

O16

**Quirks in chromosome behavior in wheat: a general rule or an exception?**

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At the present level of technology, microscopic observations provide only a coarse resolution of various aspects of chromosome behavior but they still offer some interesting insights. New genomics data and cytological observations point to a need for new definitions of old functions and structures. Particularly urgent is a new definition of the centromere. The pattern of centric fission in wheat implies that the kinetic function of the centromere, located in the primary constriction, is separated from the cohesion function, and the latter spreads out considerable distances from the kinetochore. Adhesion of sisters does not appear to be an inherent function of a specific chromosome region: it seems to radiate out of the kinetochore region wherever it happens to be located. In a chromosome with an inverted arm, sisters adhere close to the kinetochore region and not telomere; translocation of the kinetochore region to the middle of an arm brings the sister adhesion with it. It is well established that crossing over in the Triticeae concentrates in the distal regions of chromosomes. This is thought to be a direct consequence of the terminal initiation of pairing and synapsis and strong positive chiasma interference. It can also be thought of as a centromeric effect; specifically, interference from sister chromatid adhesion. Attempts at manipulation of the cross-over pattern seemed to confirm these conclusions. However, in an inverted arm chiasmata are not formed by the telomere; they are now formed in the immediate vicinity of the kinetochore, demonstrating that the proximal half of a normal arm is structurally unable to cross over; the distal part will crossover even when next to the kinetochore. If the proximal halves of all Triticeae chromosome arms are inherently incapable of crossing over, some planned research may have to devise strategies independent of crossing over. The pattern of synapsis in the inverted arm also suggests that in addition to the leptotene bouquet, another mechanism must exist for the initiation of homologous pairing. Whether these are wheat peculiarities or a general pattern for all Triticeae is far from clear at this point.

O17

**Chromosome re-assembly for wheat improvement**

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Undomesticated species from wheat's tertiary gene pool have been the source of numerous beneficial traits for wheat breeding when introduced as chromosomal translocations. However, the presence of deleterious traits, have constrained their extensive use in food production. Using the *ph1b* mutant system, a precise crossing program, and a strong selection with molecular markers and bioassays, we demonstrated that it is possible to recombine two different alien translocations and recover stable trigonomic chromosomes with useful combinations of genes. Recombinant alien translocations were selected with new combinations of genes for disease resistance derived from *Thinopyrum intermedium* and *Th. ponticum* onto wheat chromosome arm 7DL. The integrity and stability of the newly assembled chimeric chromosomes were confirmed in F2 and F3 derived populations segregating for Leaf rust (*Lr19*) and BYDV (*Bdv2*) resistance. In a similar way we are attempting to recombine another BYDV resistance (*Bdv4*) from *Th. intermedium* chromosome 2Ai-2 with rust resistances present on 2D translocations derived from S and M genomes of *Aegilops speltoides* (SS), *Ae. kotschy* (UUSS) and *Triticum comosum* (MM).

O18

**Recombination analysis on bread wheat chromosome 3B**

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Recombination plays a major role in determining the structure and evolution of eucaryotes. A better understanding of its underlying mechanisms can help improving the genetic basis of crop species and controlling more efficiently the introgression of favourable alleles in breeding programs. In wheat, it was shown that recombination increases gradually with the distance from the centromere but the resolution of the analyses remained limited until now. Here, we undertook a more precise characterization of the recombination pattern along chromosome 3B of bread wheat by comparing a very dense genetic map (102 markers) with the cytogenetic map (16 deletion bins). This showed that 90 % of the recombination occurs in the distal 40% of the chromosome. Moreover a clear recombination gradient was observed from the centromere towards the telomere, with a strong decrease at the telomeric end on the short arm. We also compared female and male recombination. At the whole chromosome level, no differences were observed but significant changes were found in specific regions at the distal part of the long arm. The physical map of the hexaploid wheat chromosome 3B (cv. Chinese Spring) was also exploited to study the relationships between genetic and physical distances at the level of megabase-sized sequenced regions. Two contigs of 1.5 and 3Mb that are located in close vicinity at the telomeric end of the short arm were used for these studies. Recombinants were isolated by screening a large F2 segregating population (1,800 individuals) derived from a cross between Renan and Chinese Spring with SSR markers derived from the ends of the contig sequences. Recombination breakpoints were identified using more than 100 markers distributed within the sequences and the relationship between recombination and the presence of genes, TEs and other sequence features was studied in detail. The results of these analyses will be presented and discussed.

O19

**It's not size but coordination that matters**

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Despite possessing multiple sets of related chromosomes, hexaploid and tetraploid wheat both behave as diploids at meiosis. Correct pairing of homologous chromosomes is controlled by the *Ph1* locus. Early studies made two distinct *Ph1* observations. Firstly *Ph1* altered the stringency at which recombination occurred between related chromosomes and secondly that B chromosomes or heterochromatin could compensate for lack of *Ph1* in hybrids in preventing recombination between related chromosomes. Later maize studies revealed that B chromosomes induce more asynchronous replication of related heterochromatin. Using cell biological approaches, we have explored further this observation. At the onset of meiosis, heterochromatin is remodelled prior to the chromosomes pairing. The subtelomeric heterochromatin on homologues needs to be identical or near identical to remodel with *Ph1*. Failure to remodel leads to failure to pair and recombine. In contrast at the onset of meiosis without *Ph1*, all related heterochromatin can remodel at the same time irrespective of their level of homology. *Ph1* also affects centromeric heterochromatin behaviour when these sites pair during replication. *Ph1* reduces synchronisation of related heterochromatin on all chromosomes to just homologues. The *Ph1* locus has now been defined to a cluster of *Cdk2-like* (CDK2L) genes containing a segment of heterochromatin. CDK2L shows homology to *Cdk2* in mammals which remodels chromatin for replication and recruits the recombinational machinery to double strand breaks during

meiosis. Disruption of its activity results in non-homologous synapsis during meiosis. Our working hypothesis is that CDK2L is functional similar to Cdk2 and with the presence of the heterochromatin explains the *Ph1* phenotypes observed.

**O20**

**Development and mapping of SSR markers for 1RS**

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The development of 74 microsatellite marker primer pairs yielding 76 polymorphic loci, specific for the short arm of rye chromosome 1R (1RS) with an average polymorphism information content (PIC-value) of 0.55 is reported. Four libraries enriched for microsatellite motifs AG, AAG, AC and AAC were constructed from DNA of flow-sorted 1RS chromosomes and 1290 clones were sequenced. Additionally, 2778 BAC-end-sequences from a 1RS specific BAC library were used for microsatellite screening and marker development. From 724 designed primer pairs, 119 produced 1RS specific bands and 74 of them showed polymorphism in a set of ten rye genotypes. It is shown that this high attrition rate was due to the highly repetitive nature of the rye genome consisting of a large number of transposable elements. The 76 polymorphic loci were mapped physically into 12 chromosomal regions (bins) on 1RS using 1RS deletion chromosomes added to wheat. Genetic mapping was done by using two F2 mapping populations with 96 genotypes in each, developed from three rye inbred lines. Results of genetic mapping will be presented.

**O21**

**Dissecting the hexaploid wheat genome by chromosome sorting**

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The presence of three homoeologous genomes and large genome size (1C~17,000 Mbp) hamper physical mapping and positional cloning in hexaploid wheat (*Triticum aestivum* L., 2n=6x=42). An attractive approach to reduce the complexity of these accomplishments is to dissect the genome to smaller parts such as chromosomes and chromosome arms. We have shown previously that laser flow cytometry is a suitable method to achieve this goal in a number of species. This approach involves a preparation of suspensions of intact mitotic chromosomes. The chromosomes in suspension are stained by a DNA-specific fluorochrome and classified according to relative fluorescence intensity. Any chromosome, which differs in relative DNA content from other chromosomes, can be discriminated and sorted at high speed. However, due to small differences in size among the wheat chromosomes, only the largest chromosome (3B) can be resolved and sorted from standard wheat lines. Analysis of several double ditelosomic (dDt) lines indicated that they could be used to isolate short and long arms of individual chromosomes. In this work we screened a full set of dDt lines and established that most of the 42 wheat chromosome arms could be sorted. The remaining arms could be sorted from isochromosome lines. Thus, chromosome sorting from cytogenetic stocks offers a powerful tool to dissect the wheat genome to fractions representing only 1 - 3% of the total. The opportunity to divide the complex genome into manageable portions helps to structure an international collaboration on wheat genome sequencing. This work was supported by the Czech Science Foundation (521/06/1723, 521/05/H013) and Ministry of Education, Youth and Sports of the Czech Republic (LC06004).