Fishing for physical genome information – *Brassica* cytogenetics past, present and future

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ABSTRACT

Until the last decade the major tool for cytogenetic analyses in *Brassica* was light microscopy to investigate meiotic chromosome pairing, and in fact in many cases this still remains the most powerful tool available to investigate chromosome and genome homologies and interactions. Investigations of *Brassica* mitotic chromosomes are limited by their small size and scarcity of useful cytogenetic landmarks, and because of this it is difficult or impossible to identify *Brassica* chromosomes using traditional cytological techniques. Today, fluorescence in situ hybridisation (FISH) techniques offer the potential not only for more reliable chromosome identification in *Brassica*, but also in terms of the information they might be able to offer regarding the integration of genetic and physical maps, for ordering molecular markers and measuring physical distances, and for structural and functional chromosome analysis. This review describes the most important past work in classical *Brassica* cytogenetics, outlines current results and looks at potential future applications of molecular cytogenetic techniques in *Brassica* genome reseach and breeding.

Key words: *Brassica* – cytogenetics – chromosomes – FISH – GISH

INTRODUCTION

After Morinaga and U discovered through cytogenetic studies in the early 1930's that amphidiploid *Brassica* species originate from diploid progenitors and contain the complete chromosome sets of their parental species, chromosome studies came to play a leading role in genome analysis among the *Brassicaceae*. The age of classical cytogenetics has however been largely superseded by the implementation of DNA techniques during the past few decades, and the difficulties associated with *Brassica* chromosomes as a cytological object – in particular their small size and lack of distinctive cytological landmarks – have made *Brassica* cytogenetics a rare art amongst the proliferating molecular marker technologies. For many years little more could be achieved than simple chromosome counts or meiotic studies of the offspring from interspecific or intergeneric crosses, giving insight into genome homologies amongst the various *Brassica* relatives. In recent years, however, advances in the molecular cytogenetic technique of fluorescence *in situ* hybridisation (FISH), which enables the direct chromosomal localisation of labelled DNA probes, have enabled a resurgence of cytogenetic analyses in plant genome research and molecular breeding.

BRASSICA CYTOGENETICS IN THE PAST

The field of "Brassica cytogenetics" dates back to the early decades of the 20th century, when a number of predominantly Asian scientists began with detailed investigations of chromosome numbers and chromosome pairing in some of the important crucifer species. The first major achievement was the publication of the chromosome number for *Brassica rapa* by Takamine in 1916, followed eight years later by the synthesis and analysis of *Raphanobrassica* by Karpechenko (1927) and the experiments of Morinaga, who began working intensively on interspecific hybrids during the 1920s and investigating chromosome pairing and homology in detail. It was during this period that others began to publish surveys of chromosome counts for large numbers of crucifer species – in particular Manton (1932), who was one of the pioneers in this area – and to investigate the somatic chromosomes in more detail. However it was the work of Morinaga (1929) and U (1935) that gave rise to another generation of researchers who began to look more deeply into genome homology in the *Brassicacaeae*. The development of ovary culture and embryo rescue techniques in the 1950s enabled enormous progress in the study of genome homologies based on chromosome pairing analyses. Additionally, technological advances in optical equipment and microscopy brought a great improvement in cytological techniques in general, and based on these techniques Röbbelen (1960) was the first to publish detailed cytological descriptions of *Brassica* somatic chromosome structure.

From a cytogenetics perspective the period between the 1960s and the end of the 80s was dominated by an intensive effort to collect and classify botanical representatives of the crucifer tribe and to study the evolutionary and genomic relationships among this array of species. One of the major personalities in this movement was Harberd (1972), whose study of chromosome pairing among a huge number of species eventually led to the classification of cytodemes describing homologous genomes. We also know now that there is extensive genome homology or homoeology throughout the entire *Brassica* coenospecies, and from a plant breeding perspective in particular it has become well-known that we consequently have the possibility to broaden gene pools for the introgression of novel genes or alleles, well beyond the species boundary (Fig 1).

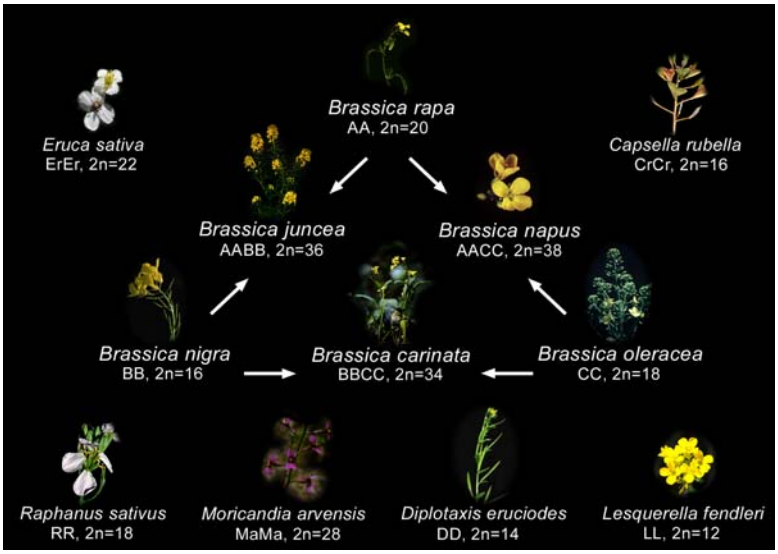

Fig 1. Cytogenetic analyses of interspecific hybrids played a major role in the elucidation of the genetic relationships among the species of the *Brassica* triangle and their near relatives.

CURRENT TECHNOLOGIES

Until the last decade the major tool for cytogenetic analyses in *Brassica* was light microscopy to investigate meiotic chromosome pairing, and in fact in many cases this still remains the most powerful tool available to investigate chromosome and genome homologies and interactions. Unfortunately investigations of *Brassica* mitotic chromosomes are limited by their small size and scarcity of useful cytogenetic landmarks, and because of this it is difficult or impossible to identify *Brassica* chromosomes using traditional cytological techniques.

On the other hand, fluorescence in situ hybridisation (FISH) techniques offer the potential not only for more reliable chromosome identification in *Brassica*, but also in terms of the information they might be able to offer regarding the integration of genetic and physical maps (see Howell et al. 2002), for ordering molecular markers and measuring physical distances, and for structural and functional chromosome analysis.

We have developed FISH methods for the accurate localisation of repetitive DNA sequences at chromosomal sub-arm level in *Brassica* species, allowing more reliable chromosome identification (Fig 2; Snowdon et al. 2002) and giving new information on genome structure and evolution. In addition we apply genomic *in situ* hybridisation (GISH) for identification and characterisation of parental genome components in oilseed rape (*B. napus*) hybrids (Fig 3; Snowdon et al. 2000). Physical localisation of transgene inserts can provide information on insert location and copy number that cannot always be obtained by molecular genetic analysis. Furthermore, high-resolution FISH can give important information about ordering and physical distances between molecular markers, which are both vital considerations for physical mapping and positional cloning.

OUTLOOK

New molecular cytogenetic and sequence information describing centromere structure and function in Arabidopsis is the first step in the development of artificial plant chromosomes, which – due their potential for introgression of entire gene suites in a stable manner without disrupting the existing genome – may represent the genetic transformation vectors of the future. Continuing developments in molecular cytogenetic techniques will doubtless enable further integration of *Brassica* physical and genetic maps in future.

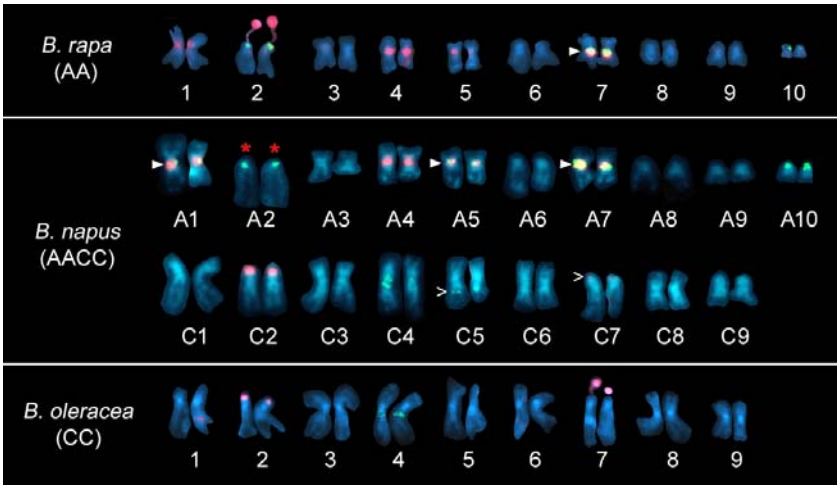

Fig 2: Karyotypes based on fluorescence *in situ* hybridisation patterns with 5S (green) and 25S (red) rDNA probes and DAPI staining (blue), for *Brassica rapa* L., *B. oleracea* L. and *B. napus* L. The *B. napus* karyotype is divided into two sets of chromosomes with differing chromatin condensation 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 (for more information see Snowdon et al. 2002).

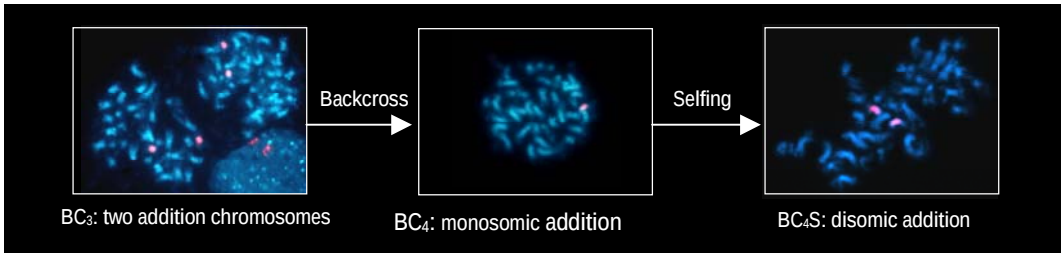

Fig 3: Example of genomic *in situ* hybridisation (GISH) for selection of backcross offspring originating from interspecific hybrids. The *B. napus* chromosomes are stained blue, while the red signals show *Raphanus sativus* addition chromosomes carrying gene(s) for nematode resistance.

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