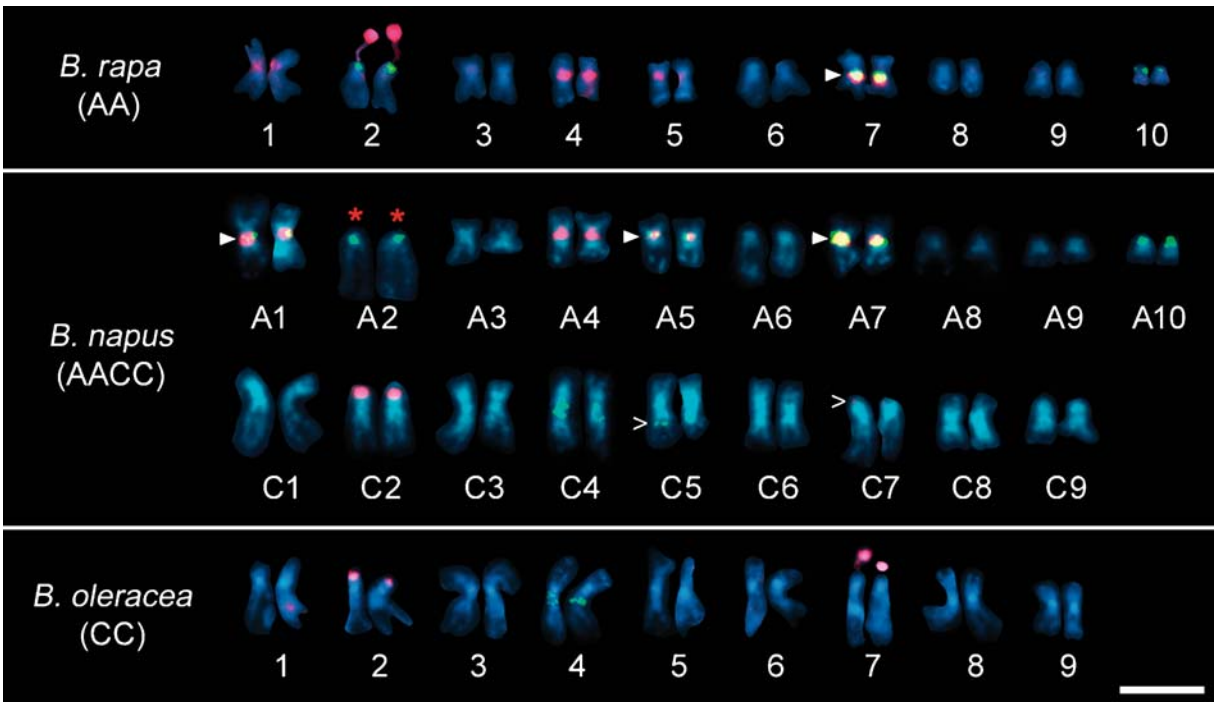

Figure 2. Karyotypes based on fluorescence *in-situ* hybridization patterns with 5S (green) and 25S (red) rDNA probes and DAPI staining (blue), for *Brassica rapa*, *B. oleracea* and their amphidiploid *B. napus*. Closed arrowheads indicate co-localization of 5S and 25S loci, whereas open arrowheads show a small 5S locus and a small 25S locus on *B. napus* chromosomes C5 and C7, respectively. The red asterisks represent the position of a large 25S rDNA locus located on the satellite of *B. napus* chromosome A2, which in this spread was lost during chromosome preparation. The *B. napus* karyotype is divided into two sets of chromosomes with differing chromatin condensation patterns resembling, respectively, those of *B. rapa* (**A**) and *B. oleracea* (**C**). Each *B. napus* chromosome is aligned and numbered in accordance with its putative homologue in the *B. rapa* or *B. oleracea* genome. Image from Snowdon *et al*. (2002).

mainly *B. oleracea* BAC clones that could be assigned to linkage map positions through development of locus-specific PCR assays. A total of 22 probes representing 19 loci were used to integrate the cytogenetic and genetic linkage maps and compare the orientation of the chromosomes with their respective linkage groups. Such cytogenetic maps represent an important new resource for locating genomic sequences with unknown genetic map positions and to analyse the relationships between genetic and cytogenetic maps. A similar strategy based on FISH localization of genetically and physically mapped BAC clones is currently being followed for integration of the genetic, physical and cytological maps of *B. rapa* within the *B. rapa* whole-genome sequencing project (Yang *et al*. 2006). Hybridization of locus-specific probes to meiotic pachytene chromosomes can considerably increase the resolution of the cytological mapping. Ziolkowski & Sadowski (2002) demonstrated the application of this technique in *B.* oleracea using ribosomal DNA probes and BAC clones from *A. thaliana*. Because of the considerably higher resolution than is possible in *Brassica* mitotic chromosomes, cytological mapping to pachytene bivalents offers the opportunity to potentially compare physical and genetic distances among selected markers. Together with synteny data and comparative genome annotations this technique should play a major role in increasing the feasibility and success of map-based gene cloning in *Brassica* crops.

Repetitive sequences and genome evolution

Centromeric regions of *B. rapa and B. oleracea* chromosomes contain two divergent 176 bp centromeric repeat sequences (Hallden *et al*. 1987, Iwabuchi *et al*. 1991, Sibson *et al*. 1991, Xia *et al*. 1993) with a degree of specificity for different chromosomes within the diploid genomes (Harrison & Heslop-Harrison 1995). Lim *et al*. (2005) identified two classes of these 176 bp

repeat sequences in *B. rapa*. Similar 176 bp repeats with more than 90% sequence similarity are also predominant in distant crucifer relatives including *Diplotaxis* (Harbinder & Lakshmikumaran 1990), although less than 80% sequence identity is found in the corresponding repeats in *Sinapis* and *Raphanus* (Capesius 1983, Grellet *et al*. 1986, Sibson *et al*. 1991, Xia *et al*. 1993). In *B. nigra* two different tandem repeat sequences specific to the B genome were identified by Gupta *et al*. (1992), one of which is unrelated to either A or C genome sequences. *In-situ* hybridization to *B. juncea* metaphase chromosomes showed that this sequence is located at all B genome centromeres and enables B genome chromosomes to be distinguished from A and C genome chromosomes by GISH (Schelfhout *et al*. 2004).

Detailed analysis of sequence diversity in repetitive sequences can offer interesting insight into the evolutionary relationships among and between *Brassica* species and their crucifer relatives, and may give useful information regarding the potential for intergeneric gene transfer to brassicas from more distant relatives via homoeologous recombination (see below). For example, Lenoir *et al*. (1997) and Tatout *et al*. (1999) studied the molecular phylogeny of short interspersed element (SINE) Sl retrotransposons in numerous different *Brassica* diploids and related crucifer species. Even among closely related species significant sequence divergence was found that appears to have arisen from sequence-specific surges in S1 amplification (Lenoir *et al*. 1997). SINE amplification near or in genes can cause post-transcriptional regulation or even give rise to novel gene domains (Bennetzen 2000). Furthermore, the amplification of SINES can result in unequal homologous recombination between cis-Sl elements (Lenoir *et al*. 1997), potentially leading to chromosomal rearangements that can be a driving factor in speciation. The genomic organization and diversity of retrotransposons in *Brassica* diploids and allotetraploids was investigated in detail by Alix *et al*. (2004, 2005). Sequence analysis revealed distinct Ty1/copia and LINE-like elements, whereby the latter were present at only very low copy numbers in the genomes investigated. A third clade could be subdivided into Ty3/*gypsy*, Athila and virus-like branches. Phylogenies based on the sequence comparisons showed no correlation with the known genome relationships among the species of the *Brassica* triangle, indicating that members of the element families were present in a common ancestor (Alix *et al*. 2004). On the other hand some sub-families appeared to be amplified in individual species. Fluorescent *in-situ* hybridization of representative reverse transcriptase domains from the different retroelements to *B. oleracea* (Alix *et al*. 2005) showed characteristic chromosomal distributions for each group, suggesting that the different retrotransposons have preferential amplification sites and possibly different insertion/excision control mechanisms.

In order to study evolutionary relationships among different members of the Brassicaceae, Lysak *et al*. (2005) performed chromosome painting with an almost 9 Mbp long BAC contig from *A. thaliana* chromosome 4 to trace homoeologous chromosome regions in 21 different species of the family Brassicaceae, including species representing *Brassica* crops. Homoeologues were identified in all three *Brassica* amphidiploids in six copies corresponding to the *Arabidopsis* segment, whereby rearrangements caused by inversions or translocations could be observed in the homoeologous copies within the *Brassica* genomes. Phylogenetic studies based on comparative sequencing of conserved genes indicated that species containing three or six copy pairs descended from a common hexaploid ancestor with basic genomes similar to that of *Arabidopsis*. The presumed hexaploidization event was shown to have occurred after the *Arabidopsis–Brassiceae* split, between 7.9 and 14.6 Mya.

Interspecific hybridization and homoeologous chromosome pairing

One strategy to broaden the genetic basis of oilseed rape breeding material is the production of resynthesized rapeseed by crossing the original ancestors, *B. oleracea* and *B. rapa*. This has the potential not only to increase genetic variability with a view to hybrid breeding, but also to broaden the genetic base with respect to pest and disease resistances in the narrow gene pool of modern oilseed rape. Interspecific and intergeneric incompatibility barriers can be successfully overcome in crosses between *Brassica* crop species and their relatives by embryo rescue techniques or protoplast fusion. In some cases resynthesized rape forms subjected to backcrossing with elite breeding material have resulted in successful release of cultivars carrying novel resistance genes from the diploid donor species. Synthetic *Brassica* polyploids can also offer important insights into the genetic and

epigenetic changes that occur during polyploidization. For example, Song *et al*. (1995) reported extensive and rapid genome change in the form of loss or gain of restriction fragments in early generations of polyploids generated from interspecific crosses among the A, B and C genome diploid species. The alterations also revealed divergence among different genotypes with the same parental origin, demonstrating the ability of *Brassica* polyploids to generate novel genetic diversity in only a few generations. By analysing the methylation status of a large number of isogenic resynthesized rapeseed lines and their two common parental genotypes, Lukens *et al*. (2006) demonstrated that this high degree of divergence is caused by extensive alteration in DNA methylation patterns after polyploidization, whereas few deletions or insertions could be detected. In other words, polyploidy in *B*. *napus* is accompanied by considerable regulation of epigenetic changes rather than by major genetic changes.

The observed variability in the degree of homoeologous pairing in different *B*. *napus* haploids demonstrates the potential for extensive homoeologous recombination after amphipolyploidization among *Brassica* diploids. By analysing different *B*. *napus* doubled-haploid (DH) mapping populations with co-dominant RFLP markers, Udall *et al*. (2005) identified chromosomal rearrangements that could be classified into *de-novo* homoeologous non-reciprocal translocations (HNRT), pre-existing HNRT and homoeologous reciprocal translocations (HRT). A total of 99 *de-novo* HNRT were identified as duplications of particular chromosomal regions in a small number of lines accompanied by a loss of the corresponding homoeologous region. These *de-novo* HNRT were more prevalent in a population that had a resynthesized *B*. *napus* as a parent, indicating a higher rate of homoeologous recombination in early generations of new polyploids. Nine pre-existing HNRT were identified by fragment duplication or fragment loss in DH lines from three populations involving natural *B*. *napus* parents, indicating a segregation of HNRT that already existed in one of the parents. This study suggests that chromosomal rearrangements caused by homoeologous recombination are apparently widespread in *B*. *napus*, and this phenomenon can be speculated to be also prevalent in the other *Brassica* amphidiploids *B*. *juncea* and *B*. *carinata*. One of the most interesting aspects of this from a crop-breeding perspective is that such non-reciprocal translocations can cause changes in allele dosage, which in some cases have been shown to have a demonstrable effect on the additive expression of important agronomic traits such as pathogen resistance (Zhao *et al*. 2005).

Other *Brassica* species and even less closely related genera are also important as potential sources of disease resistance for oilseed rape breeding. A prime example for this is the use of interspecific and intergeneric hybrids as a source for new resistance against blackleg (*Leptosphaeria maculans*), the most serious disease of oilseed rape worldwide. The B-genome

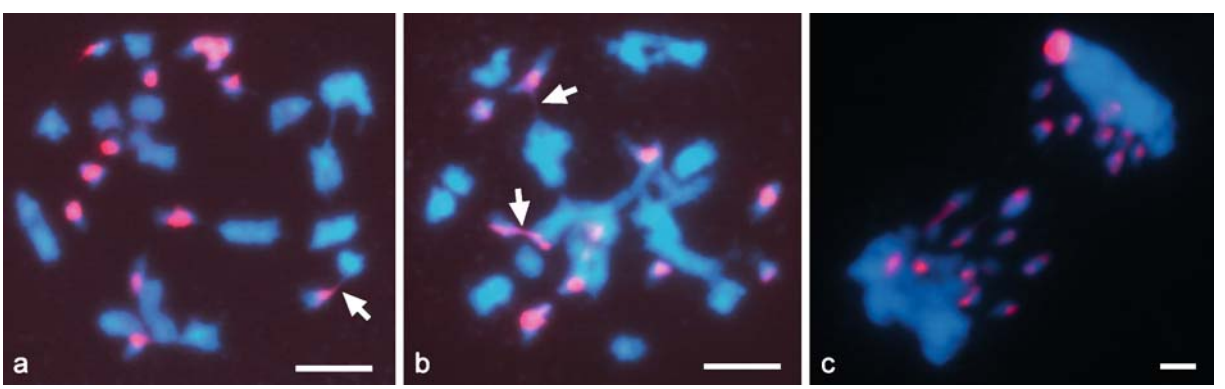

Figure 3. Detection of interspecific homoeologous recombination events (arrows) in sexual progeny of asymmetric *Brassica napus* × *C*. *abyssinica* hybrids (from Wang *et al*. 2005, used with permission from NRC Research Press, Canada) by GISH analysis in meiotic metaphase I. *Crambe* chromosomes are labelled red with Cy3, whereas non-labelled *B*. *napus* chromosomes are stained blue with DAPI. (**a, b**) In metaphase I Cy3-labelled chromatin strands (arrows) can be observed between *C*. *abyssinica* univalents and *B*. *napus* bivalents. (**c**) At early anaphase I a late disjunction of *C*. *abyssinica* chromosomes is observed; however, introgressions on *B*. *napus* chromosomes can still potentially segregate into the daughter cells. Scale bars = 10 μm.

species *B. nigra* and *B. juncea* exhibit strong resistance against blackleg and have been extensively used in an attempt to introgress resistance genes into oilseed rape (Roy 1978, Sacristán & Gerdemann 1986, Sjödin & Glimelius 1989, Chèvre *et al.* 1996, Struss *et al.* 1996). Similarly, related genera including *Sinapis* are also potential donors for transfer of blackleg resistance into *B. napus* via homoeologous recombination (Snowdon *et al.* 2000). Even distantly related crucifer genera can potentially be used for introgression of genes of interest into *Brassica* crop species via sexual or somatic hybridization, for example *Raphanus* (Voss *et al.* 2000, Benabdelmouna *et al.* 2003, Peterka *et al.* 2004), *Lesquerella* (Skarzhinskaya *et al.* 1998) or *Eruca* (Fahleson *et al.* 1997). The primary (intraspecific), secondary (intrageneric) and tertiary (intergeneric) gene pools for *Brassica* vegetable and oilseed crops contain an enormous diversity of species, and hundreds of examples have shown the potential for interspecific or intergeneric hybridizations with *Brassica* crop plants (see Warwick *et al.* 2000 for detailed information). As an example, Wang *et al.* (2005) demonstrated the successful transfer of new allelic variants of the *FAE1* gene controlling erucic acid biosynthesis from the distantly related, triploid oilseed plant *Crambe abyssinica* (n = 3x = 45) into *B.* napus by somatic hybridization. Via genomic *in-situ* hybridization (GISH) to meiotic chromosomes it was possible to detect the occurrence of homoeologous pairing between *C. abyssinica* and *B. napus* chromosomes (Figure 3), demonstrating that homoeologous recombination can potentially occur even between chromosomes from distantly related genera. On the other hand, homoeologous introgression of genes of interest even from closely related species of the same genera is not always successful, underlining the fact that pairing does not depend simply on genome homoeology.

The prerequisite for interspecific or intergeneric gene transfer is a mechanism for control of homoeologous chromosome pairing. In wheat the pairing regulation locus *Ph1* (Riley & Chapman 1958) suppresses homoeologous pairing. The successful utilization of alien introgressions in wheat breeding is particularly due to suppression of the effect of this locus. In *B. napus* the chromosomes of the A and C genomes each pair in a bivalent, disomic manner, suggesting a similar suppression of homoeologous pairing in favour of homologous pairing. On the other hand, haploid *B. napus* plants are known to show considerable genetic variation in the degree of chromosome pairing, with common formation of chiasmata among rod-shaped and ring-shaped bivalents along with multivalents (Attia & Röbbelen 1986). By investigating segregation for pairing behaviour at meiotic metaphase I in haploids produced from F1 hybrids between high-pairing and low-pairing lines, Jenczewski *et al.* (2003) found that suppression of homoeologous chromosome pairing in *B. napus* is, as in wheat, largely controlled by a single major gene, coined *PrBn* (*Pairing regulator in Brassica napus*). According to Jenczewski *et al.* (2003), however, the mode of action of *PrBn* appears to be quite different from that of the wheat *Ph1* gene. In particular, *Ph1* suppresses homoeologous pairing at both the haploid and diploid stage, but even *B. napus* haploids with a high level of homoeologous pairing show regular disomic inheritance and normal bivalent chro-

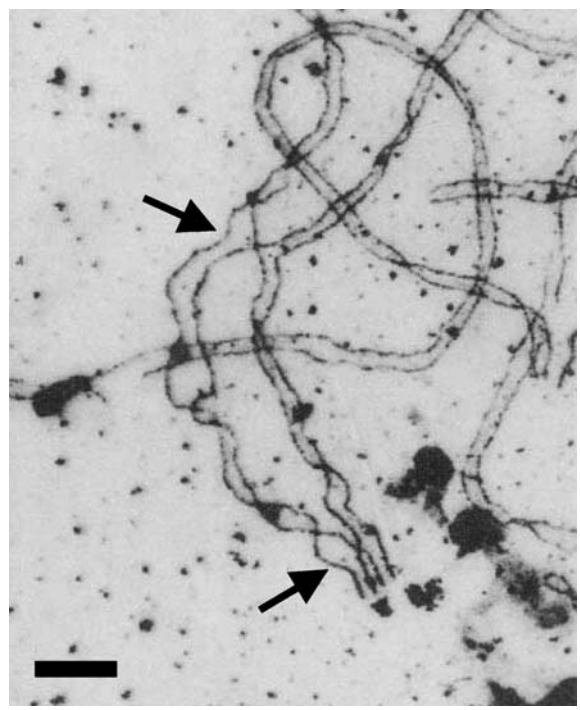

Figure 4. Cytological evidence for a reciprocal translocation between *Brassica napus* linkage groups N7 and N16 (from Fig. 3 in Osborn *et al.* 2003 , kindly provided by John Parker and used with permission from the Genetics Society of America). The image shows meiotic chromosome behaviour in part of a synaptonemal complex spread from an F1 hybrid showing quadrivalent formation between two chromosome pairs. Two synapsis exchange points are indicated by arrows. Comparative genetic mapping of N16 and analysis of marker segregation in two different crosses revealed a homoeologous reciprocal translocation with N7. Scale bar = 2 μm.

mosomes in their diploid form. Furthermore, almost no natural polymorphism has been discovered for *Ph1* in wheat, whereas allelic variability in *PrBn* is clearly detected among high-pairing and low-pairing *B. napus* phenotypes. In other words although *Ph1* is vital for chromosome fertility and stability in wheat (Sánchez-Morán *et al*. 2001), in *B. napus* the suppression of homoeologous pairing by *PrBn* is not essential. Map-based cloning on *PrBn* has the potential to offer considerable new insight into the genetic mechanisms involved in homoeologous pairing in polyploid plant species.

Homoeologous chromosomal translocations can be readily detected in *B. napus* using co-dominant marker systems for which homoeologous loci are assigned to different linkage groups in the genetic map. In this manner Osborn *et al*. (2003) detected a homoeologous reciprocal translocation between *B. napus* linkage groups N7 and N16 using segregating populations of doubled haploid lines. Pairs of homoeologous RFLP loci from the two chromosomes had identical alleles in the parental lines in regions expected to be homoeologous, and an interstitial reciprocal translocation was confirmed by cytological analysis of synaptomenal complexes in F1 hybrids (Figure 4). Although this translocation included approximately one-third of the physical length of the N7 and N16 chromosomes, few recombination events within the region were recovered in the progenies of the hybrids. Higher seed yields in offspring exhibiting the parental configurations of the rearrangement in segregating progenies point to a possible selective advantage of allopolyploidy through the fixation of intergenomic heterozygosity.

Outlook

The growing collection of physical genome resources and genomic sequence data for *Brassica* crop species, combined with the expansive genomic and transcriptomic data from the model crucifer *Arabidopsis*, today enable increasingly detailed annotation and navigation between the *Arabidopsis* and *Brassica* genomes. With the expected completion of the first complete genome sequence of a *Brassica* crop plant in the coming years, the opportunity will be greater than ever to utilize the close relationship between the model plant and its nearest crop relatives, the brassicas, for applied genomics and breeding biotechnology. Of particular interest will be the possibility this allows us to gain considerably more insight into the genetic functionality underlying intricate biochemical pathways and metabolic expression patterns involved in important *Brassica* vegetable and seed compounds – based on primary knowledge from *Arabidopsis* – and perhaps to dissect extremely complex traits such as yield or heterosis into genetic components that are manageable and usable from a practical breeding perspective. Finally, identification of the genetic factors controlling homoeologous recombination in *Brassica* chromosomes may in future enable more flexibility in the targeted introgression of novel germplasm into the gene pools of the crop brassicas, opening the way for a broadening in the genetic diversity that is available for breeding towards a sustainable production in future.

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