TRANSCRIPTIONAL ANALYSIS OF RYE B CHROMOSOMES

Dissertation

zur Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. nat.)

vorgelegt der

Naturwissenschaftlichen Fakultät
Biowissenschaften
der Martin-Luther-Universität Halle-Wittenberg

von Frau Mariana Carchilan
geb. am: 23/10/1977 in: Nisporeni, Republik Moldau

Gutachter

1. Herr Prof. Dr. Gunter Reuter, Martin-Luther-Universität, Halle-Wittenberg, Germany

2. Herr Prof. Dr. Neil R. Jones, University of Wales, Aberystwyth, UK

3. Herr Prof. Dr. Veit Schubert, IPK, Gatersleben, Germany

Verteidigungsdatum: 03.07.2008

HALLE (SAALE) 2008

urn:nbn:de:gbv:3-000014074
[http://nbn-resolving.de/urn/resolver.pl?urn=nbn%3Ade%3Agbv%3A3-000014074]

ACKNOWLEDGEMENTS

This work was performed from October 2004 till December 2007 at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben. Since October 2004 till July 2005 it was funded by German Academic Exchange Service (DAAD - Deutscher Akademischer Austausch Dienst) afterwards the financial support from IPK made possible the accomplishment of the research.

First of all I would like to express my gratitude to my supervisor Dr. Andreas Houben, the head of Chromosome Structure and Function group, for giving me the opportunity to work in his team, for continuous support and guidance, huge inspiration, and permanent encouragement.

Many thanks to Dr. Sabine Mikolajewski for shearing experience concerning cDNA-AFLP technique and Dr. Marion Röder for help in testing the rye lines with microsatellites markers to prove their isogenic nature.

I'm very thankful to Dr. Dmitri Demidov, Dr. Inna Lermontova, Dr. Margarida Rocheta, Dr. Kyotaka Nagaki for their helpful suggestions and fruitful discussions. I am also obliged to the technicians from our group Margit Hantschmann, Katrin Kumke and Oda Weiss for their perfect technical assistance.

Content

Abbreviations

bp	base pair
kb	kilo base
FISH	fluorescence *in situ* hybridization
DAPI	4',6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dTTP	2'-deoxythymidine 5'-triphosphate
RNA	ribonucleic acid
mRNA	messenger ribonucleic acid
ATP	adenosine 5'-triphosphate
CTP	cytidine 5'-triphosphate
GTP	guanosine 5'-triphosphate
UTP	uridine 5'-triphosphate
PCR	polymerase chain reaction
RT PCR	reverse transcription polymerase chain reaction
rDNA	ribosomal DNA
K4	Lysine 4
K9	Lysine 9
K20	Lysine 20
K27	Lysine 27
H3	Histone H3
H4	Histone H4
me1	monomethylated
me2	dimethylated
me3	trimethylated
EDTA	ethylenediaminotetra-acetic acid
Tris	tris-(hydroxymethyl)-aminomethan
NaCl	sodium chloride
HCl	hydrochloric acid
NaOH	sodium hydroxide
BSA	bovine serum albumin
PBS	phosphate-buffered saline
SDS	sodium dodecyl sulfate
SSC	sodium chloride sodium citrate
NEB2	New England Biolabs Buffer
min	minutes
cm	centimeters
ng	nanograms
ml	milliliters
µl	microliters
pmol	picomol
Mm	milimol
U	units
%	percent
RT	room temperature
nt	nucleotide

List of figures

List of tables

1. Introduction

1.1. B chromosomes

B chromosomes (Bs) are accessory chromosomes to the standard number of normal chromosomes (As). The main criteria which distinguish B chromosomes from normal chromosomes are: (i) they are dispensable and can be present or absent from individuals within a population; (ii) they do not pair or recombine with any members of the standard diploid (or polyploid) set of A chromosomes at meiosis; (iii) their inheritance is non-Mendelian and irregular (Jones and Houben, 2003).

1.2. Distribution of B chromosomes

B chromosomes are characterized in at least 1372 flowering plants (10-15% of studied plant species), 12 representing conifers the remaining 1360 left – angiosperms (Jones, 1982; Jones, 1995). A more recent investigation of 23,652 angiosperm species (which consist approximately 9% of total estimated 260,000 species) revealed 979 species as carriers of B chromosomes: 8% being represented by monocots and 3% by dicots (Levin et al., 2005).

To date the best characterized families regarding absence or presence of Bs are: Compositae from dicotyledons with 270 characterized species, Gramineae and Liliaceae from monocotyledons with 266 and 189 species, respectively.

In the animal kingdom, Bs were characterized in at least 263 species, mostly representing insects (Jones, 1982). Best characterized are in the group of arthropods, class Insecta, Orthoptera representing 92 species, Diptera – 42 and Coleoptera - 40 species. In mammals 55 out of 4629 species analyzed are carriers of B chromosomes, most representing rodents (Vujosevic and Blagojevic, 2004).

Pulse field gel electrophoresis, a method which allows karyotyping of chromosomes smaller than detectable by the means of light microscopy, permitted the characterization of chromosomes of fungi, which turned out to be also carriers of B chromosomes (reviewed in (Kistler and Miao, 1992; Covert, 1998)).

Within the same species from population to population the number of B chromosomes is different. In some species they vary even between the organs, for example in *Sorghum stipoideum* they are absent in stems and leaves but present in variable number in microsporocytes and tapetal cells (Wu, 1992). Absence of Bs from roots were determined in *Erianthus munja* and *E. ravennae* (Sreenivasan, 1981), *Aegilops speltoides* (Mendelson and

Zohary, 1972). In *Poa alpina* the Bs are present in primary roots and absent in adventory ones (Muntzing and Nygren, 1955). More examples are listed in (Jones, 1982) and http://www.bchromosomes.org/bdb/ (Jones and Diez, 2004). Due to their property to occur only in some individuals of a population it is possible that in some species Bs are not detected yet.

The distribution of Bs between sexes also differs from species to species. Mostly Bs occur in both sexes, however in some species the occurrence of Bs are higher in one of sexes. For example in *Astyanax scabripinnis* and in *Gastrotheca espeletia* Bs are more frequent in females than in males (Vicente et al., 1996; Schmid et al., 2002). In some species the Bs are present either in males only (eg. *Moenkhausia sanctaefilomenae* (Portela-Castro et al., 2000)) or females only (eg. *Astyanax scabripinnis paranae* (Maistro et al., 1992; Mizoguchi and Martins-Santos, 1997)).

Several factors were found to be associated with the distribution of Bs across species, namely: ploidy, genome size, breeding system, and in some mammals the A chromosome morphology (Palestis et al., 2004b; Palestis et al., 2004a; Trivers et al., 2004; Levin et al., 2005). The aforementioned factors contribute independently to the Bs occurrence. Increase of genome size correlates positively with occurrence of Bs, as reported for British flowering plant species (Palestis et al., 2004a; Trivers et al., 2004).

The analysis of 226 flowering plant species showed that Bs are predominantly present in outbreeding than in inbreeding species (Burt and Trivers, 1998). Inbreeding of *Secale cereale*, which is a outbreeder, leads to decrease in Bs' frequency (Muntzing, 1954). A similar behavior revealed the Bs in *S. vavilovii*, where they were introduced by hybridizing *S. cereale* possessing Bs with *S. vavilovii* which naturally lack Bs and backcrossing to *S. vavilovii* for six successive generations (Puertas et al., 1987).
The estimation of frequency of polyploidy among plants with Bs did not show a significant difference in comparison to the estimation among flowering plants (Jones, 1982; Jones, 1995; Trivers et al., 2004).

In mammals it was observed that Bs occur predominantly in species with mainly acrocentric A chromosomes (Palestis et al., 2004b). Study intensity in different species can influence the accuracy of association of Bs with all above mentioned factors (Palestis et al., 2004a).

Wild maize cultivars growing at higher altitudes exhibit a higher number of Bs (Lia et al., 2007). Rosato and colleagues (1998) also observed a positive correlation between the mean number of Bs per plant and altitude. In *Astyanax scabripinnis* fish, located at different altitudes along the same river, B chromosomes were found in high altitude populations and were absent in low altitude populations (Neo et al., 2000).

1.3. Size of B chromosomes

The size of B chromosomes differs from species to species. Generally Bs are smaller than A chromosomes, however B chromosomes bigger than As were detected in some species, e.g. the cyprinid fish *Alburnus alburnus* (Ziegler et al., 2003) and in neotropical fish *Astyanax scabripinnis paranae* (Maistro, 1992). In some species exist even different types of B chromosomes, for example in *B. dichromosomatica* large Bs and micro Bs can be observed (Carter and Smith-White, 1972). The marsupial frog *Gastrotheca espeletia* possesses three types of B chromosomes which differ among themselves in size and morphology (Schmid et al., 2002). Size variations of Bs were found as well in the grasshopper *Eyprepocnemis plorans* (Lopezleon et al., 1993).

1.4. DNA composition of B chromosomes

Earlier investigations on DNA composition of Bs were based on gradient density centrifugation and renaturation kinetics. In rye, the outcome of these experiments showed that the ratio and heterogeneity of repeats in DNA of B chromosomes do not differ from As. However, a slight increase in cytosine and guanine content was observed for DNA of B carrier plants (Rimpau and Flavell, 1975; Timmis et al., 1975). Boyant densities of DNA from plants with and without Bs in maize were found to be similar (Chilton and Mccarthy, 1973). Same results were reported in *Pseudococcus obscurus* (Klein and Eckhardt, 1976) and the grasshopper *Myrmeleotettix maculatus* (Gibson and Hewitt, 1970).

Later on, Amos and Dover (1981) introduced the restriction endonuclease digestion of genomic satellite DNA to characterize B chromosomes. To localize the distribution of the repeats on As and Bs they used in situ hybridization.

Molecular studies showed that in many species the B chromosomes contain sequences that originated from one or different A chromosomes (Page et al., 2001; Cheng and Lin, 2003; Bugrov et al., 2007). Mostly these are non-coding repetitive sequences or mobile elements like in *Crepis capillaris* (Jamilena et al., 1994; Jamilena et al., 1995), *Prochilodus lineatus* (de Jesus et al., 2003), *Nectria haematococca* (Enkerli et al., 1997), and *Drosophila subsilvestris* (Gutknecht et al., 1995). In maize, the sequence analysis revealed many highly repetitive sequences, including transposons, present on both A and B chromosomes, but enriched on Bs (Alfenito and Birchler, 1993; Stark et al., 1996; Theuri et al., 2005; Lamb et al., 2007b). Only a few sequences are considered B-specific though they are also present on As in minor traces. For example: Bd49 tandem repeat in *B. dichromosomatica* (Franks et al., 1996), E3900 and D1100 in *S. cereale* (Sandery et al., 1990; Blunden et al., 1993; Houben et al., 1996; Langdon et al., 2000), or the B-specific retroelement in *Alburnus alburnus* (Schmid et al., 2006). More data on characterization of DNA composition of Bs are given in (Jones, 1982; Camacho, 2005)

1.5. Chromatin structure analysis of B chromosomes based on Giemsa-banding and posttranscriptional histone modification patterns

In earlier publications B chromosomes in 50% of plant species were characterized as heterochromatic (Jones, 1975). In animal species Bs are described as mainly heterochromatic (Jones, 1982). The level of chromatin compactisation was analyzed mostly based on Giemsa-banding patterns.

The heterochromatic state is not a specific feature of Bs. The majority of B chromosomes are composed of both heterochromatin and euchromatin. In some species like *Apodemus flavicollis* (Tanic et al., 2005), *Helix pomatia* L. (Evans, 1960), *Scilla vvedensky* (Greilhuber and Speta, 1976), *Allium flavum* (Vosa, 1973) the Bs are even predominantly euchromatic.

At interphase, Bs of some species (eg. *Scilla autumnalis* (Ruizrejon et al., 1980), *Anthoxanthum aristatum* (Ostergren, 1947), *Picea glauca* (Teoh and Rees, 1977), *Clematis orientalis* and *C. hatherliensis* (Shambulingappa, 1965), *Rosa rugosa* (Price et al., 1981), *Tainia laxiflora* (Barlow and Vosa, 1969; Tanaka and Matsuda, 1972), *Puschkinia libanotica* (Barlow and Vosa, 1969)) display after Giemsa- or Feulgen staining so called heterochomatic chromocenters.

In eukaryotes, DNA is organized into chromatin by wrapping around the octamer complex formed by four core histones: H2A, H2B, H3 and H4, each being represented by two molecules. The size of DNA wrapped around the histone complex is 145-147 base pairs. The histone complex together with DNA forms the nucleosome, which represent the structural unit of the chromatin. The chromatin fibers that form chromosomes undergo several levels of folding, resulting in a more compact structure (Hansen, 2002). The core histones generally are globular, except their N-terminal ends which are less structured (Luger et al., 1997; Kornberg and Lorch, 1999).

The histones, especially the N-terminal tails are subjected to several types of post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination, glycosylation, ADP-ribosylation, carbonylation, sumoylation, deimination and proline isomerisation (van Holde, 1988). These modifications, together with DNA methylation, control the folding of the nucleosomal array into higher order structures (Strahl and Allis, 2000; Jenuwein and Allis, 2001).

The tail of histone H3 is the most subjected to posttranslational modifications (Felsenfeld and Groudine, 2003). The folding of chromatin regulates expression and maintenance (DNA replication, repair, recombination, chromosome segregation) of genetic information in a dynamic manner (Fuchs et al., 2006; Kouzarides, 2007). Most of the histone modifications are evolutionary conserved histone marks, although recently it was shown that the distribution of certain modifications (acetylation, methylation and phosphorylation) can differ among groups of eukaryotes (Fuchs et al., 2006).

The transcriptional activity of chromatin correlates with its degree of condensation. Generally, euchromatin represents the relaxed form, which contains the coding part of the genome while heterochromatin is the condensed form (Heitz, 1928). This chromatin fraction, which is considered as non-active is composed mostly of pseudogenes, tandem repeats and mobile elements. Heterochromatin can be divided in constitutive (permanent) or facultative (developmentally regulated) heterochromatin. More recent advances in chromatin characterization, in terms of epigenetic marks, showed the involvement of DNA methylation and post-translational histone modifications in chromatin assembly and maintenance (Richards and Elgin, 2002; Craig, 2005; Kouzarides, 2007).

Acetylation of histones is mainly linked with transcriptional activation, DNA recombination and repair (Grunstein, 1997; Struhl, 1998; Ikura et al., 2000; Bird et al., 2002). Phosphorylation correlates with transcription activation, apoptosis, DNA repair, chromosome condensation, sister chromatid cohesion/segregation and gametogenesis (Kaszas and Cande, 2000; Prigent and Dimitrov, 2003; Ahn et al., 2005; Krishnamoorthy et al., 2006; Houben et al., 2007). The most characterized histone modification to date is methylation. This modification occurs only on lysine (K) and arginine (R) residues of histone H3 and H4 and is performed by histone methyltransferases (HMTs) (Martin and Zhang, 2005). Each arginine can be mono- or dimethylated and each lysine can be either mono-, di-, or trimethylated (Zhang and Reinberg, 2001). There are six sites of lysine methylation (K4, K9, K27, K36 and K79) of histone H3. Histone H4 undergoes methylation on K20 (Martin and Zhang, 2005). Methylation of lysine can signal either activation or repression, depending on the position of lysine and on the number of methyl groups added. Generally it is considered that methylation of H3K4, H3K36 and H3K79 are implicated in activation of transcription and methylation of H3K9, H3K27 and H4K20 in repression of transcription (Bannister and Kouzarides, 2005). In high eukaryotes, H3K4me2,3 are marks of transcriptionally active chromatin while H3K4me1 marks the silent chromatin (Bernstein et al., 2002; Santos-Rosa et al., 2002; Schneider et al., 2004; Schubeler et al., 2004; Bernstein et al., 2005). High resolution profiling of histone methylations in human genome associate H3K27me1, H3K9me1, H3K79me1 and H4K20 with gene activation, whereas H3K27me3, H3K9me3 and H3K79me3 are correlated with repression (Barski et al., 2007).

In plants, methylation of H3K4me1,2,3 and H3K36me1,2,3 is correlated with euchromatin. The heterochromatin-specific modifications of H3K9, H3K27 and H4K20 are variable between plant species. In difference, methylation of H3K4 and H3K36 is more conserved (Fuchs et al., 2006). H3K9me3, H3K27me3 and H4K20me2,3 were found to mark the euchromatic regions in *Arabidopsis* (Soppe et al., 2002; Jackson et al., 2004; Naumann et al., 2005; Fuchs et al., 2006). Whole genome analysis revealed association of H3K27me3 in *Arabidopsis* with transcribed regions of single genes located in heterochromatic regions (Zhang et al., 2007). H3K9me1,2 and H3K27me1,2 and H4K20me are enriched on chromocenter regions (Soppe et al., 2002; Jasencakova et al., 2003; Lindroth et al., 2004).

In *Hordeum vulgare*, *Vicia faba* and *Arabidopsis* mono- di- and three-methylation of H3K4 are specific for euchromatin. The chromosomal distribution of the marks H3K9me1,2,3 H3K27me1,2,3 and H4K20me1,2,3 are variable in plants.

In *H. vulgare* H3K9me3 and H3K27me3 label the euchromatin in interphase and the gene rich regions on metaphase chromosomes. Similar pattern display H3K27me2, which in Arabidopsis is characteristic for heterochromatic chromocenter regions. H3K9me1, 2, H3K27me and H4K20me are uniformly distributed in the interphase nucleus and along the metaphase chromosomes.

In *V. faba* H3K9me1,2 , H3K27me1,2,3 and H4K20me1 show uniform distribution across the entire chromatin. Such labeling is typical for heterochromatin specific modifications in plants with large genomes. H3K9me1 and H3K27me2,3 are specific for certain heterochromatic regions (Fuchs et al., 2006). Distribution of H3K9me2 was found to depend on genome size. In species with small genome the labeling colocalized with heterochromatic chromocenters, while in species with large genomes the signals were dispersed in the nucleus (Houben et al., 2003; Fuchs et al., 2006).

In maize, the chromosomal histone methylation pattern showed association of H3K9me1 and H3K27me1,2 with heterochromatic regions, only H3K27me2 showing enrichment. H3K27me3, H3K9me2 and H2K4me2 labeled the euchromatin. Cytologically H4K20me2, 3 are undetectable in maize; the presence of H4K20me1 was demonstrated by Western analysis (Shi and Dawe, 2006).

The histones in centromeres and pericentromeric regions are marked by different modifications which are only partly conserved, mostly showing variability among both plant and animal species. In mammals, the pericentromeric heterochromatin is enriched in H3K9me3, H3K27me1 and H4K20me3 modifications (Peters et al., 2003; Schotta et al., 2004). In *Drosophila melanogaster*, H3K9me1,2, H3K27me1,2,3 and H4K20me3 are predominant marks of pericentric heterochromatin (Ebert et al., 2006). Arabidopsis pericentromeres are marked by H3K9me1,2, H3K27me1,2 and H4K20me1 (Lindroth et al., 2004; Mathieu et al., 2005; Naumann et al., 2005). In maize, H3K9me1 and H3K27me1,2 are characteristic for pericetromeric regions (Shi and Dawe, 2006).

There are only a few species where the post-translational histone modifications of B chromosomes were characterized. Histone acetylation of B chromosomes was investigated only in *B. dichromosomatica* untill now. Therefore comparative immunostaining of A and B chromosomes was performed with antibodies against H4K5Ac, H4K8Ac, H4K12Ac, H4K16Ac which are modifications specific for transcriptionally active chromatin in plants (Probst et al., 2004; Tian et al., 2005). Chromosomes A were strongly labeled, while Bs were weakly marked by H4K5Ac, H4K8Ac. The distribution of H4K12Ac and H4K16Ac modifications didn't show a such big difference between As and Bs (Houben et al., 1997). Less acetylated histones indicate very weak transcriptional activity of Bs in *B. dichromosomatica*, if any.

Histone methylation was studied in several species with B chromosomes. H3K4me2 show similar distribution on A and B chromosomes of *C. capillaries*, *B. dichromosomatica* (except micro B which are less marked by this modification) and *S. cereale* (Houben et al., 2003). More detailed analysis in *B. dichromosomatica* revealed strongly reduced level of H3K4me2,3, H3K9me3 and H3K27me3 on standard Bs and even less on micro Bs in comparison with brightly stained As. The distribution of heterochromatin specific histone modification marks H3K9me1,2 and H3K27me1 showed similar pattern on both A and B chromosomes (Marschner et al., 2007a).

1.6. DNA methylation and B chromosomes

Not only histones but also cytosine, which is one of the four essential base units of DNA can be extensively methylated. In both animals and plants, cytosine is primarily methylated at CG dinucleotide position. However in plants the methylation is not restricted to CG sequence: CNG and less abundant CNN sequence context are also possible (Gruenbaum et al., 1981). DNA methylation is involved in silencing of transposable elements and genes (Gehring and Henikoff, 2007). The DNA repeats and mobile elements on B chromosomes are also methylated, as reported for Bd49 tandem repeat in *B. dichromosomatica* (Leach et al., 1995) and E3900 repeat in *S. cereale* (Langdon et al., 2000). However, a higher DNA methylation level of B chromosomes has not been reported for any species (Houben, pers. communication).

1.7. DNA replication of B chromosomes

Replication of B chromosomes is delayed comparative to As replication, as reported in black rat *Rattus rattus* by (Raman and Sharma, 1974), in fox *Vulpes fulvus* by (Switonski et al., 1987), in fish *Astyanax scabripinnis* (Maistro et al., 1992) and in amphibia *Gastrotheca espeletia* (Schmid et al., 2002) though it is not a rule. In maize, the Bs euchromatic regions replicate simultaneously with A chromosomes, while the heterochromatin regions of As and Bs show delay in replication, the knob heterochromatin being replicated last (Pryor et al., 1980). In *B. dichromosomatica* the replication of large Bs occurs late in S phase (Houben et al., 1997), the replication of micro Bs, which are heterochromatic, occur during the entire S phase (Marschner et al., 2007a).

1.8. Inheritance of B chromosomes

1.8.1. Mitotic behavior of B chromosomes

In plants, only one third of investigated species display mitotic stability of B chromosomes (Jones, 1982). Stability in this context means the constancy of Bs within and between the roots of a plant and between the roots and aerial parts. Absence of Bs from roots were determined in *Erianthus munja* and *E. ravennae* (Sreenivasan, 1981), *Aegilops speltoides* (Mendelson and Zohary, 1972). In *Poa alpina*, the Bs are present in primary roots and absent in adventory ones (Muntzing and Nygren, 1955).

In some species the instability of the Bs is partial and strictly defined. For examples in *Sorghum stipoideum*, mosaicism of B chromosomes is found in microsporocytes and tapetal cells, while they are totally eliminated from stems and leaves (Wu, 1992). In some animals the presence of B chromosomes also exhibit irregularities. For example in *Locusta migratoria*, they show inter- and intrafollicular numerical variation (Kayano, 1971), in *Acris crepitans* the number of Bs differ between cells of the testis (Nur and Nevo, 1969). Among grasses Bs are generally stable, in *Alliums* Bs are mostly unstable (Bougourd and Parker, 1979). In *B. dichromosomatica* the large B is mitotically stable and the micro B is numerically variable from cell to cell (Carter and Smith-White, 1972).

1.8.2. Meiotic behavior of B chromosomes

One of the main characteristics of Bs is that they do not pair or recombine with any members of the standard diploid (or polyploid) set of A chromosomes at meiosis, except a few cases describing A-B translocations in rye (Pohler and Schlegel, 1990) and in maize (Beckett, 1978, 1991; Birchler, 1991; Carlson and Roseman, 1992; Auger and Birchler, 2002). Generally they do may pair among themselves, however in some species they behave as univalents even if more than one B chromosome are present in the dividing cell. There are at least 22 species where Bs are described as being non-pairing at metaphase I, however this irregularity does not lead to their elimination (Frost, 1956, 1959; Grun, 1959; Jones, 1995). Gillies (1983) first tried to characterize the pairing of Bs using electron micrograph analysis of meiotic prophase chromosomes. His result confirmed the observations obtained previously by light microscopy. Later on, pairing of Bs in other species were characterized by electron microscopy, for example in *Crepis capillaris* (Jones et al., 1989; Jones et al., 1991) and *S. cereale* (Jones, 1993). During meiosis in many species, Bs show drive as in *Lillium callosum* where the segregation distortion through the female side occurs when females are 1B carriers leading to accumulation of Bs in the progeny up to 80 % (Kimura and Kayano, 1961). In many cases transmission is non-Mendelian.

1.9. Drive of B chromosomes

The accumulation of Bs was characterised in about 70 plant species, from which only 60% showed an accumulation mechanism. The 40% left either show no accumulation, or Bs were lost in the progeny (Jones, 1995). As plants which show no accumulation of Bs can be mentioned *Guizotia scaraba* (Hiremath and Murthy, 1986), *Poa alpina* (Muntzing and Nygren, 1955), *Poa trivalis* (Bosemark, 1957a), *Centaurea scabiosa* (Frost, 1958), *Allium schoenoprasum* (Bougourd and Parker, 1979). In animals, no accumulation mechanism was detected in *Metagagrella tenuipes* (Gorlov and Tsurusaki, 2000).

If accumulation of Bs occurs, they have a non-Mendelian inheritance and their occurrence in offspring is much higher than expected. There are several mechanisms of Bs accumulation, which in its turn can occur during several stages of life cycle: mitosis (pre- or post-meiotic) and meiosis (Jones and Flavell, 1982; Jones, 1991). In different species drive occurs in different ways.

Drive of B chromosomes can be classified as: premeiotic drive, when the accumulation of Bs is a result of mitotic nondisjunction. Such mechanism was observed in grasshoppers *Calliptamus palaestinensis* (Nur, 1963), *Camnula pellucida* (Nur, 1969), *Neopodismopsis abdominalis* (Rothfels, 1950) and *Locusta migratoria* (Kayano, 1971). From plants can be mentioned *C. capillaris* (Parker et al., 1989) and *B. dichromosomatica* (Carter and Smith-White, 1972).

Meiotic drive was first found in *Lilium callosum* (Kayano, 1957; Kimura and Kayano, 1961). It was observed that the number of B chromosomes in progenies depends on which parent was carrier of Bs. For 0B♀ × 1B♂ crosses the ratio of 0B : 1B plants was 45:54. In the reciprocal cross 1B♀ × 0B♂ the ratio was 16:83. In case of 1B female, accumulation of Bs occurs in offsprings. The mechanism of accumulation is via drive of the univalent B chromosome toward a cell that becomes an egg (Kimura and Kayano, 1961). In animals, a similar phenomenon was observed in the grasshopper *Myrmeleotetix maculatus* (Hewitt, 1973; Hewitt, 1976). The meiotic spindle is asymmetrical and the Bs tend to localize mostly on the side of the egg than polar body. The degree of asymmetry correlates with the degree of B drive in females. *Pseudococcus affinis* can serve as another example. The B chromosome of this insect is heterochromatic during the cell cycle except late prophase I, when it becomes euchromatic. Bs join the maternal haploid set and in this way they are transmitted to the progeny in unreduced form (Nur, 1962). Nondisjunction is not involved in meiotic drive.

Postmeiotic drive is more frequent in plant species (Jones and Flavell, 1982; Jones, 1991). It occurs predominantly at first pollen grain mitosis via nondisjunction process and preferential migration of B chromatids to the generative nucleus, as for example in *S. cerale*. Maize is the only species where B nondisjunction occurs at second pollen grain mitosis. Differently from rye where the nonseparation of sister chromatids occur at both pollen grain and egg cell mitosis in maize this takes place only through the male side. In this species drive is characterized by three effects: a) the aforementioned nondisjunction of B chromatids, b) preferential fertilization (70% of cases) of the egg by B positive sperm, while 0B sperm fertilize the polar nuclei, and c) suppression of meiotic loss of B univalents (Roman, 1947, 1948b, a; Carlson, 1969, 1978, 1986; Carlson and Roseman, 1992; Gonzalez-Sanchez et al., 2003).

An unusual mode of drive was observed in wasps *Nasonia vitripennis* and *Trichogramma kaykai* (Werren and Stouthamer, 2003). The B-like paternal sex ratio (PSR) chromosome in these species is transmitted through sperm but after entering the egg it causes condensation and loss of the paternal chromosomes. In this way the PSR chromosome transforms a diploid (female) zygote to a haploid male carrying the B chromosome.

Elements controlling drive were determined only in a few species by indirect observations. In rye was described that the heterochromatic block on the ends of long arm of B chromosomes includes a trans-acting element which controls the nondisjunction of chromatids during first pollen grain and egg mitosis. Sister chromatids of deleted B chromosomes which lack this region separate normally. B chromosomes introduced by hybridization and backcrossing in wheat display the same nondisjunction properties, which confirm that the controlling element is not dependent on background genotype (Muntzing, 1970; Endo, 2008). In maize such element was described as the heterochromatic knob on the distal end of long arm of Bs (Roman, 1947; Carlson, 1978).

1.10. Effects associated with B chromosomes

The effects of B chromosomes are usually cumulative, depending upon their number and not their presence or absence (Jones, 1982; Green, 1990). At low number the B chromosomes do not have any influence on phenotype. Different phenotypic effects were correlated with presence of high number of Bs. In general, Bs influence negatively the fitness and fertility of the plants (Ostergren, 1947; Bosemark, 1957b; Gonzalez-Sanchez et al., 2004). In *Haplopappus gracilis*, individuals carrying Bs showed increase in pigment production in achene's walls (Jackson and Newmark, 1960). In maize, leaf stripping was correlated with the presence of Bs (Staub, 1987). In *Plantago coronopus* the presence of Bs caused the male sterility (Paliwal and Hyde, 1959), though this effect was not observed by Raghuvanshi and Kumar (1983). In grasshopper *Myrmeleotettix maculatus*, they were correlated with retardation in animal development (Harvey and Hewitt, 1979) and sperm dysfunction (Hewitt et al., 1987). In *N. vitripennis,* the effect of B-like PSR chromosome consists in changing the males into females by destroying the paternal chromosome during early embryogenesis (Werren, 1991).

Not only negative effects are associated with B chromosomes. They can act as diploidizing agents in allopolyploids (Jenkins, 1986). In rice, Cheng et al. (2000) could observe a slight

positive effect of Bs on plant height, weight of grain, length of panicle, length and weight of grain and negative effect on number of tillers and width of grain. In some species, individuals with Bs show better survival rate under certain stress conditions. For example in *A. schoenoprasum* and *A. porrum* Bs carrier seeds show better germination ability under drought conditions (Gray and Thomas, 1985; Holmes and Bougourd, 1989; Plowman and Bougourd, 1994). In *A. schoenoprasum* (Holmes and Bougourd, 1991) B positive plants showed better fitness than no carrier ones while grown under high sowing density. In *Apodemus flavicollis* was observed a positive correlation between the mean number of B chromosomes and body mass, but only in males (Zima et al., 2003). In the same species they affect mandible phenotype and the level of morphological integration was higher in animals with Bs (Jojic et al., 2007).

In Lindström wheat, the additional rye B chromosome lead to enhancement of plant vigor and straw weight and the negative effect on fertility which could be the cause of this positive effect (Muntzing, 1973).

In hybrids, Bs prevent or suppress the homoeologous pairing of A chromosomes. This effect was observed in hybrids between *Aegilops mutica* and *A. speltiodes* with *Triticum aestivum* (Dover and Riley, 1972) and in a tetraploid hybrid between *Lolium temulentum* × *L. perenne* (Evans and Davies, 1985; Jenkins and Jones, 2004).

Bs influence also the recombination frequency of As – mostly increasing, however in some cases decreasing the number of chiasmata frequency depending on species (Burt and Trivers, 2006). In maize Bs influence the recombination of As by increasing the chiasmata frequency, especially when B is univalent (Carlson, 1994).

1.11. Origin and evolution of B chromosomes

There is no definitive elucidation of provenance of B chromosomes. To date there are several hypothesis which explain the way B chromosomes originated (Jones, 1995; Camacho et al., 2000; Berdnikov et al., 2003; Jones and Houben, 2003). It is possible that in different organisms the Bs have different origin.

1.11.1. B chromosome originated from A chromosomes

In many species the repetitive sequences isolated from B chromosomes show high similarity with those of As, for example in: *C. capilaris* (Jamilena et al., 1994; Jamilena et al., 1995), *Z. mays* (Alfenito and Birchler, 1993; Cheng and Lin, 2003), *B. dichromosomatica* (Leach et al., 1995; Houben et al., 2001), *S. cereale* (Sandery et al., 1990; Blunden et al., 1993; Cuadrado

and Jouve, 1994; Houben et al., 1996), *D. subsilvestris* (Gutknecht et al., 1995). In *S. cereale* by GISH was found that the whole B chromosome, except the subtelomeric region hybridized with genomic DNA from 0B plants, which is another proof that Bs in rye originated from As (Tsujimoto and Niwa, 1992b; Wilkes et al., 1995).

In some species, rapid amplification of rDNA played a role in formation and evolution of Bs as shown in *Rattus rattus* (Stitou et al., 2000), *Trichogramma kaykai* (van Vugt et al., 2005), *E. plorans* (Lopez-Leon et al., 1994). The most convincing example was described for *Plantago lagopus* where analysis of several generations and *in situ* localization with rDNA probes demonstrated that B originated as result of massive amplification of 5S rDNA (Dhar et al., 2002).

1.11.2. B chromosome originated after interspecific hybridization of closely related species

This mode of origin was first proposed by Battaglia (1964) and latter confirmed by other scientists (Battaglia, 1964; McVean, 1995; Camacho et al., 2000). In hybrids generated after interspecific crosses between *Coix aquaticus* (2n=10) and *Coix gigantea* (2n=20, aneuploids: 2n=18 - nullisomic, 2n=24 - hexasomic) there were obtained a spectrum of individuals with following composition of *C. gigantea* (G) and *C. aquaticus* (A): 2n =14 (9G + 5A), 2n =15 (10G + 5A), 2n=16 (11G + 5A). The hybrid plants were grown together with the parents resulting after open pollination in plants with 2n = 10 up to 2 n = 21 with different number of chromosomes coming from *C. gigantean* and *C. aquaticus*. The plants with 2n=11 were analyzed and found to contain 2n = 11 (10A + 1G) or 2n = 11 (9A + 2G). The *C. gigantea* chromosomes integrated in *C. aquaticus* genome showed B chromosome-like behavior during meiosis (Sapre and Deshpande, 1987).

In the gynogenetic fish *Poecilia formosa*, initiation of embryogenesis requires sperm of males from the related bisexual species *P. mexicana* or *P. latipinna*. The paternal genome is afterwards eliminated early in development. The progeny results in a spotted pigmentation phenotype. Cytological analysis revealed supernumerary microchromosomes in *P. formosa* offsprings which appeared as result of incomplete elimination of paternal genome. They are inherited and different numbers of supernumeraries (B chromosomes) result in variable pigmentation variations which represent consequences of activity of paternal gene located on them (B chromosome) (Schartl et al., 1995).

Another example is the B-like PSR chromosome of wasp *N. vitripennis*. The phylogenetic analysis of DNA sequences of a NATE retrotransposon showed high similarity to sequences of a species in the genus *Trichomalopsis* (McAllister and Werren, 1997). B chromosomes were detected also in hybrids between two cultivated lines of some genotypes of *Pennisetum glaucum* (Le Thi et al., 1994).

1.11.3. B chromosomes derived from sex chromosomes

The similarity of meiotic behavior, morphology, and heteropycnocity between Bs and sex chromosomes in some species suggested the origin of Bs from sex chromosomes (Amos and Dover, 1981; Jones, 1982; Green, 1990). The similarity in composition and localization of two DNA probes (180 bp tandem repeat and ribosomal DNA) of B_2s, which are mostly composed of these two sequences, with those on X chromosome suggest that the B_2s in grasshoppers *E. plorans* probably originated from X chromosomes (Lopez-Leon et al., 1994). Another example can serve the B chromosomes of *Leiopelma hochstetteri* which at DNA level showed similarity to the W sex chromosome (Sharbel et al., 1998). Also morphological similarities between Bs and univalent W chromosome were observed (Green et al., 1993). In *Glossina* species was reported about the homology between B and sex chromosomes, suggesting the formation of B via Y chromosome duplication and further evolution by accumulation of repetitive DNA (Amos and Dover, 1981).

1.12. Molecular techniques for investigation of transcription

There is a large spectrum of methods available for the analysis of global transcriptional activity.

The mostly wide used methods for comparative transcription analysis are: micro- and macroarray hybridisation, differential display, cDNA-AFLP (Affinity Fragment Length Polymorphism), and SSH (Suppression Substractive Hybridization). Each method has its advantages and disadvantages. Depending on the aim, available sequence information, financial possibilities one can choose the most suitable method.

Arrays (micro and macro) are used to survey the expression of thousands of genes in a single experiment. The output of an array experiment is called a “gene expression profile”. All array

experiments rely on the core principle that transcript abundance can be deduced by measuring the amount of hybridization of labeled RNA to a complementary probe. In case of macroarray, the detection system is identical to those of Southern/Northern hybridization; in case of microarray the detection is done by measuring the fluorescence intensity. The usage of macro- or microarrays is determined by the start material available. For model organisms (e.g. *Arabidopsis thaliana,* rice), the array filters are commercially available. This method is not suitable for organisms with low sequence information.

Differential display was invented by Pardee and Liang (1992) to allow rapid, accurate, and sensitive detection of altered gene expression. The method is based on synthesis of cDNA from mRNA, PCR using single stranded cDNA as template and oligo dT and random one as primers, amplifying the 3'ends of transcripts. PCR products are then resolved in denaturing polyacrylamyde gels. Visualization can be performed by radioactive, fluorescence or silver staining detection. The band of interest can be excised, reamplified, and sequenced. The advantage of this method is that no preliminary sequence information is required, whereas disadvantages are: high background, low reproducibility and high frequency of false positives.

cDNA-AFLP is an improvement of traditional differential display techniques developed by Bachem and colleagues (1998). It is a PCR-based method which starts with the cDNA synthesis from total or mRNA using random hexamers or oligo dT as primers. The obtained fragments are digested with two restriction enzymes - normally a 4-cutter and a 6-cutter, and adapters are ligated to the ends of the fragments. In the first amplification step, only those fragments are amplified that were digested by both restriction enzymes and thus have different adapters at the end. In the following amplification steps, the complex starting mixture of cDNA is fractionated into smaller subsets by selective PCR amplification using primers on the adapters that contain one or more extra nucleotides. By increasing the stringency of PCR amplification (adding more additional nucleotides to the primers), the sensitivity of the analysis can be increased. In this way, genes with a low expression level can also be detected. The fragments that are amplified are roughly 100 to 800 bp long. These fragments are separated on high-resolution gels. The differences in the intensity of the bands that can be observed provide a good measure of the relative differences in the levels of gene expression. Further characterization of interesting transcripts often requires the identification of the corresponding full-length cDNA. The advantages of this method include a small amount of

starting material requirement, high sensitivity, simple principle, and no need of sequence information while disadvantages are: high number of false positives which can be diminished by running several control PCRs and sensitivity to SNPs (single nucleotide polymorphisms).

Suppressive Substractive Hybridization (SSH) were developed by Diachenko and colleagues (1996). SSH combines the selectivity of subtractive hybridization with the sensitivity of PCR. One of its main advantages is that it allows the detection of low-abundance differentially expressed transcripts. However, it doesn't detect small sequence differences.

1.13. Investigation of transcriptional activity of B chromosomes

Being dispensable for normal growth of individuals Bs were considered nonfunctional and not carrying any essential genes, therefore for many decades they were characterized as transcriptionally inactive. However, the transcriptional activity of Bs is of interest since they were discovered (Wilson, 1907). The approaches to study gene transcription were evolving in time according to the available techniques.

Fox and colleagues (1974) first attempted to study the transcription behavior of Bs in the grasshoppers species *Myrmeleotettix maculatus* and *Chorthippus parallelus* by monitoring the formation of RNA transcripts during diplotene using as detection the autoradiographic determination of incorporated [H^3] uridine. The labeling intensity of Bs was very low indicating little or no transcription. Comparable experiments performed by Ishak and colleagues (1991) in *Apodemus penisulae* resulted in a similar finding.

Indirect evidence of transcription of Bs was based on observation of meiotic Bs forming lampbrush structures in the frog *Leiopelma hochstetteri* (Green, 1988) and the fly *Simulium juxtacrenobium* (Brockhouse et al., 1989). Lampbrush is a special type of chromosome structure characteristic of the diplotene stage of meiosis in oocytes of a number of animal species. It consists of axial condensates of chromatin (chromomeres) and distinguishable lateral loops which are active in RNA synthesis. The RNA molecules associate with heterogeneous nonhistone proteins, forming periodic condensates of 20-30 nm ribonucleoprotein (RNP) particles. Loops are maintained by transcription and if transcription is stopped, there is no formation of a lampbrush structure (Chaumeil et al., 2006). Since transcribed coding and non-coding sequences (including highly repetitive sequences (Solovei

et al., 1996)) are organized as loops, it is likely that the lampbrush structure of frog and fly Bs is caused by active coding and/or noncoding sequences.

Other indications about transcriptional activity of B chromosomes result from biochemical analysis by comparative esterase isozyme activity of plants with and without Bs in *Scilla autumnalis* (Ruizrejon et al., 1980) and rye (Bang, 1990). In B-positive plants, an additional band was determined by protein electrophoresis. However, in both cases it is unclear whether additional band was caused by an isozyme gene which is located on Bs or Bs influenced the transcription behavior of A-located isozyme genes.

The B chromosome of pathogenic fungus *Nectria haematococca* was found to encode a family of cytochrome P-450 genes responsible for the detoxification of phytoalexin pisatin, an antimicrobial compound produced by the garden pea (*Pisum sativum L.*) (Miao et al., 1991; Han et al., 2001). This is the best example where a link can be traced between transcription of B-located genes and a defined function. B-located elements controlling the host pathogenicity were also found in other fungi, e.g. *Alternaria alternata* (Hatta et al., 2002). More examples are listed in Covert et al. (1998).

In many species B chromosomes contain nucleolar organizer regions (NORs) which contain ribosomal RNA (rRNA) genes (Green, 1990; Jones, 1995). The most common indirect technique to visualize active NORs is silver staining (Hizume et al., 1980). *In situ* hybridization has been widely used to visualize the presence or absence of B-located rDNA sequences (Maluszynska and Schweizer, 1989; Donald et al., 1995). However, not all rRNA loci are active. For example, based on RT-PCR experiments, the 45S rRNA genes of the large B chromosome in *B. dichromosomatica* were found to be inactive in leaf tissue (Donald et al., 1995). Indirect evidence of rDNA activity could serve its association with nucleolus during interphase. Micro Bs in *B. dichromosomatica* showed 70% of non-association with the nucleolus, suggesting inactivity of micro B-located 45S rDNA (Marschner et al., 2007b). The first molecular evidence of ribosomal gene activity on B chromosomes in plants was found in *C. capillaris* in which one of two B-specific members of rRNA gene families were weakly transcribed (Leach et al., 2005). In animals, activity of rDNA was demonstrated for the B-like PSR chromosome of the parasitoid wasp *Trichogramma kaykai* (van Vugt et al., 2005).

In red fox (*Vulpes vulpes*) and two subspecies of raccoon dog (*Nyctereutes procyonoides*) a proto-oncogene C-KIT gene was identified and localized on Bs by *in situ* hybridization (Graphodatsky et al., 2005). However its transcriptional activity was not yet demonstrated. In 0B and +B yellow-necked mice (*Apodemus flavicollis*) differential expression of the genes: chaperonin containing TCP-1, subunit 6b (zeta) (CCT6B), fragile histidine triad gene (FHIT) and hypothetical gene XP transcript, were identified by differential display (Tanic et al., 2005). However, B chromosome-localization of these genes was not demonstrated. Therefore it doesn't exclude the possibility that these genes are A-localized and its transcription behavior is influenced by the presence of Bs.

B chromosomes of many species are composed mainly of degenerated mobile elements, tandem repeats, and other nonfunctional DNA which are considered as transcriptionally silent (Alfenito and Birchler, 1993). However, recently in maize it was shown that parts of *StarkB,* a B-specific transposable element, are transcribed (Lamb et al., 2007a). The transcripts coming from transposable elements are claimed to be involved in regulatory processes as chromatin compactisation; they can switch on genes by read-through, either switch off or insertion into the gene during transposition.

1.14. Rye B chromosomes

First in rye (2n = 2x = 14) Bs were mentioned by Gotoh (1924). Their number varies between 0-8 Bs per plant. The size of Bs is half of the A chromosomes (Fig. 1). In the genus Secale, out of five representing species, *Secale cereale* showed the highest predisposition of carrying Bs (Jones, 1993). However in *S. segetale*, Bs are present as well (Niwa and Sakamoto, 1995, 1996). The crosses between 2B plants of *S. cereale* and 2B plants of *S. segetale* resulted in progeny of 4B individuals. Cytological investigations showed that the Bs of both species do pair at diakinesis in pollen mother cells. This demonstrates that in both species the Bs are homologous, and probably have a monophyletic origin (Niwa and Sakamoto, 1995).

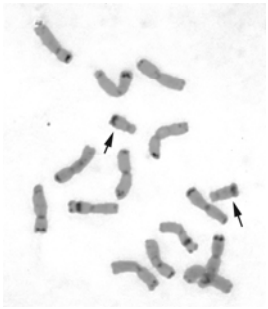


Fig. 1. Giemsa banding of rye cell with 2 Bs (arrowed).

The geographical distribution of rye Bs is all around the world: first found in Japan and later on reported in almost all wild and semi-wild studied populations from Asia, Europe and Northern America. The frequencies of plants with Bs vary from population to population: the highest are found in populations from Far East in Korea and Japan (Jones, 1993). No B

chromosomes were found in breeding cultivars as they negatively influence the fertility and for this reason during selection they are eliminated.

Rye Bs are stable at mitosis with a constant number in all somatic cells. The meiotic behavior of Bs in rye is marked by the fact that during metaphase I (MI) they do not pair with As, except A/B translocations (Pohler and Schlegel, 1990). One B in the cell behaves as univalent at meiosis: often it is lost during division. Two Bs usually pair forming a bivalent at MI, though not always. When more than 2 Bs are present in the cell they can pair as multivalents. The frequency of such pairing is variable depending on the strain. For example in Swedish varieties formation of bivalents are predominant to the multivalents (Hakansson, 1957), while in JNK strain the formation of radial multivalents are frequent (Kishikawa 1968). The pairing frequency among Bs is often determined by the genotype environment, which was classified as lines possessing high transmission rate (which tend to accumulate the Bs) or low (which tend to lose the Bs). The A- complement genotypes controls the B-transmission frequency (Romera et al., 1991).

As in many other species rye Bs have a non-Mendelian inheritance. Crosses between 0B × 1B plants result in 80% 0B and 20% 2B plants, with a few carrying only 1B (Puertas and Lacadena, 1974). Neither of 0B × 2B crosses result in 1B progeny; most of the plants are carrying 2Bs. Crosses between 0B × 2B, 2B × 0B and 2B × 2B show high numeric variation of Bs between different varieties and within them (Jones, 1993). In some 0B × 2B and 2B × 0B crosses more 0B offsprings are obtained while in other crosses 2Bs and more are predominant. The 2B × 2B crosses also result in offsprings with variable number of Bs which differ from the expected one. The transmission rate of B chromosomes is also influenced by maternal effect (Puertas et al., 1990). The crosses between 0B × 2B, 2B × 0B and 2B × 2B showed a large variation of Bs in the progeny. The 2B plants whose maternal parent had 2Bs showed high transmission of Bs. 0B plants coming from 2B maternal parent showed increased rate of acceptance of pollen from B carrier plants. Respectively the progeny of 2B males and 0B females coming from 0B maternal parent displayed lower frequency of B transmission. In 2B × 0B crosses, variations were also observed, although not correlated with the influence of the maternal parent. In 2B × 2B crosses, the pollen coming from a 2B maternal parent fertilizing the 2B mother plant resulted in increase of B chromosome frequency in the progeny (Puertas et al., 1990).

The accumulation of B chromosomes in rye occurs due to nondisjunction of sister chromatids at first pollen grain as well as first egg cell mitosis (Fig. 2) (Müntzing, 1946; Hakansson, 1948; Muntzing, 1963). The element which controls the nonseparation of sister cromatids is located on the distal end of the long arm of Bs. The chromatids of iso-B chromosomes, which lack the terminal end of the long arm, separate normally at first mitosis after meiosis (Muntzing and Lima-de-Faria, 1952). However, if an additional standard B or its distal end of long arm is present, a deleted B also undergoes nondisjunction (Lima-De-Faria, 1962; Endo, 2007; Endo, 2008). Endo (Endo, 2008) generated a series of rearranged Bs and wheat + rye Bs translocations via a gametocidal (Gc) system. In both cases: either an additional standard B present or the terminal region of long arm of Bs the chromatids of the deleted B do not separate at first mitosis after meiosis. In wheat the nonseparation occurs like in rye which means that the background genotype doesn't encode any major controlling element. Only the pericentromeric region and the trans-acting element located on the distal end of long arm of Bs are in charge for the nondisjunction in rye (Endo, 2008). A dosage effect depending on the ratio of deleted B per standard B was observed.

Fig. 2. Rye nondisjunction at first pollen grain mitosis

The effects of rye Bs are rather negative. Their presence in the plant lead to decrease in viability and fertility (Müntzing, 1943, 1963). The negative effect on fertility can be explained by the univalent formation at meiosis (Jones and Rees, 1967) and spindle abnormalities at pollen mitosis (Hakansson, 1957). The Bs in rye do not influence the sister chromatids exchange of A chromosomes (Friebe, 1980). The influence of Bs was found to be variable in different genotypes. For example in Korean rye, the negative effects of Bs are less severe (Müntzing, 1967). To study the influence of Bs excluding the variability due to genotype Puertas and colleagues (1985) compared the viability and fertility of Bs in *S. cereale* and *S. vavilovii*. In *S. vavilovii* which naturally contain no Bs, they were introduced by backcrossing with JNK rye carrier of Bs. The results lead to the conclusion that the Bs influence the fitness independently of the genotype. The Bs were found to influence mainly the fertility (grain/plant, grain/flower) and to have no influence at all on viability (spike/plant, spike/flower) (Puertas et al., 1985). Same results were obtained after introgression of Bs coming from JNK strain in other twelve rye isogenic lines (Jimenez et al., 1994). The dosage

of A chromosomes does not influence the behavior of Bs. This is because in tetraploid rye, Bs behaves in the same way like in diploid rye (Müntzing, 1963).

The rye B is mainly composed of DNA sequences in common with those of the A chromosomes (As) (Rimpau and Flavell, 1975; Timmis et al., 1975) with the exception of the terminal part of the long arm (Tsujimoto and Niwa, 1992a; Wilkes et al., 1995; Houben et al., 1996). Two B-specific families of high copy repetitive DNA, D1100 (Sandery et al., 1990) and E3900 (Blunden et al., 1993) have been isolated and mapped to this terminal domain. Both families are organized in a complex manner and neither represents a simple monotonous array of tandem repeats (Langdon et al., 2000). *In situ* hybridization shows that at metaphase the D1100 display two adjacent clusters with a small gap between them, with the more homogeneous E3900 positioned towards the telomere (Wilkes et al., 1995; Langdon et al., 2000). This B specific domain is characterized as the B heterochromatic block, which replicates late in S phase and also corresponds to the most prominent and conserved Giemsa-banding positive band discriminated by DAPI staining at metaphase (Jones, 1993; Houben et al., 1996; Langdon et al., 2000). The rye Bs carries no ribosomal DNA (Niwa and Tsujimoto, 1992; Cuadrado and Jouve, 1994).

2. Aims of the thesis

The questions addressed in this work are focused on the analysis of transcriptional activity of rye B chromosomes. To answer these questions following approaches were employed:

1. Investigation of the histone H3 and H4 methylation properties of rye Bs.

It is known that histone modifications cause the change of the chromatin structure leading to formation of euchromatin (transcriptionally active) or heterochromatin (transcriptionally inactive). To determine the chromatin structure which is in direct correlation with transcriptional activity, the histone methylation pattern of B chromosomes versus the transcriptionally active A chromosomes of rye was compared by immunostaining with antibodies against histone H3K4me1,2,3, H3K9me1,2,3, H3K27me1,2,3 and H4K20me1,2,3.

2. Investigation of transcriptional activity of previously isolated rye high copy B specific sequences and characterization of their transcripts.

The transcriptional activity of repeats D1100 and E3900 (Blunden et al., 1993) which are located in the subtelomeric region of the long B chromosome arms was investigated by Northern hybridization and RT-PCR.

To characterize D1100 and E3900 transcripts, their polyadenylation status and small RNA formation capability was investigated.

3. Identification and characterization of the general transcriptional activity of B chromosomes.

To characterize the global transcriptional activity and to identify new B specific transcripts, comparative cDNA-AFLP was performed. Plants with and without Bs from three inbred lines were used for screening to reduce the false positives due to the polymorphism. It is not excluded that B chromosomes influence the transcription behaviour of A chromosomes. Therefore, the additional bands in 0B material were also determined by cDNA-AFLP profiling.

4. Elucidation of the origin of rye B chromosomes.

The sequence analysis of newly identified B-transcripts could provide new information about the origin of B chromosomes.

3. Material and methods

3.1. Plant material and plant cultivation

Three different inbred lines of rye (*Secale cereale* L.): 7415, P 12, and 2677 (Jimenez et al., 1994; Ortiz et al., 1996) were selected for cDNA-AFLP experiments. For generation of these inbred lines, four backcrossings of the new generation with pollen collected from plants with +2B chromosomes were performed by the authors. Therefore, the B chromosomes in each line should be of the same origin (Jimenez et al., 1994).

After vernalization, the plants were grown in the same temperature, humidity, and light conditions (16 hours light, temperature 22ºC day and 16 ºC night). As the presence of Bs do not show any phenotypic effects, each plant was checked by chromosome counting, Southern hybridization using a B-specific probe and PCR using 3900 forward 5 and 3900 reverse 5 primers (Table 5) specific for B chromosome sequences.

Rye-wheat chromosome addition lines carrying chromosome 6B and a small wheat chromosome fragment translocated to one of the rye chromosomes were kindly provided by R. Schlegel (Gatersleben). The addition lines were generated by crossing rye (cultivar 'Pluto') with wheat (cultivar 'Falon') and several backcrosses of the hybrids with the same rye parent line (Fig. 3).

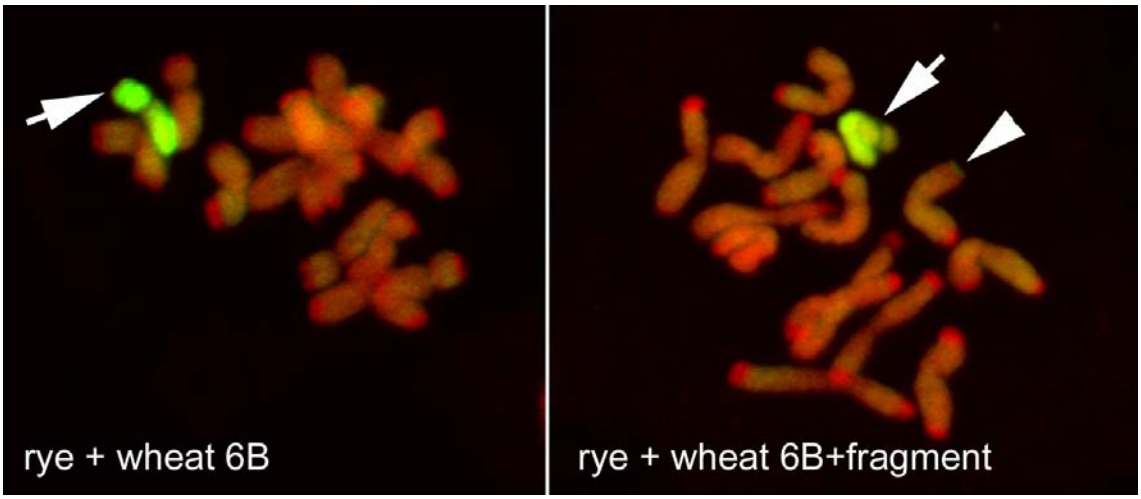


Fig. 3. Genomic in situ hybridisation (GISH) of two rye-wheat addition lines carrying the wheat chromosome 6B (in yellow, arrowed) and a translocated small wheat chromosome fragment (in yellow, indicated by an arrowhead). Pictures were obtained from A. Houben (IPK, Gatersleben).

3.2. Chromosome counting

Caryopses were germinated on moist filter paper at 22-24ºC in dark during 3-4 days. To accumulate metaphase cells, 2-3 cm long roots tips were treated in ice cold water for 16 hours. Afterwards, roots were fixed in ethanol : glacial acetic acid (3:1) solution for 2-3 days at room temperature (RT). To prepare chromosomes, roots were stained in 1% acetocarmine dissolved in 45% acetic acid and squashed between slide and cover slips. A phase contrast microscope was used for chromosome counting.

3.3. Extraction of genomic DNA

DNA was extracted from 100 mg grinded leaves in 1.5 ml prewarmed at 65ºC isolation buffer (0.1 M Tris, 0.7 M NaCl, 0.05 M EDTA, pH 8.0) for 15 minutes. After adding 0.65 ml isoamylalcohol : chloroform (1:24) the mix was centrifuged at 14,000 rpm, RT. The supernatant was transferred into a new tube, the DNA precipitated with 700 µl isopropanol, the pellet washed with 1 ml 70% ethanol, then dried and resuspended in 100 µl double distilled H_2O (Souza, 2006).

3.4. Extraction of plant RNA

Total RNA was isolated from roots, leaves, and anthers by using the Trizol method (Chomczynski and Sacchi, 1987). Contaminating genomic DNA was digested with DNase (Roche). Afterwards, DNase was inactivated by heat-treatment for 10 minutes at 70°C and removed by phenol : chlorophorme (1:1) treatment. RNA was recovered by isopropanol precipitation. The absence of genomic DNA contamination was tested by PCR using E3900-specific primers (E3900 forward 5 and 3900 reverse 5 (Table 5)) on DNAse treated RNA without reverse transcription reaction. The RNA quality was monitored by denaturing MOPS-formaldehyde gel electrophoresis (Sambrook J, 2001). The concentration was determined spectrophotometrically.

3.5. cDNA-AFLP analyses

The cDNA-amplified fragment length polymorphism (AFLP) method was performed according to Bachem (Bachem et al., 1996; Bachem et al., 1998). The cDNA was synthesized using Super SMART cDNA synthesis kit (Clonthech). 1 µg of total RNA from leaves served for synthesis of the first cDNA strand. The double-stranded cDNA was subsequently purified

with NucleoSpin columns (MACHEREY NAGEL), quantified spectrophotometrically, and the quality was checked by agarose gel electrophoresis.

3.5.1. AFLP analysis

300 ng double-stranded cDNA was restricted with 5.0 U *Pst*I (rare cutter) and 2.5 U *Mse*I (often cutter) (New England Biolabs) at 37ºC for 3 hours. To the restricted cDNA fragments were ligated 2 pmol *Pst*I adapters and 20 pmol *Mse*I adapters complementary to the restriction sites (Table 1). The ligation was performed overnight at 4ºC in 12.5 µl mix containing 1x NEB2 buffer, 1 mM ATP, BSA (0.1 mg/ml) and 1 Wise-U T4-Ligase (New England Biolab). Ligated fragments were diluted 1:4 with 0.1x TE. 5 µl was used for PCR-preamplification with 6 pmol of each primers *Mse*I 00 and *Pst*I 03 (Table 2), 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1x reaction buffer and 1U *Taq* polymerase (Qiagen). PCR conditions were: 3 minutes 95ºC; 20 cycles: 30 seconds 95ºC, 30 seconds annealing 60ºC and extension 2 minutes 72ºC. The preamplified mixture was diluted 40 times, and 3 µl was used for final selective PCR-amplification according to Vos et al. (1995). *Mse*I and *Pst*I primers containing three selective nucleotides at the 3'-prime end were used. PCR mix contained 6 pmol of each primers *Mse*I and *Pst*I (Table 2), 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1x reaction buffer and 1U *Taq* polymerase (Qiagen). PCR conditions included following steps:1) 3 minutes 95ºC, 2) 9 cycles: 30 seconds 95ºC, 30 seconds annealing (touch-down profile 65ºC-1ºC/cycle) and extension 2 minutes 72ºC, 3) 23 cycles: 30 seconds 95ºC, 30 seconds annealing 56ºC and extension 2 minutes 72ºC. 63 primer combinations were tested (Table 2, 3). PCR products were separated in 5% acrylamide gel (Roth) containing 8 M urea, 1x TBE using sequencing gel electrophoresis apparatus (Gibco BRL). To remove nonpolymerised acrylamide, the gel was first run at 1200 V, 27 mA for 30-40 minutes in 1x TBE. 3 µl of PCR products were mixed with equal volume loading dye (98% formamide, 10 mM EDTA, 0.025% bromphenol blue and 0.025% xylene cyanol), denatured for 5 minutes at 95ºC, chilled on ice and run at 1200 V, 27 mA for 2.5 hours in 1x TBE. GeneRuler™ 100 - 1000 bp range was used (Fermentas) as marker. Marker was mixed with equal amount of formamide loading dye and treated as AFLP PCR products. Negative controls (without template DNA) were included in each step.

3.5.2. Detection and recovery of electrophoresed cDNA-AFLP fragments

Silver staining, performed according to Sanguinetti et al. (1994) with slight modifications, was used to visualize cDNA-AFLP fragments. After electrophoresis, the glass plates were

separated and the acrylamide gel was fixed in 10% ethanol, 5% acetic acid solution for 5 minutes. Subsequently, the gel was stained in 0.3 % $AgNO_3$ for 15 minutes and developed in developing solution (1.5% NaOH, 0.4% formaldehyde, 100 µg/l $NaBH_3$) during 15 - 20 minutes. After each step, gels were rinsed in distilled water.

The cDNA-AFLP fragment patterns were compared between plants with and without B chromosomes. Fragments of interest (eg. fragments present in +B material only) were excised from the acrylamide gel and resuspended in 50-100 µl elution buffer (50 mM KCl, 10mM Tris HCl (pH 9.0) and 0.1% Triton-X-100) for 20 minutes at RT (Sanguinetti et al., 1994). 5 µl of eluted DNA was used for reamplification using the same PCR conditions and primer combinations as been used for selective amplification. PCR products were checked by 1.2% TAE agarose gel electrophoresis, cloned into pGEM-T Easy vector (Promega), and sequenced.

3.5.3. DNA Sequencing and sequence analysis

Sequencing was performed using facilities at IPK (Gatersleben) or at MWG Biotech AG (Ebersber).

Sequences were processed using the software 'Editseq' and aligned using 'MegAlign' (Lasergene 6). Following databases were used for sequence comparisons, BLAST (Basic Local Alignment Search Tool):

NCBI - http://www.ncbi.nlm.nih.gov/BLAST/

TIGR - http://www.tigr.org/tdb/e2k1/plant.repeats/

GrainGene - http://wheat.pw.usda.gov/GG2/blast.shtml

RT-PCR, FISH, Southern and Northern hybridization was performed for confirmation and further analysis of B-specific transcripts.

Table 1. Adaptors sequences

Adapters	Sequences 5'—3'
*Pst*I adapter forward	CTCGTAGACTGCGTACATGCA
*Pst*I adapter reverse	TGTACGCAGTCTAC
*Mse*I adapter forward	GACGATGAGTCCTGAG
*Mse*I adapter reverse	TACTCAGGACTCAT

Table 2. List of AFLP-primer sequences (Keygene).
http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html
Selective nucleotides are highlighted in red.

Pst I 5´---3´		*Mse*I 5´---3´	
P03	GACTGCGTACATGCAG**G**	M00	GATGAGTCCTGAGTAA
P47	GACTGCGTACATGCAG**CAA**	M 32	GATGAGTCCTGAGTAA**AAC**
P48	GACTGCGTACATGCAG**CAC**	M 33	GATGAGTCCTGAGTAA**AAG**
P 63/ S 11	GACTGCGTACATGCAG**GAA**	M 34	GATGAGTCCTGAGTAA**AAT**
P 65/ S 13	GACTGCGTACATGCAG**GAG**	M 47	GATGAGTCCTGAGTAA**CAA**
P 67/ S 15	GACTGCGTACATGCAG**GCA**	M 48	GATGAGTCCTGAGTAA**CAC**
P 69/ S 17	GACTGCGTACATGCAG**GCG**	M 49	GATGAGTCCTGAGTAA**CAG**
P 76/ S 24	GACTGCGTACATGCAG**GCT**	M 51	GATGAGTCCTGAGTAA**CCA**
P 79	GACTGCGTACATGCAG**TAA**	M 52	GATGAGTCCTGAGTAA**CCC**
P 81	GACTGCGTACATGCAG**TAG**	M 55	GATGAGTCCTGAGTAA**CGA**
P 85	GACTGCGTACATGCAG**TCG**	M 59	GATGAGTCCTGAGTAA**CTA**
		M 61	GATGAGTCCTGAGTAA**CTG**
		M 62	GATGAGTCCTGAGTAA**CTT**
		M 63	GATGAGTCCTGAGTAA**GAA**
		M 64	GATGAGTCCTGAGTAA**GAC**
		M 65	GATGAGTCCTGAGTAA**GAG**
		M 79	GATGAGTCCTGAGTAA**TAA**
		M 80	GATGAGTCCTGAGTAA**TAC**

Table 3. Primer combinations used for cDNA-AFLP analysis of 0B/+B plants.

Nr.	Primer combinations		Nr.	Primer combinations	
	*Pst*I	*Mse*I		*Pst*I	*Mse*I
1	S11	M48	36	S24	M59
2	S11	M79	37	S24	M60
3	S13	M32	38	S24	M61
4	S13	M33	39	S24	M62
5	S13	M34	40	S24	M65
6	S13	M47	41	S25	M65
7	S13	M48	42	P47	M47
8	S13	M49	43	P47	M49
9	S13	M55	44	P47	M55
10	S13	M63	45	P47	M63
11	S13	M64	46	P47	M64
12	S13	M79	47	P47	M80
13	S13	M80	48	P48	M32
14	S15	M51	49	P48	M33
15	S15	M52	50	P48	M49
16	S15	M53	51	P79	M48
17	S15	M55	52	P79	M55
18	**S15**	M60	53	P81	M32
19	S15	M59	54	P81	M33
20	S15	M61	55	P81	M34
21	S15	M62	56	P81	M47
22	S15	M65	57	P81	M48
23	S17	M34	58	P81	M49
24	S17	M48	59	P81	M55
25	S17	M49	60	P81	M63
26	S17	M51	61	P81	M64
27	S17	M61	62	P81	M79
28	S17	M62	63	P85	M49
29	S17	M65			
30	S24	M34			
31	S24	M48			
32	S24	M49			
33	S24	M52			
34	S24	M53			
35	S24	M55			

3.6. RT-PCR

75 ng of cDNA from roots, leaves or anthers was used for each RT-PCR reaction. Primers were designed specific for cDNA-AFLP fragments (Table 4), as well as the B-specific repeats D1100 and E3900 (Table 5). PCR mix contained 1 μM of primers, 2 mM dNTP, 1x reaction buffer, 1.5 mM $MgCl_2$, and 1U of Taq polymerase (Qiagen). The PCR conditions for D1100 were: 3 minutes 95ºC; 40 cycles: 30 seconds 95ºC, 30 seconds 65ºC , 2 minutes 72ºC. For all 3900 primer combinations the conditions were: 3 minutes 95ºC; 30 cycles: 30 seconds 95ºC, 30 seconds 65ºC , 2 minutes 72ºC. For cDNA AFLP fragments the annealing temperatures were different (Table 4), in rest the PCR condition were like for E3900. PCR was performed in Thermal Cycler (Bio-Rad). The products were analyzed in 1.2% TAE agarose gels.

Table 4. Primers specific for cDNA AFLP fragments

Nr.	Name of primers	Sequence 5'-----3'	Annealing temperature
1	2452 forward	AGTTACCATCCTCTTTCGCCACCCGCACG	71.6ºC
	2452 reverse	AAATCATTGGGGGGTTGCCGTCCCTTCG	
2	2448 forward	ATGAGGGGGAACGCAGACAACAAC	66.5ºC
	2448 reverse	TCGCTGGCTGAGAACAATGATAAC	
3	1553 forward	ACTATTACAGTGGCAACAGC	64.5ºC
	1553 reverse	ATGAGGTGGGGGCTTCAGCG	
4	1559 forward	ATTATTTCGTTCGCCTACACACACAGC	68.8ºC
	1559 reverse	TGCTACATCCTCCTCTCCCTCATCTCG	
5	8149 forward	AGCTCCAACACAGCACCAAGGGCATCCG	72.5ºC
	8149 reverse	AGCCAACCAAACGCCAGGCACCACCAGC	
6	4747 forward	TGGCCTTACGGAACCCATCTTGC	66ºC
	4747 reverse	TGTCACCACTGGCTTGAACAGGC	
7	2565 forward	GTGAATGATGCTTGTGTCTTCG	61.5ºC
	2565 reverse	AGAGGGCGCAAAAGATCCTAC	
8	2453 forward	AATGACTGGATTGACGTGAAGTG	61.5ºC
	2453 reverse	CAAGCCATAAAGAGGCACCAC	
9	2465 forward	TGCCGTAGAAGATGAGGTTCCCGC	66ºC
	2465 reverse	GTGTCGTTCTGATTGACCAAAGAGC	
10	1334 forward	AACATCATACAAAACATCACG	58.8ºC
	1334 reverse	CCGGGAGTCCGATGAAGATC	

Table 5. Primers specific for D1100, E3900 and barley-eEF1alpha sequences.
Primer positions are based on the published sequences: Z54196 for D1100 (Sandery et al., 1990; Langdon et al., 2000) and AF222021 for 3900 (Blunden et al., 1993; Langdon et al., 2000) and Z50789 for eEF1alpha (Nielsen et al., 1997).

Name of primers	Sequence (5'--3')	Position (bp)
1100 forward	GCTTGCACCGGCTTCGTCCCG	57-77
1100 reverse	GTGTACTTGAGACACGCAAGC	857-837
3900- forward 1N	CTAGCCCGTCGTCCTATTTCCG	84-105
3900 forward 1	GAGGGGGTAATCTTTGCTCAGC	253-274
3900 forward 2	ATAGAACGGCTGGGATGAGACG	1559-1580
3900 forward 3	ACCGTCGTTGCCGCCGTTCG	2427-2446
3900 forward 4	AGATGATGGTTTTGATGACGGCG	2888-2910
3900 forward 5	CCGACGACTTGGCGAGGTATG	3231-3251
3900 forward 6	TGTTTGATGGTGATGGTGATGCG	3597-3619
3900 reverse 1	TCCTGTTCGTCTCATCCCAGC	1587-1567
3900 reverse 2N	GAAACCAAAGGAGTAGACTTGC	2364-2343
3900 reverse 2/3	TCTCTTTCTTCTCCCTACGTGC	2667-2646
3900 reverse 4	GCAAAGTCCTCGACCAACTGC	3409-3389
3900 reverse 5	GAGCGGGCGGAGGAGTTTCT	3688-3669
3900 reverse 6	CTGCAACTGTCTCCACATACACG	3940-3918
barley-eEF1alpha-forward	AGGAGAAGACTCACATCAACATC	64-86
barley-eEF1alpha-reverse	TGGGCTCGTTGATCTGGTCA	735-716

3.7. Northern hybridization

For Northern analysis, 20 μg of total RNA was separated in denaturing MOPS-formaldehyde agarose gel, and blotted onto Hybond-N^+ membrane (GE Healthcare) by capillar transfer in 20x SSC overnight (Sambrook J, 2001). RNA was crosslinked to the membrane using UV transiluminator (auto crosslink setting). Prehybridisation and hybridization were performed in Church buffer (7% SDS, 10 mM EDTA, 0.5 M phosphate buffer (pH 7.2)), at 64 °C. Probes (100 ng) were labeled with 5 μl [α-^{32}P] CTP (125 μCi P^{32} (GE Healthcare)) using HexaLabel™ DNA Labeling Kit (Fermentas). After overnight hybridization, membranes were washed twice in 40 mM phosphate buffer (pH 7.0) containing 1% SDS and 2 mM EDTA at 65°C. Radioactivity was vizualised using either X-ray films or the phospho-imager (Fuji).

3.8. Restriction digestion, gel electrophoreses of genomic DNA and Southern hybridization

Southern hybridization was performed according to (Sambrook J, 2001). 10 µg of genomic DNA from plants with and without Bs was restricted overnight with the restriction enzymes: *Eco*RI, *Pst*I, *Dra*I or *Xba*I (Fermentas) according to the manufacture instruction. The digested DNA was size-fractionated by gel electrophoresis (0.8% agarose in TAE buffer, 18-20 hours at 25V). The gel was denatured in denaturation buffer (1.5 M NaCl, 0.5 M NaOH) for 30 minutes, neutralized in neutralization buffer (1.5 M NaCl, 1 M TrisHCl (pH7.2), 0.001 M EDTA) 15 minutes twice then, blotted onto Hybond-N^+ membrane (GE Healthcare) by capillar transfer in 20x SSC overnight. The DNA was fixed to the membranes by incubating for 5 minutes in 0.4% NaOH. Prehybridisation, probe labeling, and hybridization were performed in the same conditions as described for Northern hybridization.

3.9. Fluorescent in situ hybridization (FISH)

FISH was performed according to Houben et al. (2006).

3.9.1. Preparation of chromosome spreads

Caryopses were germinated on moist filter paper at 22-24ºC in dark during 3-4 days. Root tips (1.5 - 2 cm) were treated with ice cold water for 16 hours to accumulate metaphases, then fixed in ethanol:acetic acid (3:1) for 2-3 days at room temperature. To remove cell walls root meristems were treated with an enzyme mixture of 2.5% Pectinase, 2.5% Cellulase R-10, 2.5% Pectolyase Y-2 dissolved in 1x PBS at 37ºC until the material become soft (about 50 minutes). Alternatively, roots were treated for 30 minutes with 1% aceto-carmine (prepared in 45% acetic acid) at room temperature. The root tips were squashed in a drop of fresh 45% acetic acid between glass slide and cover slip. The preparation was checked under a phase contrast microscope. Slides with good chromosome spreads were immersed into liquid nitrogen and after immediate removal of cover slip air dried. Dried slides were stored desiccated at room temperature or –20°C if for a longer time.

3.9.2. Probe preparation

The probes were either excised from plasmids by restriction digestion, separated in gel electrophoresis and purified using QIAquick gel extraction kit (Qiagen) or generated by PCR. The probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP by nick-translation.

Therefore, 1 µg DNA was mixed with 4 µl of biotin or digoxigenin-nick-translation mix (Roche), the volume adjusted with water up to 20 µl, and incubated at 15°C for 90 min. The reaction was stopped by adding 1 µl 0.5 M EDTA (pH 8.0) and heating to 65°C for 10 minutes.

The probe was further precipitated by adding 1/10 volume 3 M sodium acetate and 2.5 volume ice-cold 95% ethanol and kept for at least 30 min at –80°C or overnight at –20°C. After centrifugation, the precipitate was washed with 0.5 ml 70% ethanol, dried and resuspended in 20 µl water. Per slide, 50 ng of labelled probe plus 500 ng of sonicated salmon sperm DNA was dissolved in 6 µl deionised 100% formamide and 6 µl 20% dextran sulfate in 4x SSC was added. The hybridization mix was denatured at 80°C for 10 min and cooled immediately on ice to avoid renaturation.

3.9.3. *In situ* hybridization

Before hybridisation, slides were first baked at 60ºC for 30 minutes, washed for 3x 5 minutes with 2x SSC, and then treated with 100 µl of 100 µg/ml RNase A (in 2x SSC) under a piece of parafilm for 1 hour at 37°C in a humid chamber. After RNase treatment, the slides were washed 3x 5 minutes with 2x SSC in a coplin jar and treated with pepsin (100 µg/ml in 0.01 M HCl) for 5-15 minutes at 37°C under a piece of parafilm and washed for 3x 5 minutes with 2x SSC. Then the slides were fixed in 4% paraformaldehyde (dissolved in 1x PBS) at RT for 10 minutes, washed for 3x 5 minutes with 2x SSC, and dehydrated by incubation for 5 minutes in 70%, 96% and 100% ethanol. Next, the chromosomal DNA was denatured by immersion the slides in 70% formamide containing 2x SSC at 70°C for 2 minutes, followed by dehydration for 3 minutes in 70%, 96% and 100% ice-cold ethanol. The probe was applied on prewarmed to 45°C slides, covered with pre-warmed cover slips, avoiding air bubbles. The edges of the cover slips were sealed with rubber cement to avoid evaporation and the slides were incubated at 37°C overnight in a humid chamber.

3.9.4. Detection of hybridization sites

After overnight incubation cover slips were removed, slides were washed 15 minutes at 42ºC in 2x SSC containing 50% formamide, and then 3x 5 minutes in 2x SSC at 42°C with agitation. Next, slides were incubated with 100 µl blocking solution (5% BSA, 0.1% Tween 20, 4x SSC) under parafilm for 30 min at 37°C. After removing the blocking solution, slides were probed with 50 µl of rhodamine conjugated anti-DIG antibodies raised in sheep and/or of

Alexa 488 conjugated streptavidin (diluted 1:100 in 1% BSA, 4x SSC, 0.2% Tween 20), covered with parafilm and incubated in the dark for 1 h at 37°C in a humid chamber followed by washing 3x 5 minutes with 4x SSC, 0.1 % Tween 20 at 42°C. To amplify DIG-signals, slides were incubated with 50 µl of rhodamine labeled donkey anti-sheep antibodies under parafilm in the dark for 1 hour at 37°C in a humid chamber, washed again 3x 5 minutes with 4x SSC, 0.1% Tween 20 at 42°C, and then the buffer was drained using a blower. Slides were then mounted with 10 µl of antifade containing 10 µg/ml DAPI (4´, 6-diamidino-2-phenylindole), covered with cover slips, and observed under the microscope. To observe each fluorescence, specific filters were used: DAPI filter (excitation wavelength 359 nm, emission wavelength 461 nm), FITC filter (excitation wavelength 490 nm, emission wavelength 525 nm) or rhodamine filter (excitation wavelength 540 - 560 nm, emission wavelength 580 nm). Pictures were taken by cooled sensitive charged-couple device (CCD) camera and pseudo-colored using Photoshop program (Adobe).

3.10. Indirect immunostaining

3.10.1. Chromosome spreads preparation

For immunostaining, the seeds were germinated in the same conditions as for FISH. The root tips were fixed in freshly prepared 4% paraformaldehyde solution (dissolved in 1x PBS) for 20 minutes on ice, then washed 3x 15 minutes in 1x PBS on ice. Cell walls were digested by treating the root meristems with an enzyme mix (2.5% Pectinase, 2.5% Cellulase R-10, 2.5% Pectolyase Y-2 dissolved in 1x PBS) at 37ºC until the material become soft (about 50 minutes). The macerated material was several times washed in 1x PBS. The squashing was performed in 1x PBS between slide and cover slip, then slides were immersed into liquid nitrogen and after immediate removal of the cover slips collected in a coplin jar with 1x PBS. For longer storage, slides were kept in 100% glycerol at 4°C.

3.10.2. Detection of antigens

First, slides were incubated with 100 µl blocking solution (8% BSA, 0.1% Tween 100, 1x PBS) under parafilm for 30 min at RT, and then washed 2x 5 minutes in 1x PBS. The slides were left to drain for a short moment and then probed with the primary antibodies (Abcam Ltd, UK and Upstate Biotechnology, USA) diluted in 1x PBS, 1% BSA in the following dilutions: 1:2000 (anti-H3K4me3), 1:500 (anti-H3K9me2), 1:200 (anti-H3K4me1,2; anti-

H3K9me1,3; anti-H3K27me1,2,3), and 1:100 (anti-H4K20me1,2,3) overnight at 4°C. After washing 2x 5 minutes in 1x PBS, slides were treated with the secondary antibodyies conjugated to Cy3 for 1 hour at 37°C under parafilm in a humid chamber followed by washing 3x 5 minutes in 1x PBS in the dark. Afterwards, slides were mounted with 10 µl of antifade containing 10 µg/ml DAPI (4′, 6-diamidino-2-phenylindole) covered with cover slips and observed under the microscope. Slide observation and signal capture were performed as was described for FISH.

3.11. Small RNA analysis

Small RNA analysis was performed according to Mette et al. (2005)

3.11.1. Enrichment of small RNA

Total RNA isolated from 1g leaves or anthers by standard Tryzol method (Chomczynski and Sacchi, 1987) was dissolved in 3.87 ml double distilled water. The large RNA was separated from small one by adding 0.5 ml 5M NaCl and 0.63 ml of 40% polyethylenglycol MW8000. The mixture was kept on ice for 30 minutes and centrifuged at 9000 rpm. Small RNA which remained in supernatant was precipitated with 100% ethanol in presence of 1/10 3M sodium acetate at -20°C for 2 hours. After centrifugation the pellet was washed with 70% ethanol, dried and resuspended in 60 µl H_2O. Small RNA was checked by MOPS-formaldehyde gel electrophoresis and quantified spectrophotometrically at 260 nm.

Equal amounts of small RNA (60 µg) were mixed with formamide loading buffer, heated at 95°C for 5 min, and resolved in 15% denaturing acrylamide 0.225x TBE gel electrophoresis under denaturing conditions (7M Urea). Decade RNA markers (Ambion) were used as size marker. To equalize the migration speed of markers and probes, 60 µg of commercial tRNA (Sigma) was added to markers. The gel was stained with ethidium bromide washed in 0.5x TBE for 20 min. The RNA was further electroblotted to a Zeta-Probe nylon membrane (Bio-Rad) for 1 hour at 10V using Trans-Blot semidry transfer cell (BioRad). After transfer, the membrane was briefly washed in 0.5x TBE, air dried and vacuum-dried at 80°C for 2 hours to fix the RNA to membrane.

3.11.2. Labelling of small RNA probes

RNA probes were synthesized according to the RNA in vitro transcription kit (Fermentas). Each reaction contained 1 µg plasmid DNA, 7µl 5x transcription buffer, 20mM rNTP (N=A,

G, C), 12.5 µl [α-^{32}P] UTP 125 µCi (GE Healthcare), 3 µl RiboLock RNase inhibitor (20U/µl), 2 µl T7 RNA polymerase(20U/µl), and H_20 up to 35 µl. The synthesis was performed at 37° C for 2 hours. The plasmid DNA template was removed by incubation for 15 minutes with 1µl of RNase-free DNAse I (10U/µl, Roche). The probes were further hydrolyzed in 500 µl carbonate buffer (120mM Na_2CO_3/ 80mM $NaHCO_3$) for 4 hours at 60°, and neutralized by adding 35 µl 3M sodium acetate.

3.11.3. Small RNA hybridization

The hybridization solution contained 125 mM sodium phosphate buffer (pH 7.2), 250 mM NaCl, 7% SDS and 50% deionized formamide. Membranes were prehybridized for 2 hours at 42°C, followed by hybridization with the probe overnight under the same conditions. After hybridization, the membranes were washed 2x for 30 minutes in 2x SSC, 0.2% SDS at 42°C and exposed to X-ray film overnight. To remove unspecific hybridization signals, the membranes were treated with RNase A and then exposed again to X-ray film for 5 days.

D1100 positive control was prepared with ShortCut RNAi Kit (New England Biolabs). Therefore, RNA first was synthesized from both strands of the fragment inserted in pGEM-T Easy vector by in vitro transcription. The RNA was further mixed and reanealled by heating at 90ºC for 3 minutes and slow cooling. The double-stranded RNA was processed by ShortCut RNase III digestion in the presence of manganese resulting in a heterogeneous population of short (18-25 bp) small interfering siRNAs. Different amounts of control siRNA were loaded in parallel to the experiment to make sure that the hybridization is specific and to quantify approximately the amount of small RNAs formed.

4. Results

To study the transcription behavior of rye B chromosomes, we employed three different approaches. (1) The chromatin properties of B chromosomes were analyzed. Therefore, the epigenetic marks (histone H3 and H4 methylation) of A and B chromosomes were compared by indirect immunofluorescence. (2) To assess the transcription-status of the predefined B chromosome-specific repeats D1100 (Sandery et al., 1990) and E3900 (Blunden et al., 1993), we used Northern hybridization and RT-PCR. (3) In order to identify novel B-specific transcripts, cDNA-AFLP analysis was conducted on 0B and +B rye plants.

4.1. The chromatin properties of A and B chromosomes are partly different

The structure of B chromosomes was examined in terms of epigenetic marks by examining the methylation status of certain lysine residues in the amino terminal tails of histone H3 and H4. Cells were stained with antisera that discriminate between mono-, di- and trimethylated lysines (K4, K9 and K27 of H3 and K20 of H4). The results are summarized in table 6 and fig. 4 and 5.

The chromosomal distribution patterns observed for the heterochromatin marks H3K9me1 and H3K9me3 were similar to those reported for H3K9me2 for both A and B chromosomes, with a uniform distribution throughout chromosome arms; and this is characteristic for plants with large genomes (Houben et al., 2003). While the terminal heterochromatic regions of As and Bs were H3K27me1 deficient, the same chromosomal regions were enriched in di- and trimethylated H3K27 (Fig.4). Staining specific for H4K20me1,2,3 resulted in a weak and disperse labeling (Fig. 5). These patterns are independent of the number of Bs present.

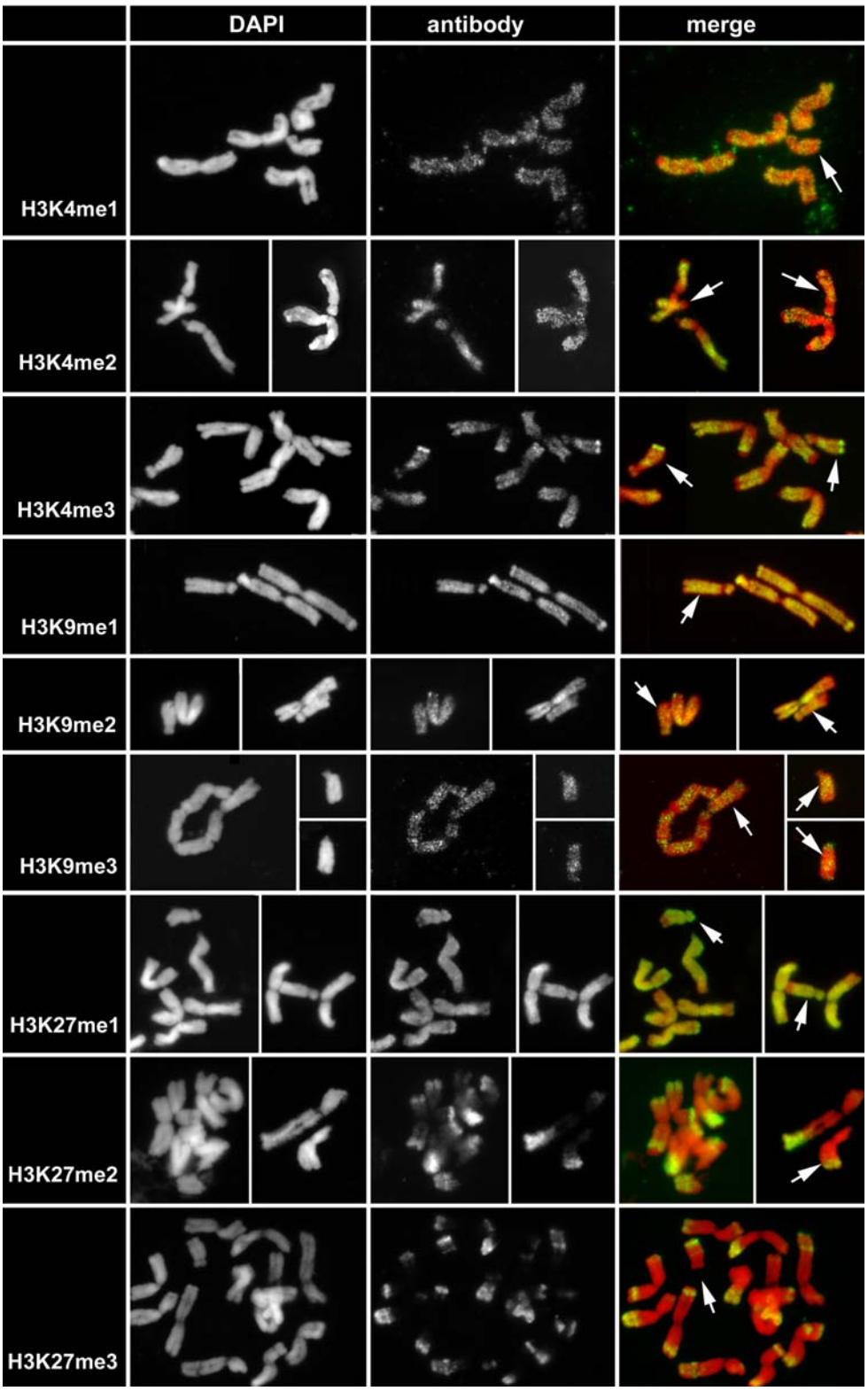


Fig.4. Histone marks of A and B chromosomes. Metaphase cells of rye with Bs (arrowed) after immunostaining with antibodies specific for histone H3K4me1,2,3; H3K9me1,2,3 and H3K27me1,2,3.

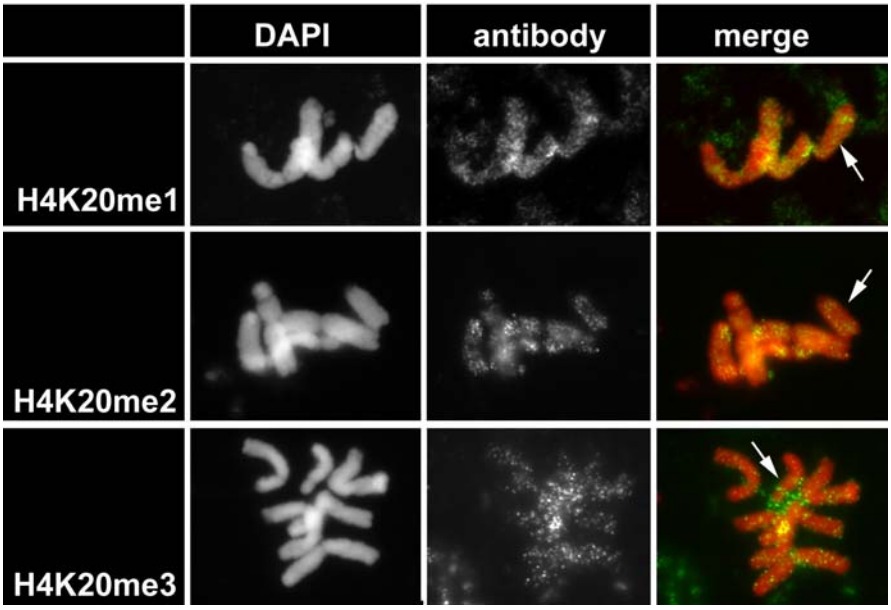


Fig. 5. Metaphase cells of rye with Bs (arrowed) after immunostaining with antibodies specific for histone H4K20me1,2,3.

Table 6. Spatial relationship of a variety of histone H3/H4 and DNA modifications with the mitotic rye B chromosome.

	B-terminal domain	**B without terminal domain**
H3K4me1	dispersed	dispersed, except pericentromere
H3K4me2	dispersed	dispersed, except pericentromere
H3K4me3	strongly increased	dispersed, except pericentromere
H3K9me1	dispersed	dispersed, except pericentromere
H3K9me2	dispersed	dispersed
H3K9me3	weakly dispersed	weakly dispersed
H3K27me1	deficient	dispersed
H3K27me2	dispersed	deficient
H3K27me3	dispersed	deficient
H4K20me1	weakly dispersed	weakly dispersed
H4K20me2	weakly dispersed	weakly dispersed
H4K20me3	weakly dispersed	weakly dispersed

The highly conserved euchromatin-specific methylation mark at lysine 4 of H3 revealed a B-domain-specific distribution pattern. H3K4me1,2 preferentially mark euchromatin of A and B chromosomes, although the heterochromatic B subterminal domain displays some level of

labeling. There is a higher density of labeling in chromosome arms, a decrease in pericentromeric regions and an absence of signal in heterochromatin. This is particularly evident in the distal heterochromatic blocks of rye A chromosomes that are brightly DAPI-stained (Fig. 4). Surprisingly, the largest signal of H3K4me3 was seen in the terminal part of the long arm of the Bs. To be more precise, it superimposes with the distal block of the D1100/E3900 positive region that forms the most prominent DAPI stained region in these chromosomes. This immunostaining pattern is dependent on the presence of the B-specific domain; since morphological variants of the B chromosome that lack the terminal part of the long arm do not show any particular enrichment in H3K4me3. At interphase no pronounced H3K4me3-labelling was found, most likely due to decondensation and therefore reduced intensity of immunosignals. Alternatively, but less likely, a cell cycle-dependent B-domain-specific demethylation of H3K4me3 occurs. The H3K4me3 distribution pattern is identical for plants with different numbers of Bs.

In conclusion, we can summarize that investigation of comparative histone modification patterns of A and B chromosomes revealed that the subtelomeric region of long arm of B chromosomes, which according to Giemsa banding are strong heterochromatic domains, displayed association with euchromatin specific modification H3K4me3. This was the first indirect evidence of B chromosomes activity in rye and led to investigate the activity of the sequences located in this region.

4.2. B-specific high-copy sequences D1100 and E3900 are transcriptionally active

The abundance of trimethylated lysine 4 of histone H3 and the decondensed structure at interphase of the B-subtelomeric domain (Langdon et al., 2000; Carchilan et al., 2007), prompted us to investigate whether transcriptional activity of this region could be detected. The B-terminal region is mainly composed of arrays of E3900 and D1100 repeats (Langdon et al., 2000) and these were used as probes in a Northern blot analysis of total RNAs prepared from plants with and without B chromosomes. As shown in Fig. 6A probe E3900-0N, which covers the entire length of E3900, detected a continuum of transcripts ranging in size from 6.5 kb to less than 0.2 kb. These transcripts were mainly present in plants containing B chromosomes. In addition, bands of 2, 3 and 5 kb were detected over the smear.

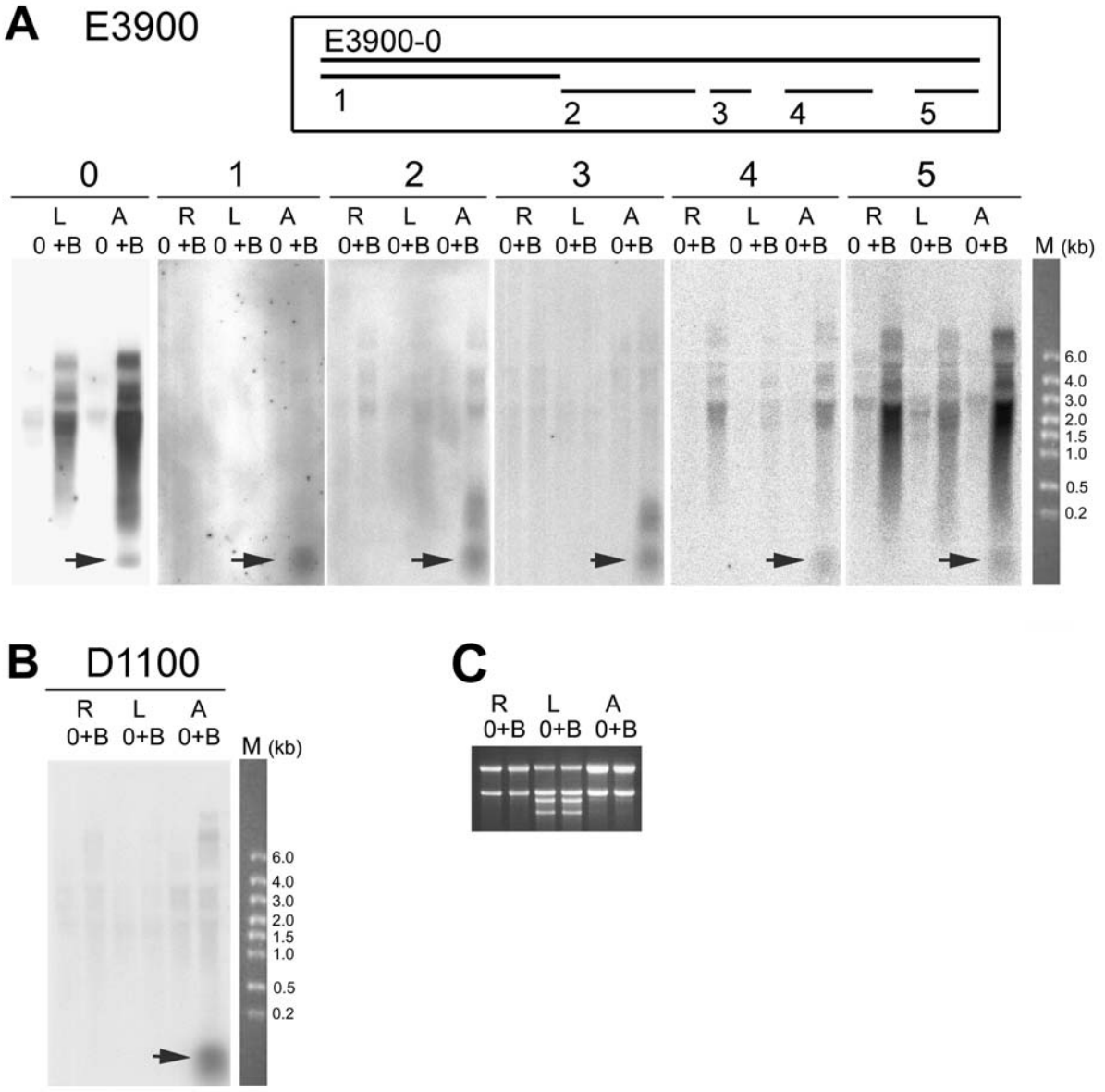


Fig. 6. Transcriptional analysis of E3900 and D1100.

RNA gel blot analysis of (A) E3900 and (B) D1100 transcripts isolated from roots (R), leaves (L) and anthers (A) of 0B/+B rye. Schemata indicates the different regions (0N – 5N) of E3900 used as hybridization probes. Arrows indicate B-specific signals of small size (< 200 bases). (C) Loading control, ethidium bromide staining of the RNA gel. Size marker according RiboRuler RNA Ladder (Fermentas).

Weak hybridizing bands in 0B material could be explained by cross hybridization with homologous transcribed A chromosome sequences. In order to analyze whether the entire E3900 repeat is transcriptionally active or only parts of it, five subregions of E3900 were used as Northern probes. All regions revealed cross hybridization with RNAs of small size (< 200 bases, arrowed) derived from anthers with B chromosomes; with the highest level of

transcription at the end of the 3900 repeat (region 5N). Weak hybridizing bands in 0B material could be explained by cross hybridization of short microsatellite sequences located within the 3'-region of E3900 (region 5N) (Langdon et al., 2000).
Anther- and B-specific hybridization of small size transcripts were also found for D1100 (Figure 6B, arrowed).

To further characterize the transcribed sequences, RT-PCR experiments were conducted on total RNA isolated from roots, leaves and anthers of 0B and +B rye and wheat Lindström plants. E3900-transcripts were amplified from all subregions of the repeat (Fig. 7A). The primer pair D1100-1RT allows the amplification of a D1100-specific fragment of expected size (Fig. 7B). In each cDNA sample derived from RNA of B-containing plants a product of the expected size, which was not present in any sample derived from the RNA of plants lacking Bs, was amplified. As indicated by Northern hybridization (Fig. 6), there were differences in yield in many cases, mostly showing a higher abundance of B-transcripts in anthers rather than in roots and leaves.
As control, RT-PCR with primers specific for the elongation factor eEF1 alpha designed on barley sequence with accession number Z50789 published by Nielsen et al. (1997) was performed. It showed a comparable yield in all three tissues, and the differences were not due to unequal RNA loading. Notably, although the same number of PCR cycles were used to amplify the different E3900-subregions, as was demonstrated by Northern hybridization (Fig. 6), regions RT4 to RT6 revealed the highest level of transcription with less tissue type specificity.

Control experiments (Fig. 7C) with RNA of all samples without an initial reverse transcription step (lanes -RT) and on genomic template DNA (lane g) confirmed that the PCR products are unequivocally dependent upon reverse transcription of RNA molecules originating from transcribed E3900/D1100-repeats on the B chromosomes. This result confirms that the B-located E3900/D1100 repeats are transcribed. Hence, both types of B-specific repeats are transcriptionally active, although with different tissue-type dependent activity.
RT-PCR products amplified from anthers were sequenced. All of the sequences obtained (Table 7) shared a high similarity with either D1100 or E3900 repeats. Both repeat transcripts are at least partly polyadenylated, since all D1100/E3900 RT-PCR products were obtained from cDNA synthesized with poly-dT-primers. In addition, Northern hybridization of both

repeats showed cross hybridization with fractions enriched for polyadenylated RNA; although the E3900 revealed a significantly stronger signal in the polyadenylated fraction than the D1100 (Fig. 8).

Table 7. Sequence comparison of RT-PCR products with published E3900 and D1100 genomic sequences.

	Positions	Similarity[1]	Accession numbers
E3900 1RT	253-1587	99%	EF566937
E3900 2RT	1606-2610	98%	EF566938
E3900 3, 4, 5RT	2427-3688	97%	EF566939
E3900 6RT	3597-3940	96%	EF566940
D1100 1RT	57-857	92%	EF538668

[1]RT-PCR E3900 sequences were compared to the sequence AF222021.
[1]RT-PCR D1100 sequence was compared to the sequence Z54196.

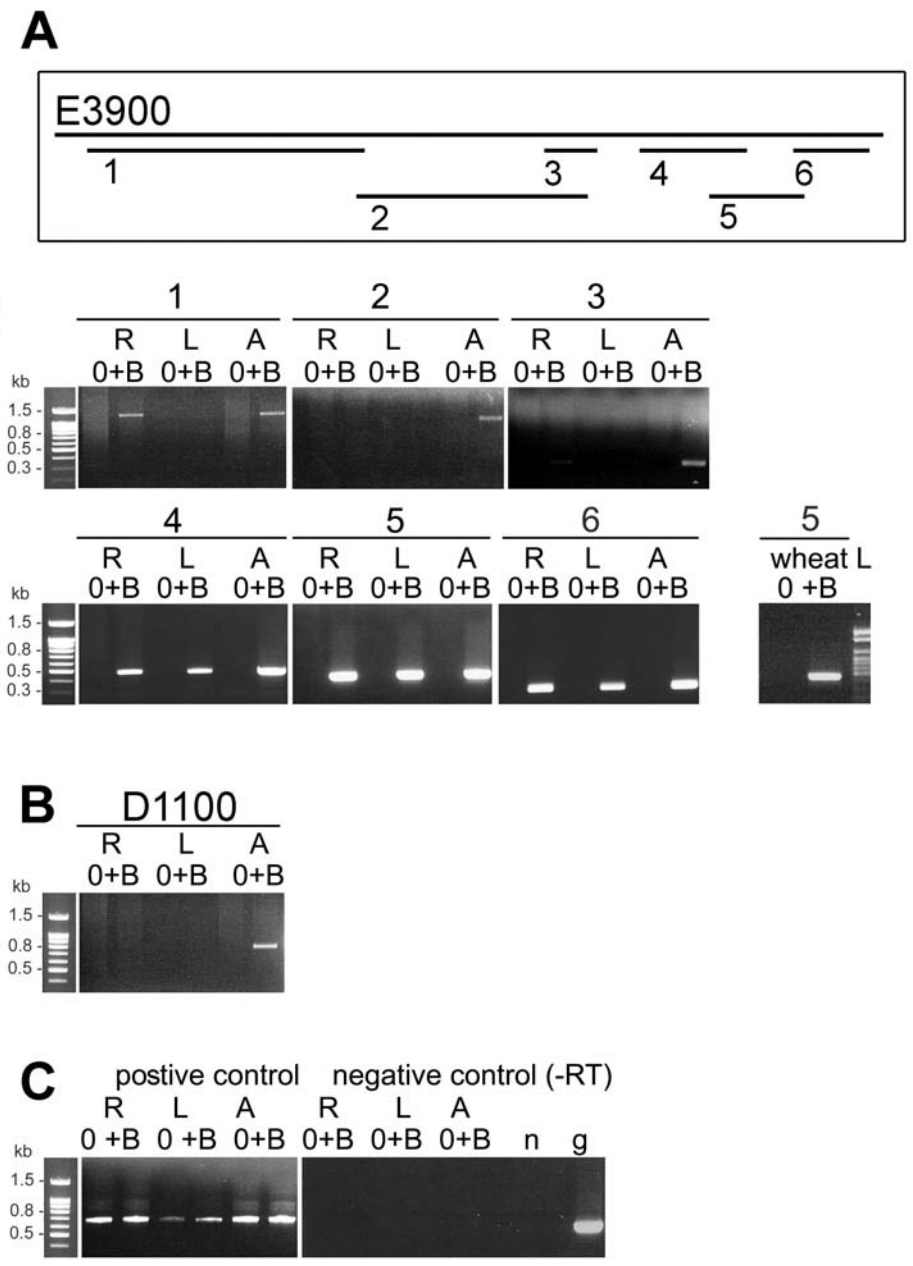


Fig. 7. Transcriptional analysis of E3900 and D1100.

(A) E3900 and (B) D1100 RNA abundance assessed by semi-quantitative RT-PCR on root (R), leaf (L), and anther (A) tissue of 0B/+B rye and 0B/+B wheat. Schemata indicate the different regions of E3900 and D1100 amplified by RT-PCR. (C) Controls: Positive control using B chromosome independent primers (eEF1 alpha elongation factor). Negative control using RNA of all samples without an initial reverse transcription step to demonstrate the absence of genomic DNA contamination, (n) PCR without template DNA and (g) PCR on genomic +B DNA. Each sample contained approximately the same amount of RNA.

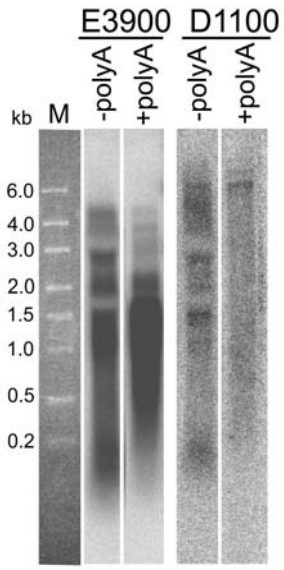


Fig. 8. D1100 and E3900 undergo polyadenylation. Northern hybridization of D1100 and E3900 with total RNA separated into poly(A)+ and poly(A)- fractions. A probe specific for actin was used as a poly(A)+ positive probe.

4.3. D1100 transcripts are coming mostly from one strand, while E3900 is transcribed from both strands generating heterogeneous products

To determine whether transcription of E3900 and D1100 occurs from one strand or both strands, strand specific RT-PCR was performed on +B rye cDNA isolated from anther tissue. Therefore, for synthesis of the first strand sequence specific primers F5, R5, F3 for E3900 and F and R for D1100 were used instead of oligodT primers, for each primer performing a separate reaction. Primer combinations used for second strand amplification included one primer used for first strand synthesis and another designed on the complimentary strand (indicated in parenthesis Fig. 9). The PCR products were separated in TAE agarose gel electrophoresis. E3900 F5R5 strand specific PCR should result in products of 460 bp and E3900 F3R5 1200 bp. For D1100 FR strand specific PCR products of 800 bp were expected. The expected RT-PCR products are indicated with arrows (Fig. 9). Analysis of the products indicates that the transcripts of E3900 are coming from both strands. D1100 is transcribed predominantly from forward strands generating a number of fragments different in size. The amplification from the reverse strand resulted in a very faint band of expected size. The heterogeneous size of PCR products could be explained by the complexity of the repeat organization. Both repeat families are composed of different copy numbers of tandemly

organized repeats which are intercalated with degenerated mobile elements (Sandery et al., 1990; Blunden et al., 1993; Langdon et al., 2000).

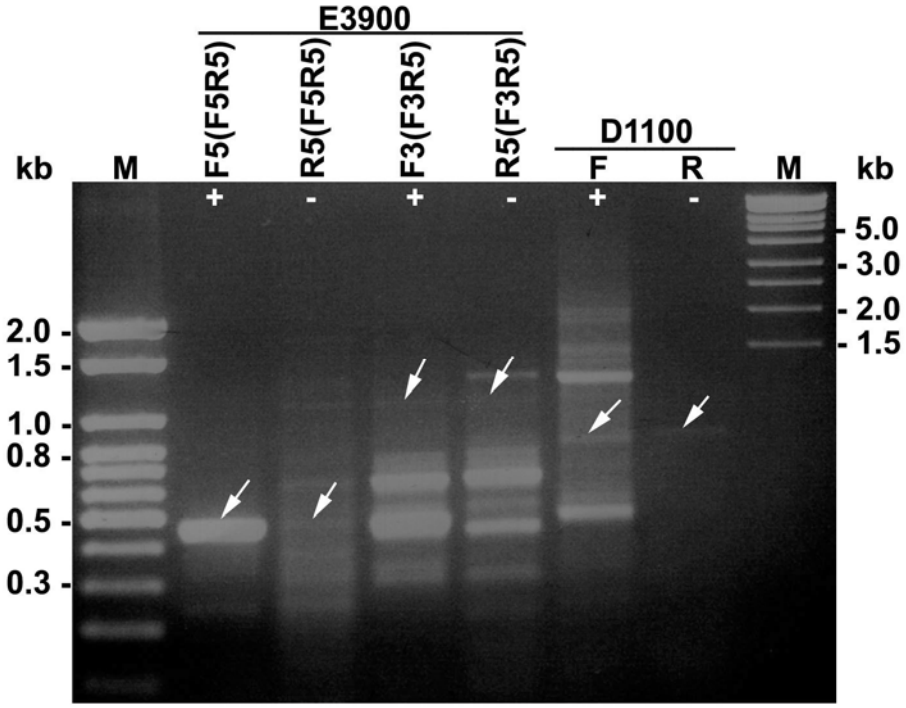


Fig. 9. Strand specific RT-PCR of E3900 and D1100. With "+" are marked the PCR products analysis for which the cDNA was synthesized from forward strand and "-" from reverse strand.

From strand specific PCR results we can conclude that the transcription of E3900 occurs from both strands; D1100 is mostly transcribed from one strand though the second strand also showed very weak transcription. These RNAs could lead to the formation of double stranded RNA which can be further processed into small RNA.

4.4. E3900 and D1100 transcripts are not processed into small RNA

In order to determine whether the D1100 and E3900 transcripts are processed into small RNA (smRNA), we performed Northern analysis, using low molecular weight RNA isolated from anther and leaf tissue of plants with and without Bs. The blots were hybridized with the E3900-specific probe 3N and the cloned RT-PCR product of D1100 using the primer pair combination 1100 forward and 1100 reverse (Table 5).

Neither probe detected any B-specific smRNAs. However, Bs-independent hybridization signals corresponding to 21 to 24 nucleotides were observed for anthers but not for leaves RNA (Fig. 10). This result implies that the majority of D1100 and E3900 transcripts escaped

processing by the RNAi machinery in leaf and anther tissue. In order to check probe quality the D1100 probe was hybridized with artificially generated smRNAs, and hybridization of 21 nucleotides was found, depending of the amount of control smRNA loaded (Fig. 10A, arrowed).

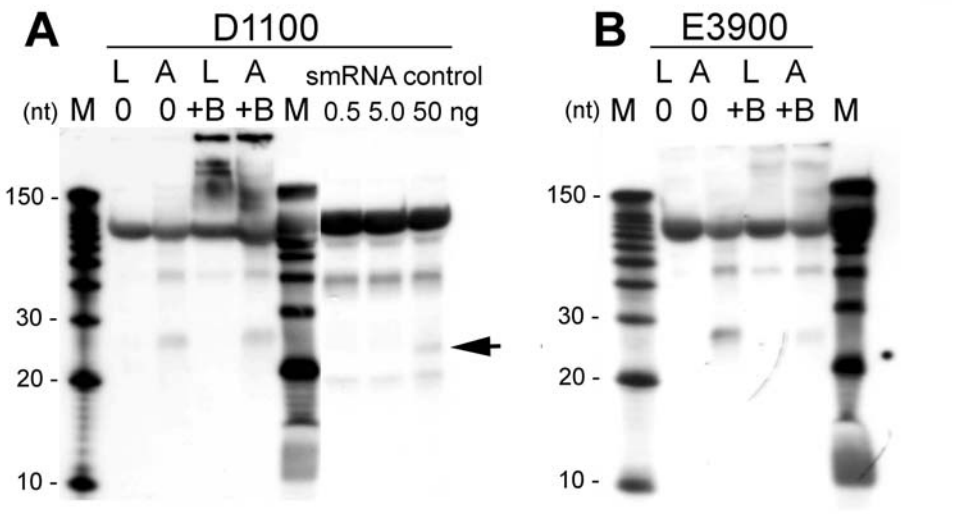


Fig. 10. Analysis of small RNA of (A) D1100- and (B) E3900-B chromosome-specific sequence in leaf (L) and anther (A) tissue. Positive control with artificially generated small RNA of D1100 demonstrates cross hybridization with a 21 nucleotide long fragment (arrowed).

B specific small RNAs were detected neither for E3900 nor for D1100. However for both repeats, hybridization with RNAs bigger than 100 nt occurred in + B material (Fig.10) similarly to previous findings by Northern analysis (Fig. 6).

4.5. Comparative cDNA-AFLP analysis of 0B/+B rye plants reveals weak B-specific activity

To determine the general transcriptional activity of B chromosomes comparative cDNA AFLP analyses was performed on following three different nearly isogenic rye 0B/+B lines: 1 - 7415, 2 - P 12 and 3 - 2677 (Jimenez et al., 1994; Ortiz et al., 1996). This method allows identification of differentially expressed sequences without prior sequence information. The differences in the intensity of bands provide a good measure of the relative differences in the levels of gene expression.

First RNA was isolated from leaf and anther tissue using the Trizol method. For RNA isolation, young leaves (first and second leaf from top) coming from several (7-10) plants with

or without B chromosomes of the same inbred line were used. The anthers were first staged microscopically. All meiotic stages including tetrads were considered. Staged anthers were collected and pooled from several plants for RNA isolation. The RNA quality was checked by formaldehyde gel electrophoreses (Fig. 11) and further quantified spectrophotometrically. Double stranded cDNA was synthesized using SuperSmart cDNA synthesis kit (Clontech). The quality of cDNA was checked by TAE agarose gel electrophoresis.

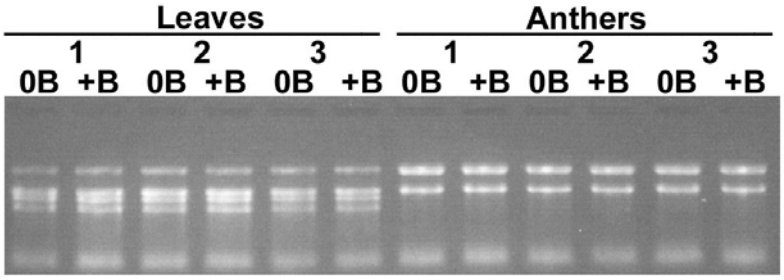


Fig. 11. Quality check of RNA isolated from 0B/+B leaf and anther tissue of the rye lines: 1 - 7415, 2 - P 12 and 3 – 2677.

63 primer combinations (Table 2 and 3) were used for the AFLP analysis. For analysis, transcription profiles of plants with and without B chromosomes were compared for each individual line and across the three different rye lines.

The patterns obtained after gel separation of the PCR products amplified from plants with and without B chromosomes didn't show major differences. The uniform profile of main transcripts between different lines proved that the cDNAs were equalized. The degree of variability of transcripts due to the presence or absence of B chromosomes was lower than the variability between the three inbred lines due to the different origins of the rye lines (Fig. 12).

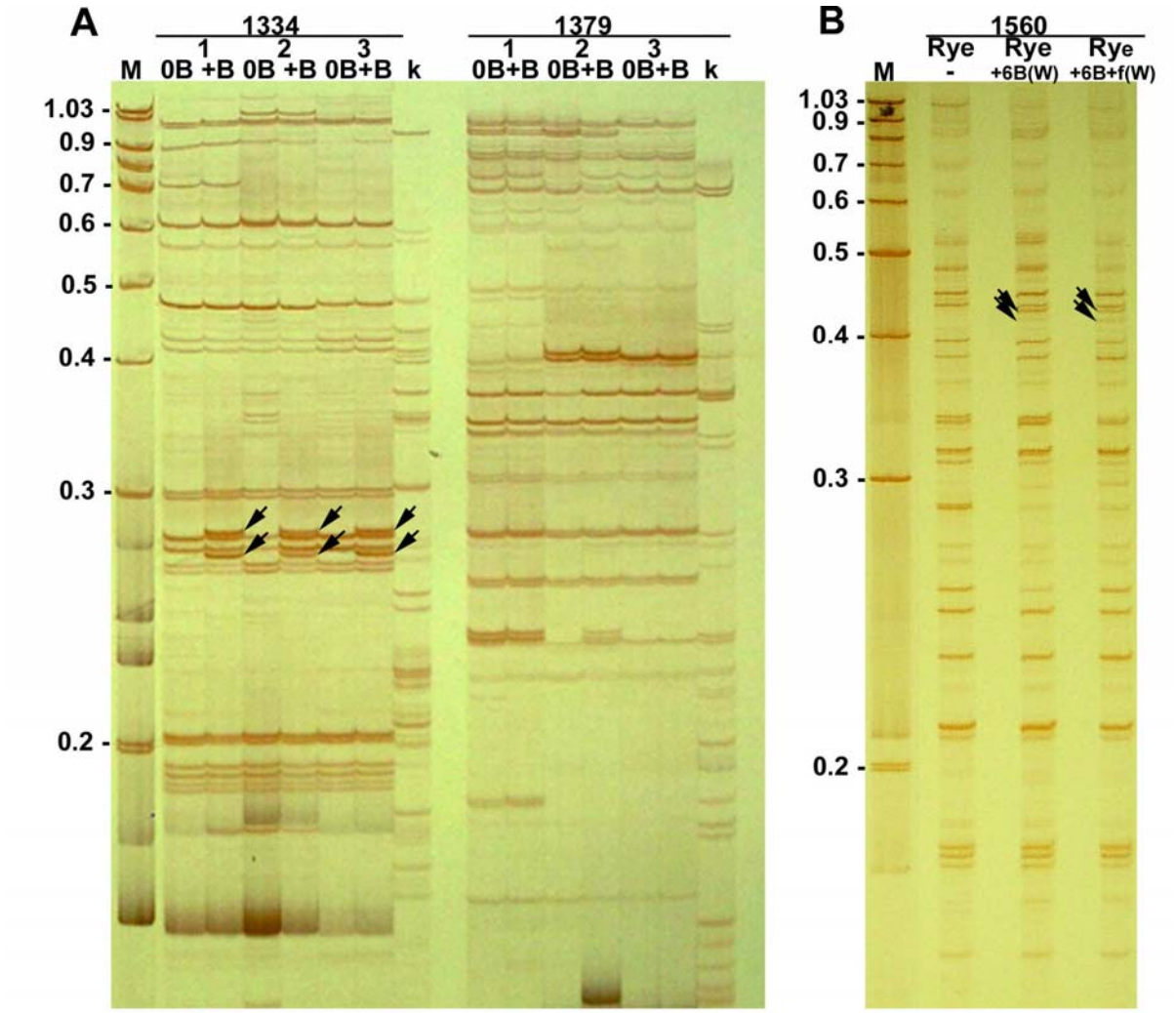


Fig. 12.
A - cDNA AFLP fingerprint obtained after separation of PCR products amplified by primer combinations 1334 and 1379. 1, 2, 3 correspond to the different rye inbred lines used without (0B) or with addional B chromosomes (+B). k – negative control. The positions of B-extra bands are arrowed.

B - cDNA AFLP fingerprint analysis of rye lines with and without an additional wheat chromosome 6B (6B(w)) and small wheat fragments (f(w)) obtained using the primer combination 1560. 1, 2, 3 correspond to the different inbred lines used. Additional bands which are visible in rye-wheat addition lines (6B) are arrowed.

The adaptors and primers present in the restriction-ligation mixture, preamplification and amplification reaction can result in a number of unspecific PCR products. Therefore, for each restriction ligation and amplification step, negative controls without template DNA were included (see Fig. 12**A**, lane (k)). The control PCR for each primer combination was run in parallel with the experimental probes. For counting, only clearly visible bands were

considered; for example 43 bands were counted for the primer combination S13M34 shown in Fig.12**A**. The major bands amplified by each primer combination in all three inbred lines were screened and the additional bands present in +B material and 0B material were counted (Table 8). A PCR product was not considered if it coincided in size with a band of the negative control PCR.The size range of amplified cDNA-fragments was between 80 and 1200 base pairs.

Table 8. Total number of cDNA AFLP bands and of additional bands present in 0B or +B-positive plants only.

Number	Primer combination		Average number of bands per line*	Additional B bands			Additional bands in OB only		
				in 1 rye line	in 2 rye lines	in 3 rye lines	in 1 rye line	in 2 rye lines	in 3 rye lines
1	S11	M48	58	2	1		2		
2	S11	M79	32		2		1		
3	S13	M32	33	2					
4	S13	M33	56				1	1	
5	S13	M34	43			2***	3		
6	S13	M47	32				1		
7	S13	M48	31	3				1	
8	S13	M49	45		1		1		
9	S13	M55	38						
10	S13	M63	45			1*	1		
11	S13	M64	41		2				
12	S13	M79	42	1					
13	S13	M80	52				4		
14	S15	M51	45		1		3		
15	S15	M52	21	7					
16	S15	M53	53	1	3		1		
17	S15	M55	41	5			2		
18	S15	M59	31			1**		1	
19	S15	M60	40	1			2		
20	S15	M61	35				2		
21	S15	M62	40					1	
22	S15	M65	34			1***			1
23	S17	M34	41	2		1**	3	2	
24	S17	M48	39	3		1**	2		
25	S17	M49	41	1	2		3		
26	S17	M51	36	1			2	1	
27	S17	M61	27		1				
28	S17	M62	31				4	2*	
29	S17	M65	26						
30	S24	M34	40	1	2		2	1	
31	S24	M48	39	5	1		5	1	
32	S24	M49	18				1		
33	S24	M52	55	5		1*	1		
34	S24	M53	47	7		1*	3	1	
35	S24	M55	49	5	2		11	1	
36	S24	M59	40	4	3		5	1	
37	S24	M60	27				1	3	
38	S24	M61	45				3	1	
39	S24	M62	41	2	1		6	1	1
40	S24	M65	40	6		2***		1	
41	S25	M65	31			1**	1		
42	P47	M47	42		1	1**			
43	P47	M49	28				1		
44	P47	M55	48	1		1***	1	1	
45	P47	M63	34				1		
46	P47	M64	33				1		
47	P47	M80	44		1				
48	P48	M32	41						
49	P48	M33	35				2		1
50	P48	M49	31				1		

Number	Primer combination		Average number of bands per line	Additional B bands			Additional bands in OB only		
				in 1 rye line	in 2 rye lines	in 3 rye lines	in 1 rye line	in 2 rye lines	in 3 rye lines
51	P79	M48	30	1	2		1		
52	P79	M55	20				1		
53	P81	M32	21	1			2		1
54	P81	M33	20						
55	P81	M34	34			1**	1		
56	P81	M47	37						
57	P81	M48	18						
58	P81	M49	19			1*	3		
59	P81	M55	36	1			4		
60	P81	M63	25				3		
61	P81	M64	36				2		
62	P81	M79	19	1	1		3		
63	P85	M49	34		1	1	3		
Total			2286	69	27	16	107	21	4
			100%	3.01%	1.18%	0.7%	4.68%	0.92%	0.18%

* weak , ** middle, *** strong intensity of band

colored in gren - additional fragment analyzed in 0B material

colored in red – additional fragments analyzed in +B material

Between 18 and 58 cDNA-AFLP bands were observed per primer pair combination and in total around 2286 bands present in all three lines (0B/+B material) were detected. 112 extra bands (4.9% of average number of bands per rye line) were found in B chromosome containing material of either 1, 2 or all 3 rye lines. However, only 16 B-extra bands (0.7%) of different intensity were consistently found in all three rye lines. These 16 bands were considered as putative B chromosome-derived transcript candidates. B-extra bands, which were detected in one or two rye lines only, were not further analyzed since the chance of having a truly B chromosome derived transcript was low. Since the B chromosome also could influence the transcription behavior of the A chromosome complement as postulated by Delgado and colleagues (1995; 2004), the number of additional bands in 0B material was determined too. 132 extra bands (5.8%) were found in material without B chromosomes of 1, 2 or all 3 rye lines. However, only four 0B extra bands (0.18%) of different intensity were consistently found in all three rye lines.

The cDNA-AFLP data suggests that additional B chromosomes are capable of influencing (but weakly) the transcription behavior of the genome.

4.6. cDNA-AFLP analyses of rye-wheat addition lines confirms activity of an extra A chromosome

To test whether the number of cDNA-AFLP bands would increase if a transcriptionally active chromosome has been added to the rye genome, the transcription profile of rye was compared

to the profiles of two rye-wheat chromosome addition lines carrying chromosome 6B (here 6B means chromosome 6 of wheat B genome) and small wheat fragment translocated to one of the rye chromosomes (Fig. 3). Using four primer pair combinations (S15M51, S15M53 S15M60 and P81M49) the analysis revealed a number of additional bands if the extra wheat chromosome 6B was present (Fig. 12 B, Table 9). Although the number of cDNA-AFLP experiments performed to study the effect of an additional wheat chromosome was lower than to analyse rye B chromosomes (4 versus 63), the outcome of cDNA-AFLP analysis indicates a higher number of extra bands if the transcriptionally potent wheat chromosome 6B co-exists.

Table 9. Comparative cDNA-AFLP analysis of rye and a rye-wheat chromosome addition lines carrying chromosome 6B and a small wheat chromosome fragment translocated to one of the rye chromosomes.

Number	Primer combination		Average number of bands per line	Additional bands in both +wheat 6B addition lines
1	S 15	M 60	46	4
2	S 15	M 51	20	-
3	S 15	M 53	28	3
4	P 81	M 49	50	3
Total			144	10
			100%	6.9%

To analyze whether the observed B-linked extra transcripts are encoded by the B chromosomes itself, additional bands which were present in the +B material of all three inbred lines were further analyzed. Therefore, 9 out of 16 +B-extra bands were isolated from the silver-stained gel and the DNA was eluted and reamplified using corresponding PCR primers. Afterwards, the PCR products were cloned, sequenced and compared to the published sequences (Table 10).

The rye B 1334 clone showed 93.5% similarity to the transposase region of a rye retrotransposon (AB124645). The sequence of rye B 8149 candidate is very close to a *Triticum aestivum* cDNAs (CK215639, DR739424) and a LINE non LTR transposon (55%). Rye B 2465 clone sequence gave 95.8% similarity to a *Triticum aestivum* clone which contain the repetitive sequence pSc 119.1 intercalated with transposable elements (Table 10).

Table 10. Sequence similarity comparison of B-specific candidate.

Name of clones	Primer combination	Similarity to published sequences and sequence type	Length of transcript
B 1334	S13 M34 and P81 M34 amplified the same sequence	*Secale cereale* Revolver-cDNA mRNA for transposase, complete cds. AB124645 (93,5%)	272 bp
B 8149	P81 M49	*Triticum* LINE element (55%), nonLTR retrotransposon, *Triticum aestivum* cDNA CK215639, DR739424 (85%) cold treatment	571 bp
B 1553	S15 M53	*Triticum aestivum* Zeananthin epoxidase AF384103 (89,5%), *Triticum aestivum* after different stress BG909303, CJ639347, BG905687, CJ664007 (91%)	291 bp
B 1559	S15 M59	*Triticum aestivum* cDNA DY761155 (96%) abiotic stress, unknown function	286 bp
B 2448	S24 M48	*Triticum aestivum* cDNA CD884985 (91%) unknown function	261 bp
B 2442	S24 M52	*Triticum aestivum* Mal-like protein mRNA AF538039 (87.8%)	280 bp
B 2453	S24 M53	*Triticum aestivum* cDNA DN829487 (97%) waterlogging-stressed seedlings, BI750881 (96%) Fusarium graminearum srayed, Hordeum vulgare subsp. spontaneum cDNA AV938960 (97%)	630 bp
B 2465	S24 M65	*Secale cereale* clone L155-119.1 pSc119.1-1 repeat sequence. EF165546 (95.8%) *Triticum monococcum* gypsy-like retrotransposon AF326781 (89.5%)	1444 bp
B 2565	S25 M65	*Zea mais* transposon-like sequence (59%) gi|25992760|gb|AF546187.1|[25992760]	242 bp

To further characterize 3 (B 1334, B 8149, B 2465) of the 9 cloned putative B-specific transcripts RT-PCR, Southern, Northern and *in situ* hybridization were performed. First Southern hybridization was performed to determine the relative copy number and organization of the isolated sequences. Further, if the abundance of the candidate sequences was sufficient, *in situ* hybridization was performed to localize their chromosomal position. To reconfirm the B-specific transcriptional activity of the candidate sequences, Northern hybridization and RT-PCR were performed. As the sequences located on B chromosomes share high similarity with those located on rye A chromosomes (Timmis et al., 1975), a wheat-rye B chromosome addition line (Lindström line) with and without B chromosomes was analyzed in parallel with those of rye (Müntzing, 1970).

4.7. Characterization of B chromosome-derived transcriptionally active sequences

4.7.1. Genomic organization of B chromosome-associated transcriptionally active sequences analyzed by Southern hybridization

The outcome of Southern hybridization experiments indicate that B 1334, B 8149 and B 2465-like sequences are members of high-copy sequence families with multiple locations in the genome of rye (Fig. 13-18).

The hybridisation patterns of rye B 1334 and rye B 2465 with digested genomic DNA of rye with and without Bs were comparable (Fig.13, 17). In addition to major hybridisation bands weaker signals ranging from 19.3 kb to less than 0.9 kb were found and no extra band was detected in genomic samples with B chromosomes. No cross-hybridisation of rye B 1334 was detected with genomic DNA of wheat (Fig. 14). However, genomic DNA of wheat with additional rye Bs displayed a comparable hybridisation pattern as those of rye, although of lower hybridisation intensity (Fig. 14). Therefore, it can be concluded that similarly organized B 1334-like sequences are located also on A chromosomes of rye.

Southern hybridisation of rye B 2465 revealed major bands and weaker signals ranging from 19.3 kb to less than 0.9 kb. Figure 17 also shows weak cross-hybridisation with wheat and different patterns for rye and wheat as well as between wheat material with and without B chromosomes. Several extra bands were detected in wheat with B chromosomes restricted with *Bam*HI (Fig. 18, arrowed).

The Southern hybridization with rye B 8149 revealed hypervariable hybridization patterns ranging between 19.3 kb and 0.9 kb (Fig. 15, 16). The patterns differed among the rye inbred lines as well as Lindström wheat irrespectively of the presence or absence of Bs. In addition, a few additional bands were consistently found in rye and wheat with Bs (Fig. 15, 16 arrowed). The polymorphic hybridization pattern suggests a high mobile activity of B 8149-like sequences in the genomes of rye and wheat.

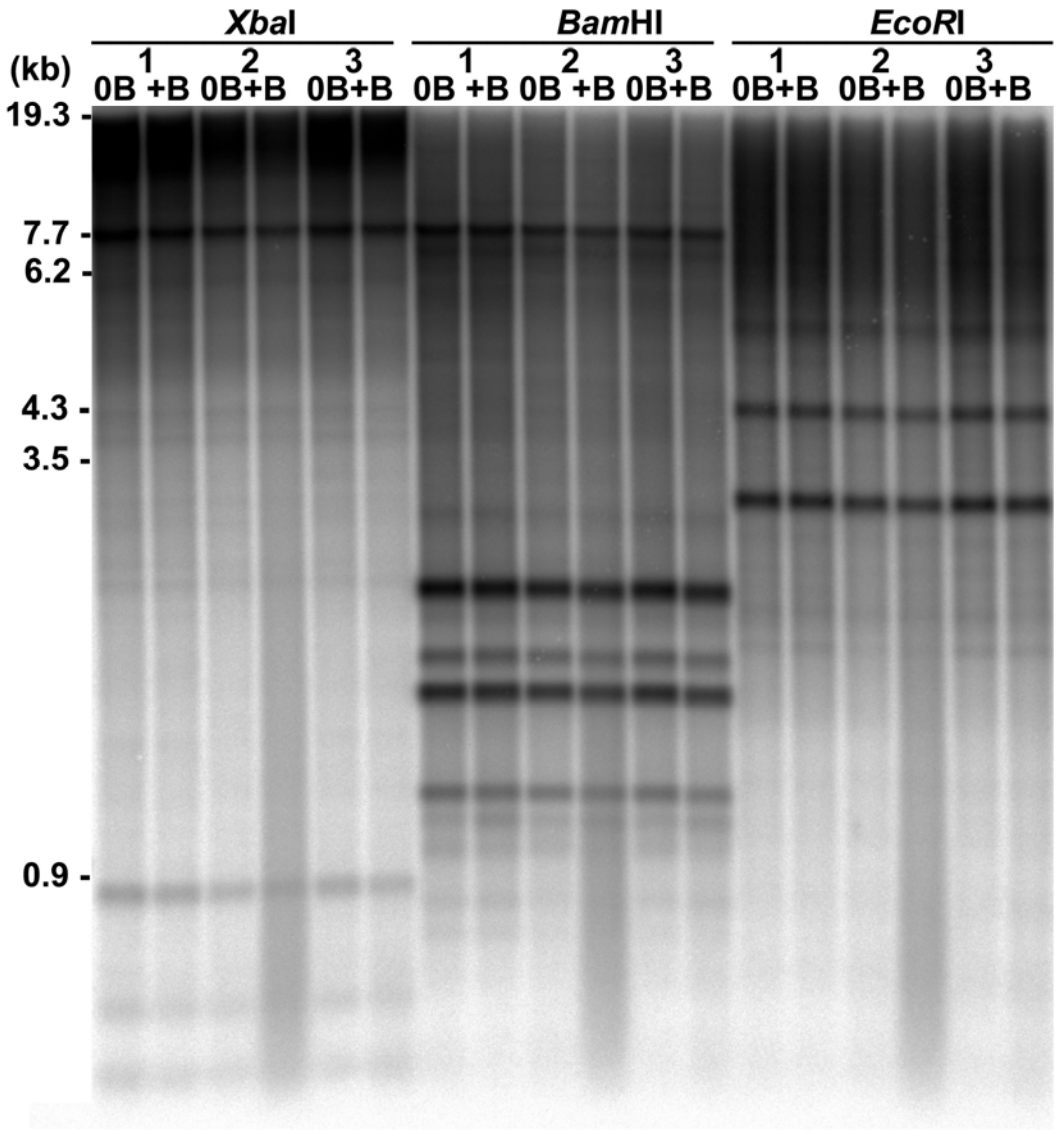


Fig. 13.
Southern hybridization of probe B 1334 with *Xba*I, *Bam*HI and *Eco*RI-digested genomic rye DNA from plants with (+B) and without Bs (0B). 1, 2, 3 are the three different rye inbred lines. Note identical hybridization patterns of rye plants with and without B chromosomes.

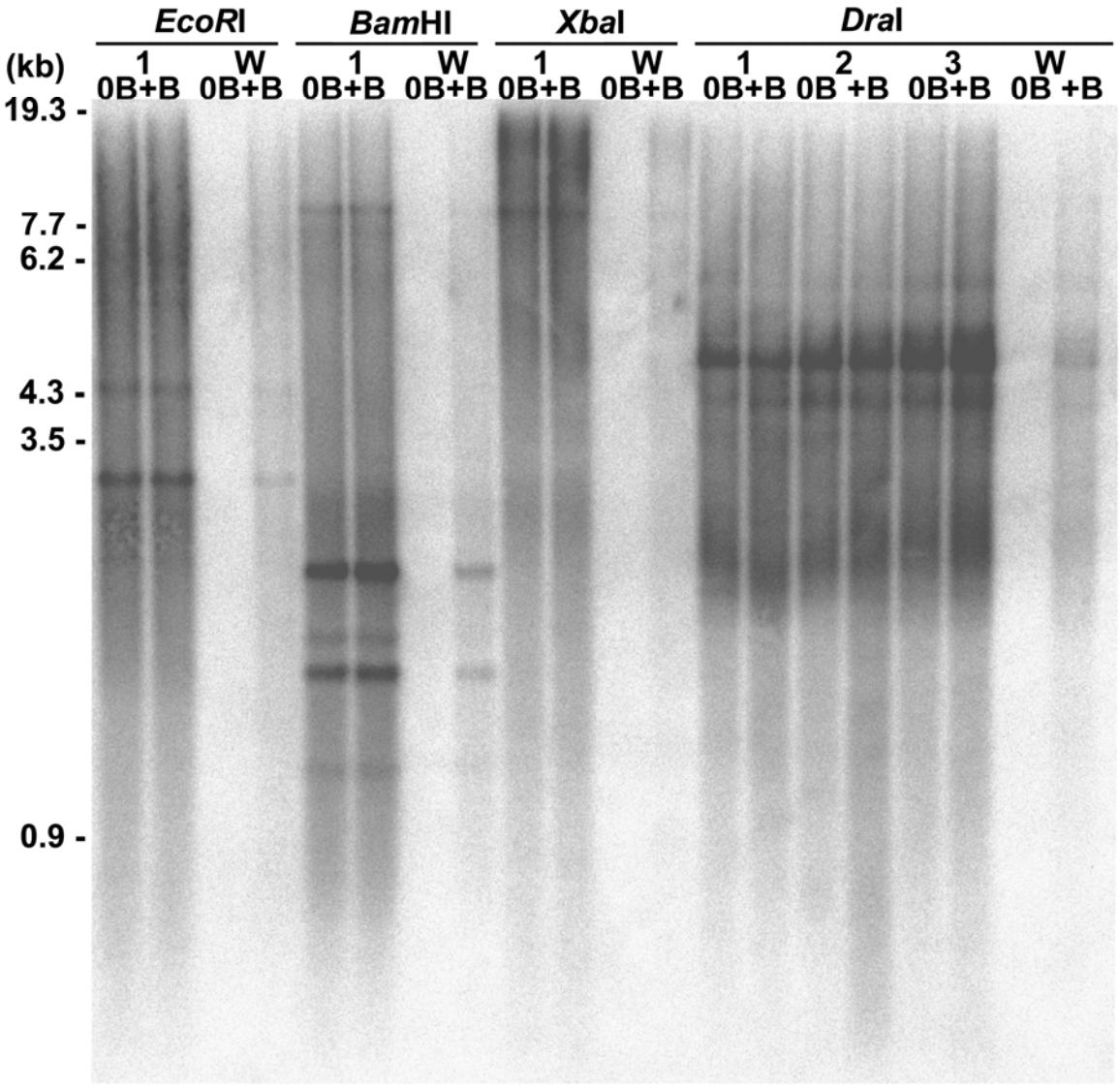


Fig. 14.
Southern hybridization of probe B 1334 with *EcoR*I, *Bam*HI, *Xba*I and *Dra*I-digested genomic rye and wheat DNA from plants with (+B) and without Bs (0B). 1, 2, 3 are the three different rye inbred lines. W – Lindström wheat with (+B) and without Bs (0B). Note identical hybridization patterns of 0B and +B rye and wheat plants with B chromosomes.

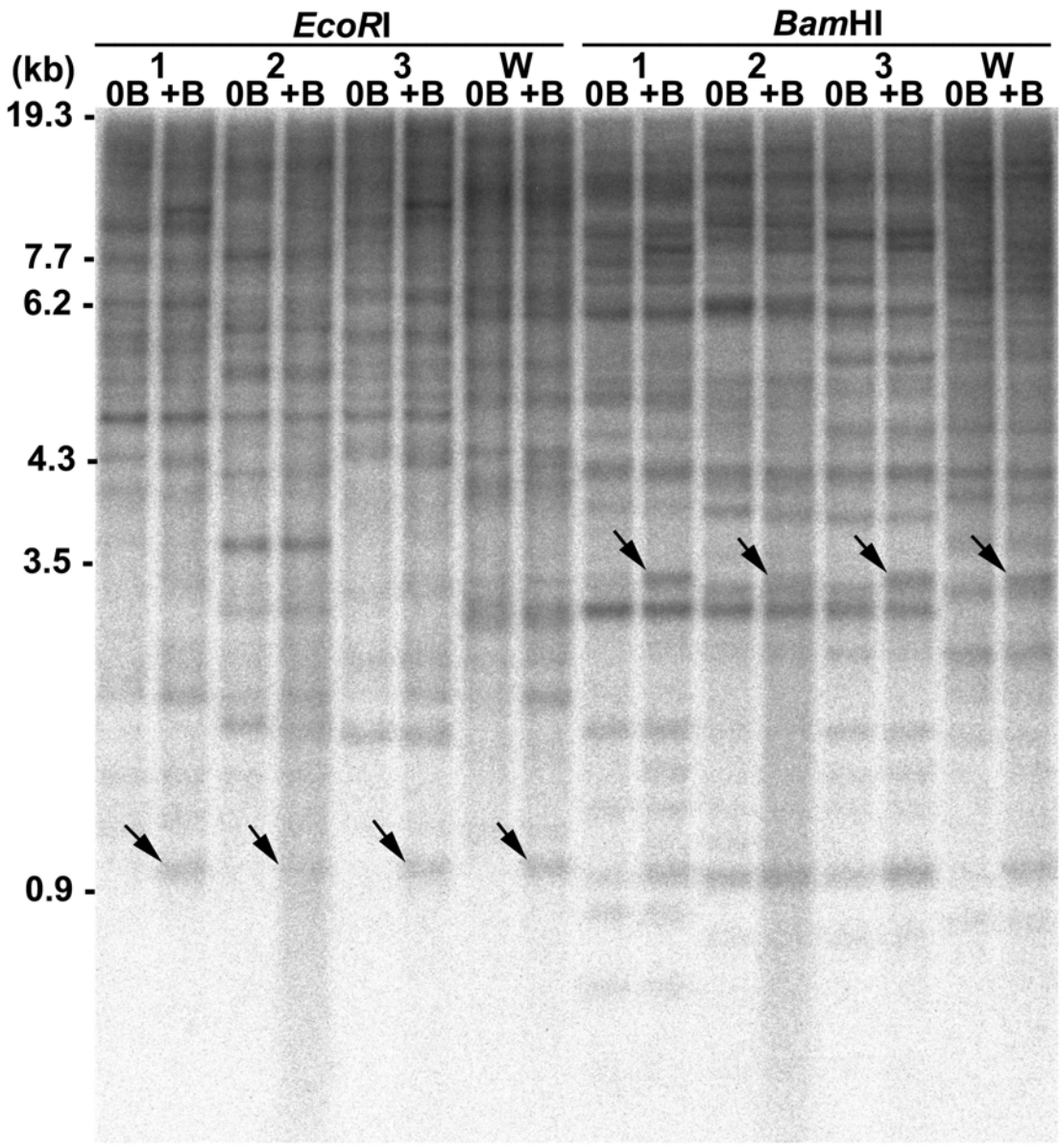


Fig. 15.
Southern hybridization of probe B 8149 with *Eco*RI and *Bam*HI -digested genomic rye and wheat DNA from plants with (+B) and without Bs (0B). 1, 2, 3 are three different rye inbred lines. W – Lindström wheat with (+B) and without Bs (0B). Note the highly polymorphic pattern. Additional B-specific restriction bands are arrowed.

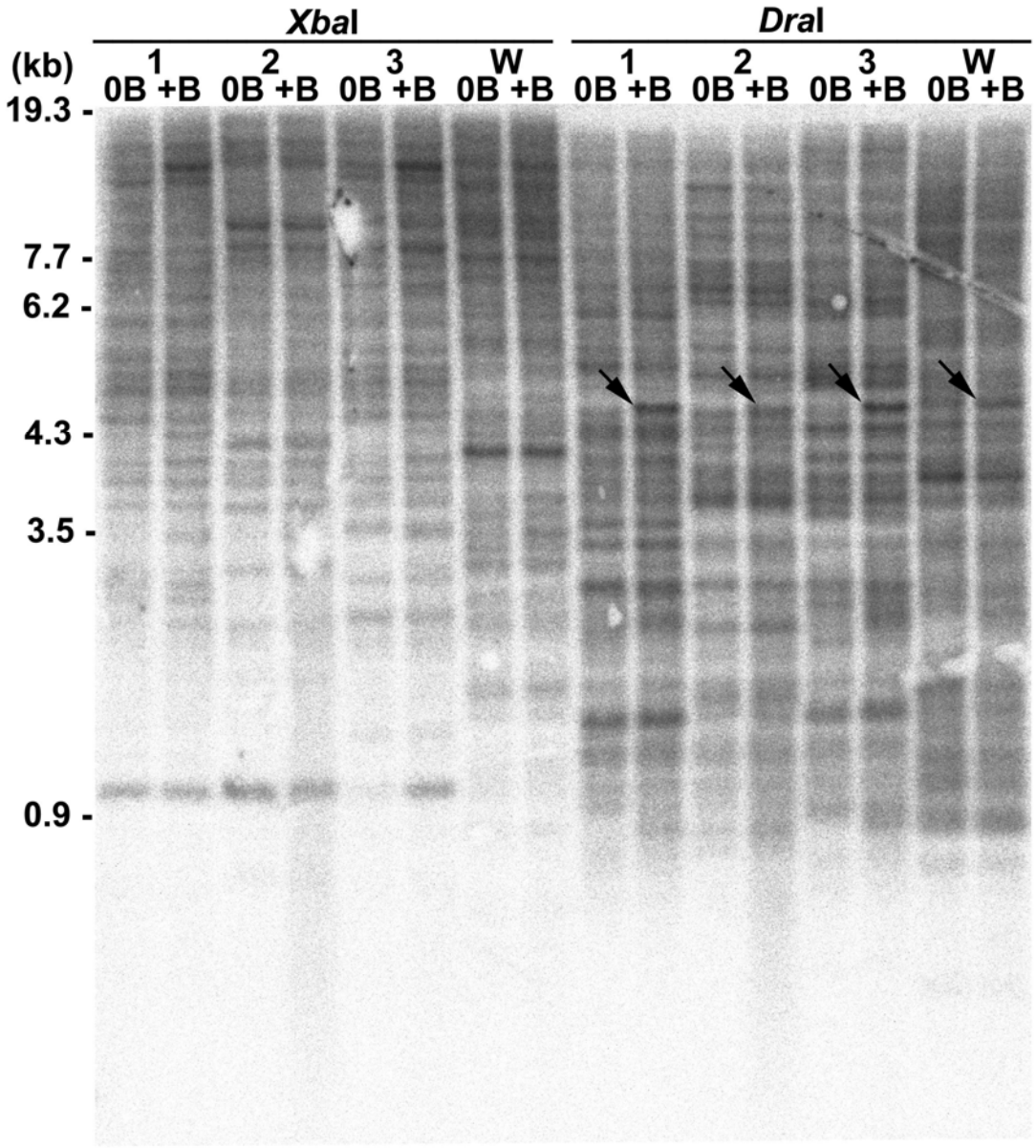


Fig. 16.
Southern hybridization of probe B 8149 with *Xba*I and *Dra*I-digested genomic rye and wheat DNA from plants with (+B) and without Bs (0B). 1, 2, 3 are three different rye inbred lines. W – Lindström wheat with (+B) and without Bs (0B). Note the highly polymorphic hybridization pattern. Additional B-specific restriction bands are arrowed.

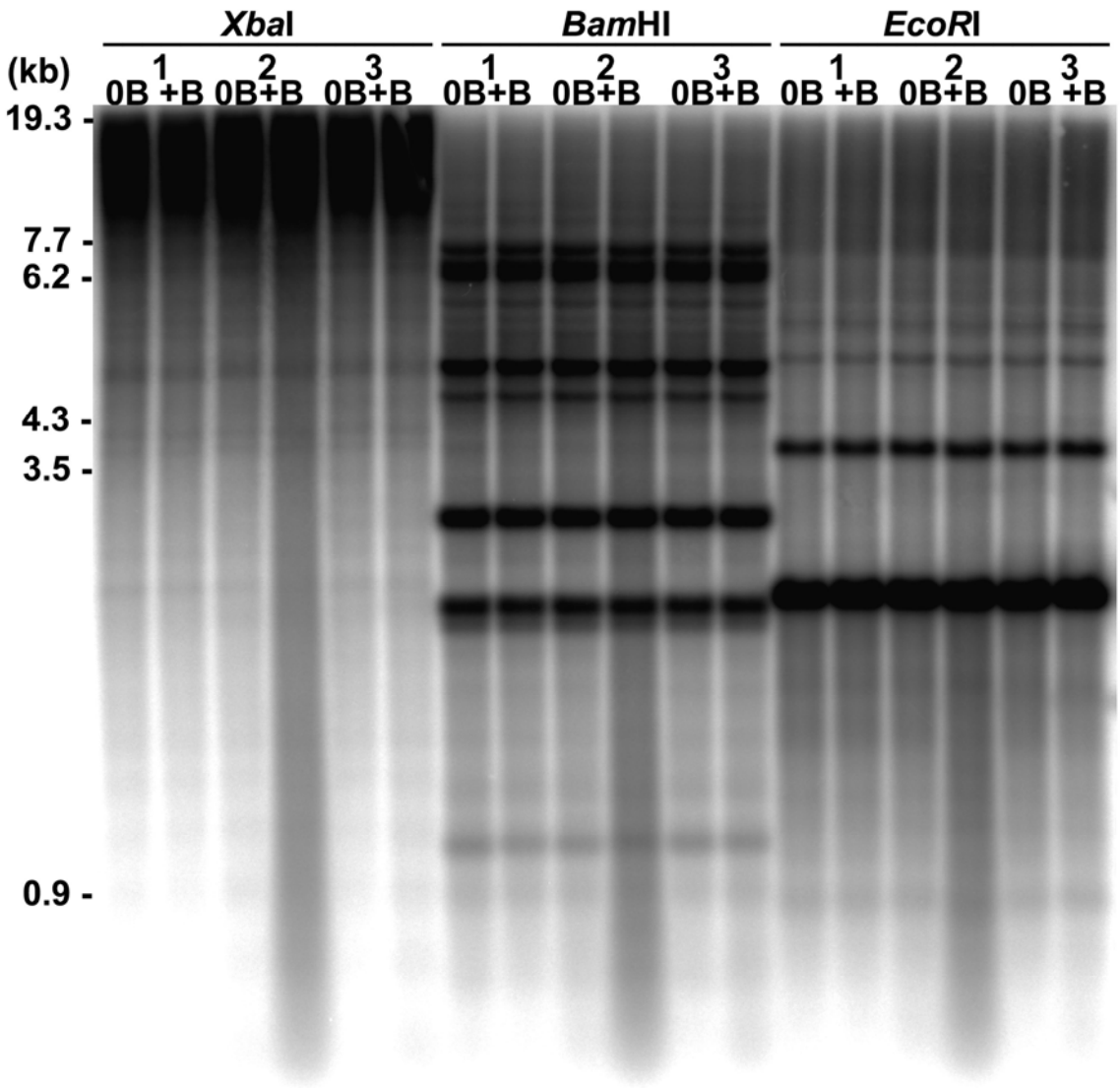


Fig. 17.
Southern hybridization of probe rye B 2465 with *Xba*I, *Bam*HI and *Eco*RI-digested genomic rye DNA from plants with (+B) and without Bs (0B). 1, 2, 3 are the three different rye inbred lines. Note identical hybridization patterns of 0B and +B rye plants.

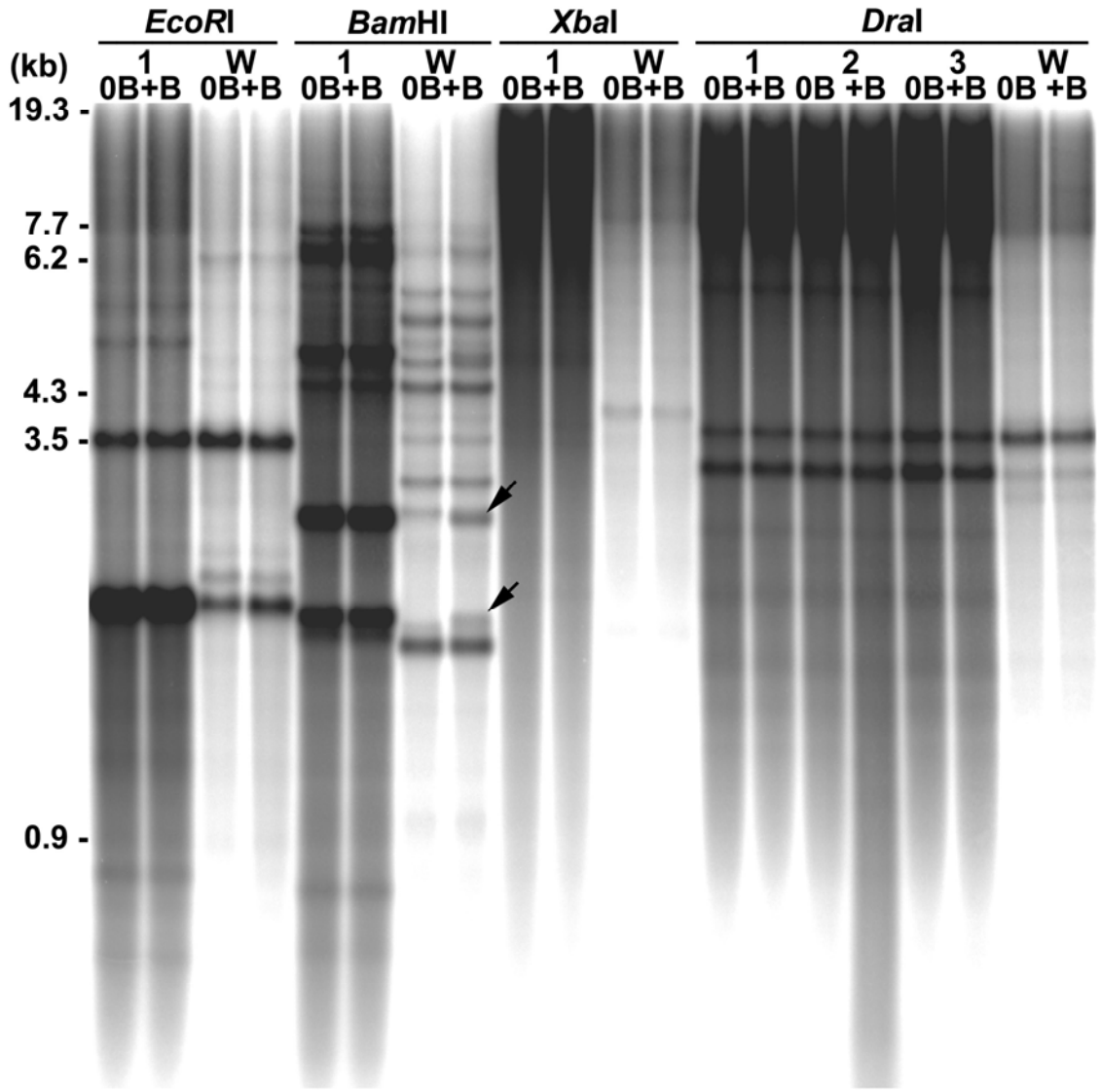


Fig. 18.
Southern hybridization of probe rye B 2465 with EcoRI, *Bam*HI, *Xba*I, and *Dra*I-digested genomic rye and wheat DNA from plants with (+B) and without Bs (0B). 1, 2, 3 are three different rye inbred lines. W – Lindström wheat with (+B) and without Bs (0B). Note identical hybridization patterns of 0B and +B rye and additional bands in wheat plants with B chromosomes (arrowed).

4.7.2. Chromosomal distribution of B chromosome-associated transcriptionally active sequences analyzed by fluorescence *in situ* hybridization (FISH)

Mitotic rye cells prepared from root tip meristems of seedlings with B chromosomes were *in situ* hybridized with the digoxigenin-labelled probes B 1334, B 8149 and B 2465. Simultaneous *in situ* hybridization with the biotin B-specific high repeat D1100 allowed the identification of the terminal region of the long B chromosome arm. B 1334-, B 8149- and B 2465-specific hybridization signals were found along all A and B chromosomes (Fig. 19). Reduced cross-hybridization of B 1334 and B 8149 were found in the terminal heterochromatic regions of A and B chromosomes. The enhanced B1334-specific hybridization signals along B chromosomes suggest a slight accumulation of this sequence in B chromosomes. The hybridization patters of the digoxigenin-labelled probes are typical for dispersely organized high-copy sequences such as some types mobile elements like described for Ty1-copia (Pearce et al., 1996; Brandes et al., 1997; Heslop-Harrison et al., 1997; Pearce et al., 1997). The chromosomal distribution of FISH signals reconfirms the existence of B 1334-, B 8149- and B 2465-similar sequences in A and B chromosomes of rye.

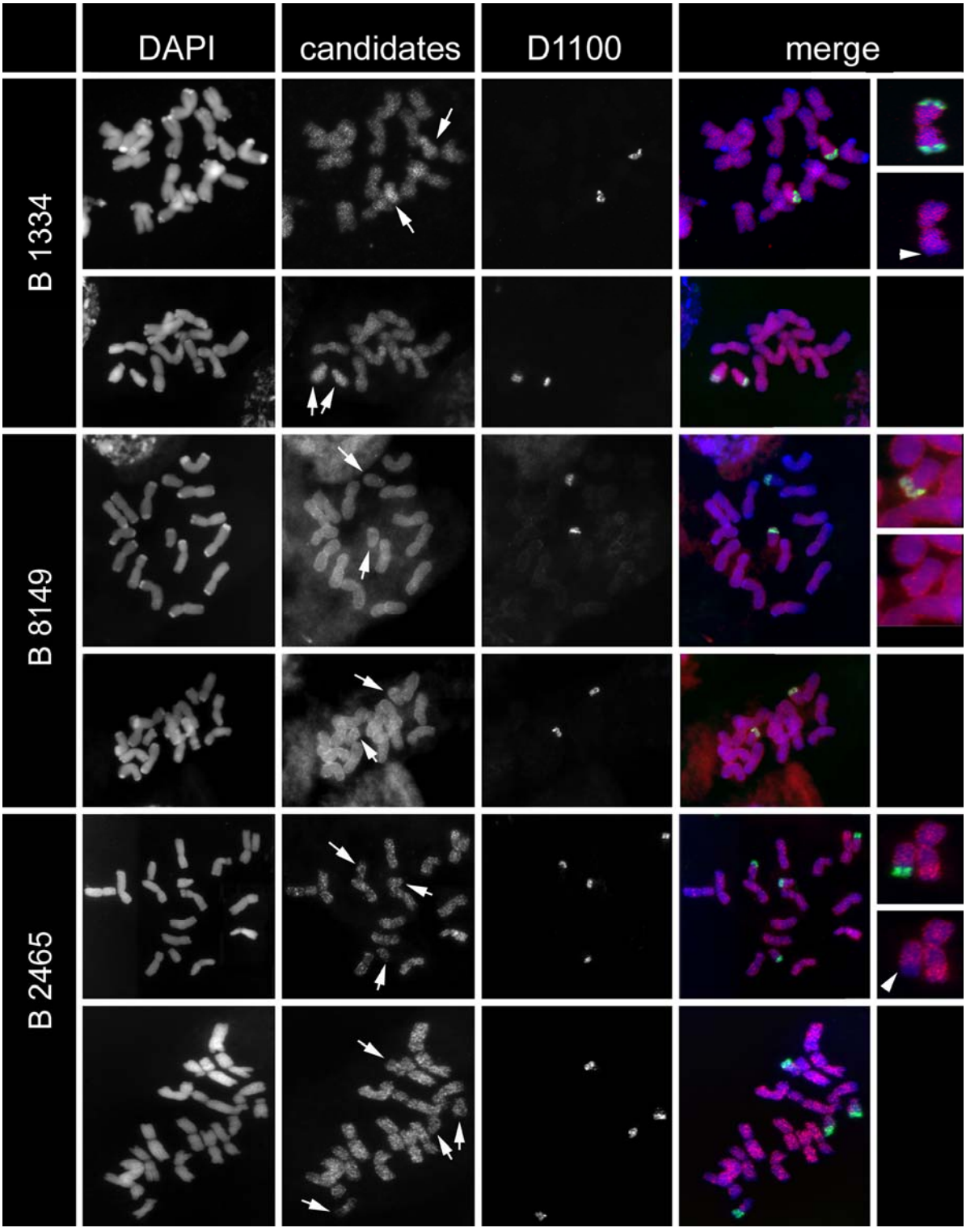


Fig. 19.
Multicolor fluorescence in situ hybridization of mitotic metaphase cells of rye containing B chromosomes (arrowed) with the B-specific repeat D1100 (in green) and with the probes: B 1334, B 8149 and B 2465 (in red). The chromosomes are counter-stained with DAPI (in blue). The insets (in merge) show enlarged Bs and the chromosomal position of the D1100-positive region are indicated by arrowheads. Note very weak cross-hybridization of B 1334 and B 8149 with the D1100-positive region and stronger hybridization signals of B1334 along B chromosomes.

4.7.3. Confirmation of transcriptional activity of the B-located sequences B 1334, B 8149 and B 2465

Southern and *in situ* hybridization showed that similar sequences to B 1334, B 8149 and B 2465 are located also on A chromosomes. To confirm transcription activity and B-specificity of these three candidates Northern analysis and RT-PCR were conducted.

RT-PCR was performed with primers which are complementary to regions with the lowest level of similarity between B 1334, B 8149, B 2465 and the corresponding published database sequences with high similarity (listed in Table 4). PCR was conducted on cDNA obtained from leaf material of rye (lines 1, 2, and 3) and wheat (Lindström) plants with and without Bs. To demonstrate equal amounts of cDNA, RT-PCR was performed with primers specific for the elongation factor eEF1 alpha (designed on a barley cDNA sequence with accession number Z50789; published by Nielsen and colleagues (1997)). Elongation factor-specific RT-PCR resulted in a relatively uniform yield of PCR products irrespectively of the genotype or presence or absence of B chromosomes (Fig. 20).

RT-PCR using B 8149-specific primers yielded in products of expected size (499 bp) in cDNA obtained from material with B chromosomes of all three rye inbred lines and Lindström wheat addition line (Fig. 20). The almost equal amounts of PCR products suggest a comparable level of B chromosome transcription irrespectively of the host genotype or species.

RT-PCR of B 1334 also confirmed B chromosome specificity. Amplification products of the expected size of 159 bp were detected when cDNA synthesised from +B chromosomes plants were used. The transcription level of the rye lines was comparable. In difference, B 1334-activity of Bs added to wheat was significantly reduced, only a weak RT-PCR band was detectable (arrowed in Fig. 20). For all RT-PCR experiments cDNA for +B samples was synthesised in all three lines from plants with 2 Bs.

The RT-PCR with primers designed on B 2465 sequence gave mixed results. PCR products of expected size were observed in +B cDNA of rye line 1 and 3 and Lindström wheat addition line. However, additional products were observed in 0B material of rye line 1 and 2. This

unexpected result could be explained by a genotype-dependent RNAi effect of this sequence, which might results in variability of transcription activity in all three lines.

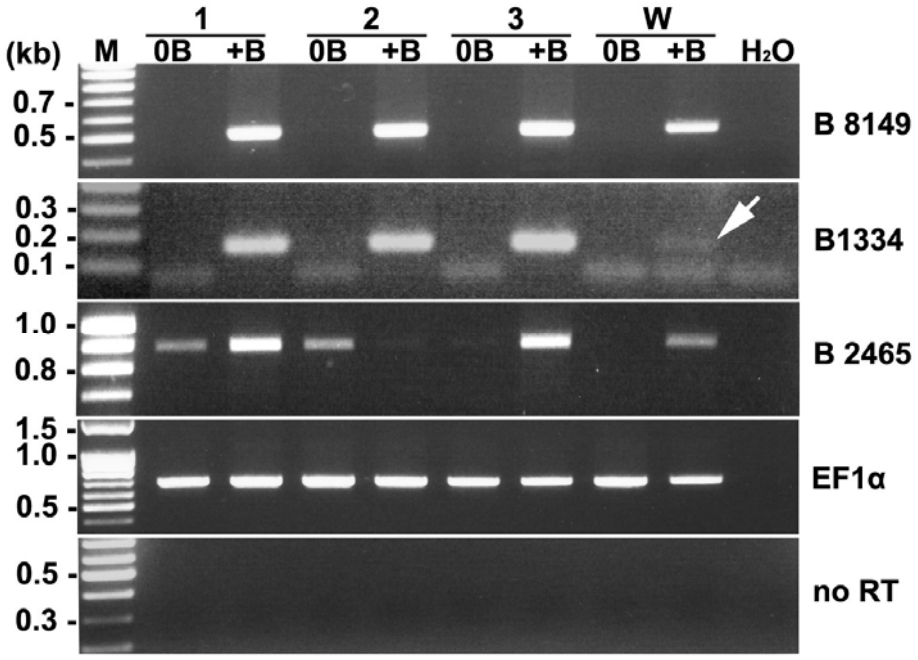


Fig. 20.
RT-PCR analysis of B 8149, B 1334 and B 2465 on RNA isolated from leaves of the three rye inbred lines (1- 7415, 2 - P 12 and 3 - 2677) and Lindström wheat (W) with (+B) and without B chromosomes (0B). Note the faint band in Lindström wheat with Bs (in B 1334, arrowed).

RT-PCR was conducted with a EF1alpha-specific primer pair to demonstrate equal amounts of cDNA. To show absence of genomic DNA contaminations control PCR without reverse transcription was performed (Fig. 20).

In conclusion, we can affirm that B 8149 and B 1334 sequences are transcribed from B chromosomes. Decreasing of the RT PCR annealing temperature resulted in the amplification of 0B products as in +B material. Sequencing of RT-PCR products coming from 0B cDNA showed high similarity (98-99%) with the ones derived from B 1334 and B 8149, though with many point mutations (Anex1, 2). Therefore, it is likely that the A chromosome located B 1334- and B 8149-like sequences are also transcriptionally active.

In order to determine if particular B specific sequences are located only on Bs or on A chromosomes as well, genomic PCR was performed. The results displayed clear band in +B material only for B 8149 (Fig. 21). For B 1334 and B 2465 bands could be observed in both

0B and +B. By increasing the stringency of PCR conditions, the amount of PCR product in 0B material could be reduced though not completely. This indicates that B1334 and B 2465 are located on both As and Bs, though the sequences on Bs display high transcriptional activity compared with sequences located on As. The specific products in case of B 8149 could be also explained by more specific primers.

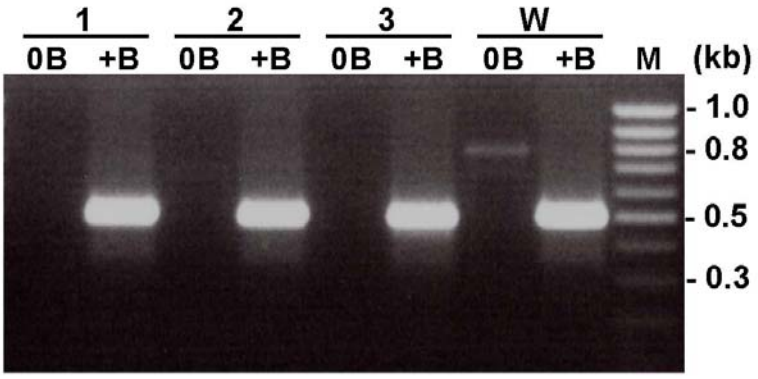


Fig. 21.
Genomic PCR analysis of B 8149 on DNA isolated from leaves of the three rye inbred lines (1- 7415, 2 - P 12 and 3 - 2677) and Lindström wheat (W) with (+B) and without B chromosomes (0B).

To confirm this assumption Northern analysis was performed with B 1334, B 8149 and B 2465 probes on RNA isolated from several tissues namely roots, leaves and anthers of rye plants with and without Bs of line 7415. This experiment confirmed that sequences similar to those isolated from B chromosomes are transcribed from A chromosomes as well (Fig. 22). The hybridization patterns of B 1334 with and without Bs were comparable, although a slightly increase of signal intensity was found in +B leaf and anther material. B 1334-like sequences are transcribed in all analyzed tissues. The highest level of transcription was found in anthers (Fig. 22A). B 8149-like sequences showed highest transcriptional activity in leaf tissue. Differently from B 1334 probe the B 8149 hybridized very weakly with RNA of anther tissue. No hybridization bands of big size were detected (Fig. 22B). Northern hybridization of B 2465-like sequences showed that this sequence is transcriptionally active in all tissue types analyzed with the highest activity being in leaf tissue (Fig. 22C). B 1334 and B 2465 displayed hybridization with transcripts of big size (about 9kb) in anther tissue (Fig. 22A, C). Loading control of RNA samples is shown in figure 5**C.**

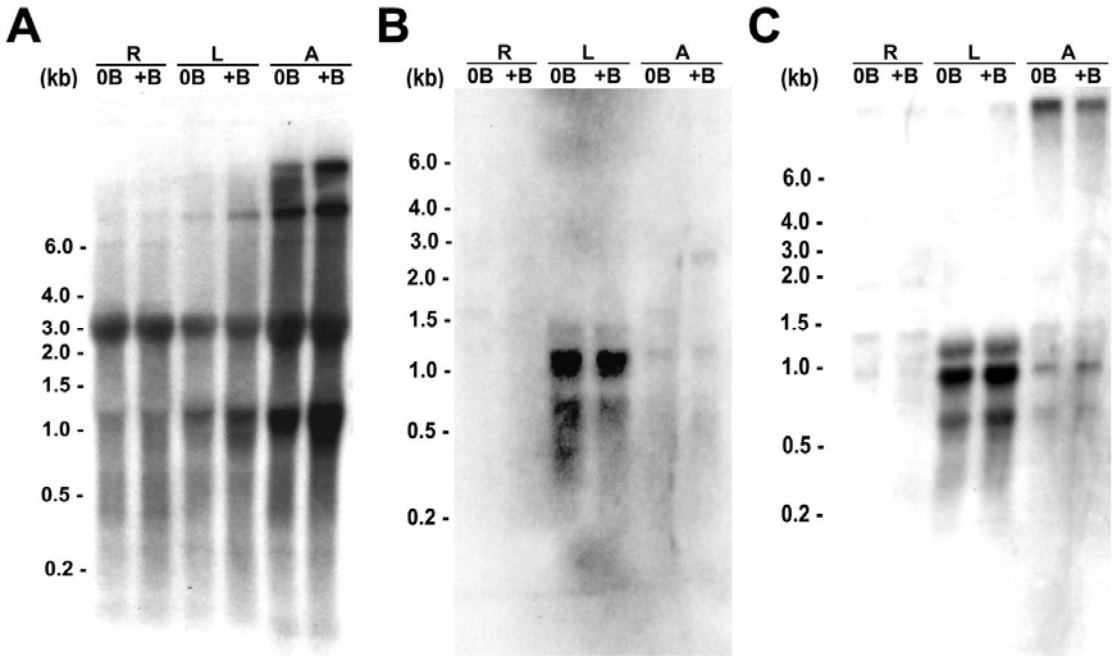


Fig. 22.
Northern hybridization of probes B 1334 (**A**), B 8149 (**B**) and B 2465 (**C**) with equal amounts of RNA isolated from roots (R), leaves (L) and anthers (A) of rye with (+B) and without B chromosomes (0B). Loading control of RNA is shown in figure 5C.
The membranes were exposed to X ray films at -80ºC for 3 days for B1334 probe and 5 days for B 8149 and B 2465 probes.

5. Discussion

5.1. Histone modifications analysis indicate on potential activity of B chromosomes

The two repeats D1100 and E3900 which compose the main part of the Giemsa banding-positive heterochromatic subterminal domain of rye Bs undergoes decondensation during interphase (Morais-Cecilio et al., 1996; Langdon et al., 2000; Carchilan et al., 2007). Contrary to the heterochromatic regions of A chromosomes this domain is simultaneously marked by trimethylated H3K4 and trimethylated H3K27 – an unusual combination of apparently conflicting histone modifications (Carchilan et al., 2007). Notably, both types of B-specific high copy repeats (E3900 and D1100) of the subtermianl domain are transcriptionally active, although with different tissue-type dependent activity. Both D1100 and E3900 transcripts are partly polyadenylated, the fraction of polyA E3900 transcripts being significantly higher than the polyA D1100. The transcription of E3900 occurs from both strands; D1100 is mostly transcribed from one strand though the second strand also showed very weak transcription activity. This RNAs could lead to formation of double stranded RNA which can be further processed into small RNA. However, no small RNAs were detected specifically for the presence of B chromosomes. The lack of any significant open reading frame and the highly heterogeneous size of mainly polyadenylated transcripts indicate that the non-coding RNA may function as structural or catalytic RNA.

5.1.1. No major differences between histone modification pattern of rye A and B chromosomes, except histone H3K4me3

H3K27 methylation shows a species-specific chromosomal distribution. The euchromatic regions of rye A and B chromosomes are uniformly H3K27me1-labeled. In contrast, the same modification in Arabidopsis and barley seems to be a heterochromatin mark (Fuchs et al., 2006). H3K27me2 is typical for heterochromatin in Arabidopsis and rye but characteristic for euchromatin in barley, while H3K27me3 is euchromatin-specific in Arabidopsis and barley, but clusters at certain heterochromatic position in *Vicia faba* (Fuchs et al., 2006) and rye A and B chromosomes.

The peculiarity of the terminal B-region lies in the fact that, contrary to the Giemsa-positive telomeric heterochromatic regions of A chromosomes, this domain is simultaneously marked by trimethylated H3K4 and methylated H3K27 – an unusual combination of apparently

conflicting chromatin modifications. Conversely, detection of mono-, di- or trimethylated H3K9, H4K20 and methylated cytosine residues show no discrimination for the B-terminal domain. Methylation of histone H3 at lysine residues 4, 9 and 27 has become one of the most consistent epigenetic marks to differentiate euchromatin and heterochromatin across a wide range of species (reviewed by Martin and Zhang, 2005). Histone lysine residues may be mono-, di-, or trimethylated *in vivo*, and the fully functional significance of these three states remains unclear (Rice et al., 2003). Both dimethyl and trimethyl states of H3K4 have been described as being associated with active euchromatic regions. Although the trimethylated, rather than the dimethylated, form of K4 in H3 marks the transcription start site of eukaryotic genes. However, only the H3K4 trimethylated state has been related to gene transcription in yeast (Santos-Rosa et al., 2002) and mammals (Miao and Natarajan, 2005). The presence of H3K4me3 in a chromatin fraction enriched in heterochromatic histone modifications and DNA methylation was only recently reported for the first time in the urochordate *Oikopleura dioica* (Spada et al., 2005). The occurrence of H3K4me3 in domains with heterochromatic features might therefore be a more common situation than hitherto realized. Moreover, the terminal domain of the rye B seems to have a high specificity for the trimethylation status of H3K4, since no enhanced labeling was found with antibodies directed against the mono- and dimethylated state of H3K4.

Further work is required to resolve whether non-repressive (H3K4me) and repressive (H3K27me) histone modifications coexist within the same nucleosome, or whether they occupy alternate nucleosomes of the terminal heterochromatic but transcriptionally active B region.

5.1.2. Giemsa banding-positive heterochromatic region is transcriptionally active

The fact that only the subtelomeric domain is highly enriched in trimethylated H3K4 shows a direct correlation with dynamic chromatin decondensation. The decondensation of that chromosome domain was consistently observed in all cell types analysed, and is particularly striking in pachytene cells in contrast with the behavior of other repetitive DNA sequences observed in the same cells, namely the subtelomeric heterochromatic blocks in the A chromosomes and the rDNA cluster, that remain tightly condensed at this stage (Cunado et al., 2000). The maintenance of a condensed state during meiotic prophase and low levels of recombination are general features of repetitive DNA sequences (Schwarzacher, 2003).

Interestingly, an extensive analysis at the molecular level revealed a high degree of instability in this region and a potential for amplification especially associated with the E3900 sequence (Langdon et al., 2000). These authors suggest that sequence polymorphism may be involved in the B transmission equilibrium, since small changes in relative sequence amount may alter the balance between condensed and decondensed forms affecting meiotic pairing. Variation in the B chromosome ability for forming bivalents at metaphase I is one of the main features modulating their transmission rate (Jimenez et al., 1997; Jimenez et al., 2000), since the mitotic drive affected by nondisjunction occurs at a constant and high frequency. This strongly supports the hypothesis that the chromatin conformation of the B-terminal domain is a crucial feature for B transmission, and for the maintenance of rye Bs in natural populations.

An intriguing result of our analyses is the identification of transcripts arising from the B-specific tandem repeats in the termimal region. Satellite DNA is generally considered not to be transcribed. However, various examples of transcribed tandem repeats have recently been reported in several organisms, including plants (May et al., 2005; Zhang et al., 2005). These examples suggest an active role for tandem repeat transcripts in several regulatory layers from chromatin modulation to transcription, RNA maturation, translation (reviewed in Ugarkovic, 2005), and even to centromere function (Bouzinba-Segard et al., 2006).

5.1.3. The putative functions of B specific transcripts

The function of B chromosome transcripts and the mechanism of transcription of B-tandem repeats are unknown at present. Their transcription may be due to read through from other active sequences such as mobile elements. Recently it has been shown that transcription of centromeric satellite DNA (May et al., 2005), or heterochromatin-located genes, is driven by adjacent regulatory elements of retroelements (Dimitri et al., 2005). Both B repeats undergo polyadenylation, at least in part, as also shown for noncoding RNA transcribed in stressed human cells (Rizzi et al., 2004), and the highest transcription activity was found in anthers. The size heterogeneity and the lack of any significant open frame is compatible with the idea that these molecules are in fact composed of a variable number of repeats of E3900 and D1100 sequences, arguing against a possible coding function. We hypothesize that these transcripts could serve some structural function in the organization and regulation of B chromosomes. It is tempting to speculate that the unique chromatin conformation and transcription activity of the B-terminal region could be involved in the trans-acting mechanism of directed non-disjunction of the rye B at pollen mitosis. Although no controlling

element for this process has been identified in the B-specific domain, the deletion of this region leads to a loss of the non-disjunction indicating its direct involvement in chromatid non-disjunction (reviewed in Jones, 1995). The process of non-disjunction is highly regulated and only occurs in the gametophytes, but we cannot exclude that it might be related to the presence of a unique combination of chromatin marks and non-coding RNA in diploid cells. In maize, it has been shown that non-coding RNA molecules play a role in establishing centromeric heterochromatin domains (Topp et al., 2004). In Arabidopsis, double-stranded RNA molecules arising from centromeric repeats may direct the formation and maintenance of centromeric heterochromatin through RNA interference (May et al., 2005)

5.2. The general study of transcription did not result in a bona fide B chromosome-specific active gene

5.2.1. cDNA-AFLP - an efficient method for the analysis of the transcriptome

cDNA-AFLP is an efficient method for gene expression analysis, especially for non-model organisms for which micro- and macroarrays or sequence data are not available yet. This method permits to identify differentially expressed sequences by isolating and sequencing the transcripts of interest. Thousands of transcripts can be detected by this method. The number of bands per cDNA-AFLP profile can vary depending of the nucleotides added to the selective primers, primer sequence and restriction enzyme used. Primers with 1 to 4 additional nucleotides usually are used for selective amplification. Primers with more additional nucleotides amplify more selectively. In the presented study were chosen primers with 3 additional selective nucleotides. Since this method is based on PCR, even small amounts of tissue are sufficient for cDNA AFLP analysis. Therefore its application is possible for gene expression studies as for example in different plant organs (Cnudde et al., 2006) and cell cycle gene expression studies, as performed on synchronized tobacco 'Bright Yellow-2' suspension cells (Breyne et al., 2002).

cDNA-AFLP is a very sensitive method, both quantitatively as well as qualitatively. It can discriminate single nucleotide polymorphism (SNP), if the SNP is in the restriction site region of the restriction enzyme used. It allows detecting more differences in transcriptional profile

than by substractive hybridization screening. However, cDNA-AFLP does not reflect the transcription of the entire genome. Comparison of theoretical calculations with the experimental data obtained after screening with half of all possible primer combinations specific for *Bst*YI and *Mse*I restriction enzymes leads to the conclusion that only 30% of transcripts can be visualized by cDNA-AFLP technique (Cnudde et al., 2006).

Both differential display and cDNA-AFLP are methods based on fingerprinting the semirandom PCR amplified transcriptome. cDNA-AFLP amplifies selectively the regions delimited by the restriction enzymes digestion whereas, in case of differential display, amplification occurs mostly near the 3' end, and one primer is oligo-dT derived. Both methods have a certain degree of false positive and the candidate transcripts have to be reconfirmed.

5.2.2. Identification of novel rye B-specific transcripts

In this study the general transcription activity of B chromosomes was analyzed by comparative cDNA-AFLP of plants with and without B chromosomes from three rye lines isogenic for its Bs. Rye is an outbreeder and additional polymorphic transcripts coming from genetically diverse A chromosomes could be expected. Therefore, three lines were employed to better distinguish B-specific transcripts from those coming from A chromosomes polymorphism. In total, using 63 primer combinations, 2286 bands were detected in all three lines in 0B/+B material. 112 extra bands (4.9% of average number of bands per rye line) were found in plants with B chromosomes of either 1, 2 or all 3 rye lines. However, only 16 B-extra bands (0.7%) of different intensity were consistently found in all three rye lines. This finding indicates a very low transcription activity of B chromosomes.

Indirect evidence that Bs could influence the transcription behavior of A chromosomes has been reported by Delgado and colleagues (1995; 2004). The authors observed that in interphase nuclei, A chromosome-localized ribosomal DNA displays a more compact distribution in cells with Bs compared to cells without Bs. A more compact distribution of rDNA sites suggests a lower level of rRNA gene activity. Therefore, the number of additional bands in 0B material was also determined. 132 extra bands (5.8%) were found in either 1, 2 or all 3 rye lines. Only 4 (0.18%) 0B extra bands were detected in all three lines. However,

whether these A chromosome-derived transcripts are down-regulated due to B chromosomes is not clear and requires further investigation.

Notably, comparative cDNA-AFLP analysis of a rye line with an additional wheat chromosome 6B (here, B means coming from the B genome of wheat, not B chromosomes) displayed 6.9% additional bands which indicate as expected higher transcriptional activity of the A chromosome 6B in comparison to the less active B chromosome of rye. However, this finding should not be overestimated because the cDNA-AFLP result on the rye-wheat addition line is based on 4 different primers only.

9 B-specific candidate transcripts were isolated and sequenced. Out of 9 candidates two, B1553 and B2442 showed DNA sequence similarity to known genes such as the Zeaxanthin epoxidase and the Mal-like protein (of *T. aestivum*), respectively. Southern hybridization showed that these sequences are present in a very low-copy number in genome of rye and no B-specific extra band was revealed. Based on Southern hybridization, Northern or RT-PCR it was impossible to verify B-specificity of these clones. One reason could be that similar sequences are located on A chromosomes and discrimination of the A- and B-located sequences was not possible due to high sequence similarity. In future, Single Strand Conformational Polymorphism technique will be employed to detect sequence differences between A and B located B1553 and B2442-similar sequences. In addition, quantitative Real Time RT-PCR could be used to verify differential expression of these candidates. After confirmation of B-specificity, these clones will be used for the analysis of the B chromosome origin in future. Therefore, wheat-rye chromosome addition lines will be employed for the identification of the A chromosome(s) with the highest similarity to the rye B chromosome.

Four of the identified B candidate transcripts: B1334, B8149, B2465 and B2565 revealed sequence similarity to mobile elements. B 2465 showed also partly similarity to the repetitive sequence pSc 119.1. The remaining clones B 1559, B 2448, and B 2452b revealed similarity to ESTs of wheat from libraries obtained after different biotic and abiotic stresses (Table 9) without indication to putative gene function.

Three candidates analyzed in details: B1334, B8149, and B2465 are present in high copy number in the rye genome. B1334 showed similarity to the transposase region of a mobile

element called 'Revolver'. 'Revolver' is a transposon-like element of 3041 bp length, dispersedly distributed in rye genome. It is flanked with 14 bp terminal inverted repeated sequences on both ends, like class II mobile elements and is transcriptionally active like class I of transposable elements (Tomita, 1998, 2003). Interestingly, the entire structure of 'Revolver' does not share clear identity neither with class I nor with class II of transposable elements (Tomita, 2003). It is also abundant, though exhibiting quantitative variations, in the genomes of wheat ancestor species *Triticum monococcum* (AA), *T. turgidum* (AABB) and *T. tauschii* (DD). However surprisingly, this element is not detectable in *T. aestivum* (AABBDD) (Tomita, 2003). B 1334 sequence also showed similarity to a rye hypervariable sequence F17 which was identified by RAPD analysis of rye plants regenerated from immature embryos and inflorescences (Linacero et al., 2000).

As expected, the hybridization of B1334 to DNA of Lindström wheat without B chromosomes did not result in cross-hybridization. However, hybridization B1334-specific signals were found if an additional B chromosome was present, confirming that B 1334 sequences are located on rye Bs. *In situ* hybridization revealed a slight accumulation of B 1334 on B chromosomes.

Sequence analysis of the identified B 8149 candidate revealed 55% DNA similarity to a nonLTR LINE retrotransposon of *Triticum* and to other ESTs (85%) of *T. aestivum.*
The high copy number of B 8149 supports the assumption that this sequence is a mobile elements derivative. Furthermore, B 8149 showed a hypervariable Southern hybridization pattern in rye and wheat, suggesting that the rate of transposition activity of B 8149-like sequences in the three rye lines analyzed is higher than of B 2465 and B 1334. Additional bands in +B material in both rye lines and wheat Lindström verified that B 8149 B-specific sequences are located on B chromosomes. The variable hybridization patterns of this element could potentially be used as rye line specific markers.

B 2465 showed similarity to the gypsy-like retrotransposon called 'Daniela' from *Triticum monococcum* (Wicker et al., 2001) and to the rye repetitive sequence pSc 119.1 (McIntyre et al., 1990). Gypsy-like retrotransposons have the youngest origin among LTR and non-LTR retrotransposons (Xiong and Eickbush, 1990). We can conclude that B 2465 belongs to an

older class of mobile elements. The Southern hybridization patterns are similar between 0B/+B rye but differ between rye and wheat lines.

Mobile elements on B chromosomes were found in many species (Stark et al., 1996; Fregonezi et al., 2007; Lamb et al., 2007b; Lamb et al., 2007a). Mostly these elements are present on both A and B chromosomes. The retrotransposon-derived repetitive 203 bp-long DNA sequence of *Alburnus alburnus* which is present only on B chromosomes (Ziegler et al., 2003) can be considered as an exception because no similar sequences were detected on As of the same species or B chromosomes of other related species. A tendency to accumulate mobile elements has been reported for plant and animal sex chromosomes (Steinemann and Steinemann, 1992; Sakamoto et al., 2000; Steinemann and Steinemann, 2005; Hobza et al., 2006). This occurs because of low gene dosage and low recombination of sex chromosomes. Similar features could be attributed to B chromosomes.

It was possible to confirm by RT-PCR that B 1334 and B 8149 sequences are transcribed from Bs only, though *in situ*, Southern and Northern hybridizations indicate that similar sequences are located and expressed on A chromosomes as well. For B 2465 in line 2, the result of RT-PCR experiment was rather opposite than expected: no band in +B and an additional band in 0B material were found. This could be explained by heterogeneity of this particular sequence, and namely its predominance on A chromosomes in rye line 2. Another explanation could be that B-specific expression of B 2465 in rye line 2 results in an RNAi effect and therefore transcripts were only found on 0B plants.

From these results, it can be concluded that B 2465, B 1334, and B 8149 are members of high copy repetitive sequence families. However, particular members of these families display chromosome type specificity. The RT-PCR results of B 2465 point out on genotype influence on transcription of certain B located sequences, which is an obvious prove of a cross talk between A and B chromosomes at molecular level.

In plants, mobile elements and repetitive DNA constitute a large fraction of the genome (Kumar and Bennetzen, 1999; Schmidt, 1999). The activity rate of mobile elements is very different. It can vary in different tissues, during periods of vegetation, environmental conditions, and type of mobile element (Kumar and Bennetzen, 1999). The transcription of

many retrotransposons is activated by various biotic and abiotic stress factors (Grandbastien, 1998). In case of B-specific candidates B 1334, B 8149, and B 2465 transcription variation was found in roots, leaves and anthers.

As retrotransposons are not able to transpose without reverse transcription, a correlation between transcription and transposition should exist. For tobacco Tto1 (Hirochika, 1993) and rice Tos17 (Hirochika et al., 1996a) an increase in transposition concomitant with increase of corresponding RNA were demonstrated.
For B 1334, the transcriptional activity is high in all analyzed tissues. This can suggest that this element is transposing actively and further accumulation of these elements is expected. However, it is not known if the transposition still occurs. For B 2465 and B 8149 the transcription level was much lower and mostly specific for leaf tissue. Most likely they are degenerated, not functional mobile elements. Alternatively, transcription of B 2465, B 1334, and B 8149 could also be explained by a read through of these sequences next to other active transposons. This scenario was reported for the transcription of a repeat family in Arabidopsis which is regulated by the Athila2 retrotransposon (May et al., 2005).

5.2.3. Analysis of B-transcripts supports A-derived origin of rye B chromosomes

The cDNA-AFLP result supports the hypothesis of Tsujimoto and Niwa (1992a), Wilkes et al. (1995), Houben et al. (1996) that the rye B chromosome is originated from A chromosomes of the same species. Because, all identified B transcripts revealed high similarity with sequences located on A chromosomes too.

The accumulation of mobile elements on Bs can have functional and evolutionary implications. Because B chromosomes encode none or a low number of genes, the accumulation of Bs does not interfere with the normal life of the plant. Furthermore, a rapid accumulation of repeats and transposons on a *de novo* formed B chromosome could reduce the recombination ability with the A chromosome complement and therefore lead to a separate evolution of the B chromosome.

Five cDNA-AFLP candidates, identified in the present work, showed similarity to cDNAs obtained from gene expression analysis of plants under stress conditions like draught, cold

treatment, or fungi infection. The function of these sequences is unknown; these could be ESTs of upregulated stress genes or activated mobile elements. So even if the plants tolerate the presence of B chromosomes, the latter a stress factor for the genome. Also, it is known that transposons are predisposed to stable mutations accumulation under stress conditions like tissue culture (Hirochika et al., 1996b).

Mobile elements are known to act in regulatory processes. For example, upregulation of LINE elements promote the spreading of Xist mRNA and hence X chromosome inactivation (Lyon, 1998). Human and mouse X chromosomes are two fold enriched in L1 repetitive elements in comparison with autosomes (Bailey et al., 2000). It is known that transposable elements can inactivate genes, for instance a tandem insertion of P transposon into Drosophila euchromatin leads to heterochromatin formation and gene silencing (Dorer and Henikoff, 1994). However, for D1100 and E3900 B specific repeats which are located in heterochromatic region, it was shown that they undergo partial decondensation during interphase (Langdon et al., 2000; Carchilan et al., 2007).

6. Summary

I.

Epigenetic marks in the chromosome complement of rye (*Secale cereale*) were studied using immunofluorescence to detect histone modifications. In particular, the euchromatic and heterochromatic components were compared in supernumerary B chromosomes versus A chromosomes of the basic diploid set, in order to provide further insights into the enigmatic properties of these 'genetically silent' B chromosomes. Only the B-specific subtelomeric, heterochromatic domain which undergoes cell-cycle dependent decondensation displayed enrichment in histone H3K4me3 – a strong mark for active chromatin. Interestingly the heterochromatic, subtelomeric regions of A chromosomes did not display any labeling by H3K4me3. No differences between A and B chromosomes were found for the heterochromatic marks H3K9me2 and H3K27me2, indicating that less-transcriptionally active Bs are not marked by an enriched level of heterochromatic histone marks.

II.

The histone H3K4me3-positive B-terminal region is mainly composed of the B chromosome-specific arrays E3900 and D1100. Abundant E3900-specific transcripts were found in somatic and even more often in anther tissue. D1100 displayed activity in anthers only, indicating tissue-specific expression. All subregions of E3900 also revealed cross hybridization with RNAs of small size (< 200 bases) derived from anthers with B chromosomes; with the highest level of transcription at the 3'end of the repeat. The majority of D1100 and E3900 transcripts escape processing by the RNAi machinery. The lack of any significant open reading frame and the highly heterogeneous size of mainly polyadenylated transcripts indicate that the non-coding RNA may function as structural or catalytic RNA.

III.

Comparative cDNA-AFLP experiments using three nearly isogenic rye lines (7415, P12, 2677), containing plants with and without B chromosomes were performed to characterize the transcription behavior of B chromosomes. 63 primer combinations were tested and 2286 different transcripts visualized.

Despite that the transcription profile of the three inbred lines with and without B chromosomes is highly similar, sixteen putative B chromosome-associated transcripts were

identified and nine of them isolated and sequenced. This consists 0.7% of total number of transcripts which is an indication of very low activity of B chromosomes.

For all identified B-specific transcripts, similar A chromosome-encoded sequences were found. The B-specific transcribed sequences B 1334, B 8149 and B 2465 are members of a high copy repeat family with sequence similarity to mobile elements, such as Gypsy like transposons and LINE elements – non LTR transposons. Southern, Northern, RT-PCR, FISH, and sequence analysis demonstrate that similar sequences are encoded on A chromosomes. These results support the hypothesis that the rye B chromosomes are originated from A chromosomes of the same species.

7. Zusammenfassung

I.

Bisher ist wenig bekannt über die Chromatinzusammensetzung der genetisch inaktiven B-Chromosomen and ob diese sich von derjenigen der aktiven A-Chromosomen unterscheidet. Um zu prüfen, ob die A- und B-Chromosomen des Roggens (*Secale cereale*) in ihrem Gehalt an Euchromatin- und Heterochromatin-spezifischen Histonmarkierungen variieren, wurden diese mit Hilfe der indirekten Immunfluoreszenzmarkierung vergleichend analysiert. Die Beobachtungen zeigten, dass die subtelomere, heterochromatische Domäne des langen B-Chromosomenarms besonders intensiv durch die Histonmarkierung H3K4me3 gekennzeichnet ist. Die subtelomeren, heterochromatischen Bereiche der A-Chromosomen zeigten dagegen nicht diese, ansonsten für transkriptionsaktives Chromatin typische Histonveränderung. Keine Unterschiede zwischen A- und B-Chromosomen wurden für die heterochromatischen Markierungen H3K9me2 und H3K27me2 gefunden. Dies deutet darauf hin, dass inaktive B-Chromosomen nicht spezifisch durch heterochromatische Histonmarkierungen gekennzeichnet sind.

II.

Die H3K4me3-positive B-Chromosomendomäne des Roggens ist durch die B-spezifischen, repetitiven Sequenzfamilien E3900 und D1100 gekennzeichnet. E3900-spezifische Transkripte wurden in somatischen und insbesondere meiotischen Geweben nachgewiesen. Dagegen konnten D1100-Transkripte nur in Antheren detektiert werden. Alle E3900-Unterabschnitte zeigten Kreuzhybridisierung mit <200 Nukleotiden langen RNA-Fragmenten. Die höchste Transkriptionsaktivität zeigte das 3'-Ende von E3900. Der Hauptanteil der D1100- und E3900-Transkripte wird nicht RNAi-prozessiert. Das Fehlen von offenen Leserahmen, die Größenheterogenität der hauptsächlich polyadenylierten Transkripte deutet darauf hin, dass es sich hierbei um nicht-kodierende RNA handelt, welche gegebenenfalls eine katalytische oder strukturelle Funktion besitzt.

III.

Um das generelle Transkriptionsverhalten von B-Chromosomen zu charakterisieren, wurden cDNA-AFLP Experimente an Roggenpflanzen mit und ohne B-Chromosomen durchgeführt. Dazu wurden drei semi-isogene Roggenlinien (7415, P12, 2677) eingesetzt. 63 Primerkombination wurden getestet und 2286 unterschiedliche Transkripte vergleichend

analysiert. Wie erwartet unterschieden sich die Transkriptionsprofile von Genotypen mit oder ohne B-Chromosomen minimal. Somit kann die Annahme bestätigt werden, dass B-Chromosomen weitgehend genetisch inaktiv sind.

Trotzdem konnten 16 B-assoziierte Transkripte (0,7% aller Transkripte) identifiziert werden, von denen 9 feincharakterisiert wurden. Für alle B-spezifischen Transkripte konnten mit Hilfe der Southern-, Northern-Hybridisierung, RT-PCR und Fluoreszenz *in situ* Hybridisierung ähnliche A-Chromosomen-lokalisierte Sequenzen nachgewiesen werden. Die B-spezifischen Transkripte B 1134, B 8149 und B 2465 sind Mitglieder repetetiver Sequenzfamilien. Diese zeigen Ähnlichkeit zu mobilen Sequenzelementen wie, *Gypsy*-Retrotransposons und *LINE*-Elementen. Diese Ergebnisse bestätigen die Annahme, dass Roggen B-Chromosomen aus den A-Chromosomen der gleichen Art entstanden.

8. Outlook

Rye plants with up to 2 B chromosomes were included in the cDNA-AFLP study described. In future experiments it would be of interest to perform cDNA-AFLP analyses on plants with a higher number of B chromosomes. This is because plants with more than 4 Bs are smaller and display disturbances in development and fertility (Jones, 1993). Therefore it is likely to find a higher number of B-specific transcripts in plants with a higher number of Bs.

The B chromosomes in rye can occur either in odd or even number. The negative impact caused by Bs presence was found to be stronger in case of odd number than an even one. Total amount of proteins and RNA were more affected in plants with 1B and 3B than in those with 2B, and 4B respectively (Kirk and Jones, 1970; Jones, 1982; Jones, 1995). The negative influence of odd and even on straw weight and tiller number of different rye varieties was also observed by Müntzing and colleagues (1963). Since there is no molecular prove for odd and even effect, in future it would be interesting to investigate its influence on transcription profile of rye B chromosomes.

The distribution of histone methylation pattern was characterized in root meristems. As the nondisjunction process of Bs occurs during first pollen grain and first egg cell mitosis, the investigation of histone methylation should be performed on anthers as well. The status of phosphorylation of H3 at S10/28 should be tested, because this cell cycle dependent modification correlates with the process of sister chromatid cohesion in plants.

The function of D1100 and E3900 B specific transcripts and the mechanism of their transcription are unknown at present. It is tempting to hypothesize that these transcripts could serve some structural function in the organization and regulation of B chromosomes. In order to test whether D1100 and E3900 transcripts are involved in the regulation of B-nondisjunction it would be of interest to perform RNA-FISH experiments with D1100 and E3900 RNA probes on pollen at the first pollen grain mitosis.

To identify additional putative transcripts involved in the process of B chromosome nondisjunction comparative cDNA-AFLP analysis should be performed on meiotic tissue.

9. Literature

Ahn, S.H., Cheung, W.L., Hsu, J.Y., Diaz, R.L., Smith, M.M., and Allis, C.D. (2005). Sterile 20 kinase phosphorylates histone H2B at serine 10 during hydrogen peroxide-induced apoptosis in *S. cerevisiae*. Cell **120,** 25-36.

Alfenito, M.R., and Birchler, J.A. (1993). Molecular characterization of a maize B chromosome centric sequence. Genetics **135,** 589-597.

Amos, A., and Dover, G. (1981). The distribution of repetitive DNAs between regular and supernumerary chromosomes in species of *Glossina* (Tsetse) - a two-step process in the origin of supernumeraries. Chromosoma **81,** 673-690.

Auger, D.L., and Birchler, J.A. (2002). Maize tertiary trisomic stocks derived from B-A translocations. Journal of Heredity **93,** 42-47.

Bachem, C.W.B., Oomen, R.J.F.J., and Visser, R.G.F. (1998). Transcript imaging with cDNA-AFLP: A step-by-step protocol. Plant Molecular Biology Reporter **16,** 157-173.

Bachem, C.W.B., vanderHoeven, R.S., deBruijn, S.M., Vreugdenhil, D., Zabeau, M., and Visser, R.G.F. (1996). Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development. Plant Jounal **9,** 745-753.

Bailey, J.A., Carrel, L., Chakravarti, A., and Eichler, E.E. (2000). Molecular evidence for a relationship between LINE-1 elements and X chromosome inactivation: The Lyon repeat hypothesis. Proc Natl Acad Sci USA **97,** 6634-6639.

Bang, J.-W., Choi H. W. (1990). Genetic analysis of esterase izozimes in rye (*Secale cereale* L.). Korean J. Genetics **12,** 87-94.

Bannister, A.J., and Kouzarides, T. (2005). Reversing histone methylation. Nature **436,** 1103-1106.

Barlow, P.W., and Vosa, C.G. (1969). Chromosomes of *Puschkinia libanotica* during mitosis. Chromosoma **27,** 436-&.

Barski, A., Cuddapah, S., Cui, K.R., Roh, T.Y., Schones, D.E., Wang, Z.B., Wei, G., Chepelev, I., and Zhao, K.J. (2007). High-resolution profiling of histone methylations in the human genome. Cell **129,** 823-837.

Battaglia, E. (1964). Cytogenetics of B chromosomes. Caryologia **17,** 245-&.

Beckett, J.B. (1978). B-A translocations in maize .1. Use in locating genes by chromosome arms. Journal of Heredity **69,** 27-36.

Beckett, J.B. (1991). Ctogenetic, genetic and plant breeding applications of B-A translocations in maize. Gupta PK, Tsuchiya T, eds. Chromosome Engineering in Plants: Genetics, Breeding, Evolution. Part A. Oxford: Elsevier, 493-529.

Berdnikov, V.A., Gorel, F.L., Kosterin, O.E., and Bogdanova, V.S. (2003). Tertiary trisomics in the garden pea as a model of B chromosome evolution in plants. Heredity **91,** 577-583.

Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T., and Schreiber, S.L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad Sci USA **99,** 8695-8700.

Bernstein, B.E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D.K., Huebert, D.J., McMahon, S., Karlsson, E.K., Kulbokas, E.J., 3rd, Gingeras, T.R., Schreiber, S.L., and Lander, E.S. (2005). Genomic maps and comparative analysis of histone modifications in human and mouse. Cell **120,** 169-181.

Birchler, J. (1991). Chromosome manipulations in maize. Gupta PK, Tsuchiya T, eds. Chromosome Engineering in Plants: Genetics, Breeding, Evolution. Part A. Oxford: Elsevier, 531-559.

Bird, A.W., Yu, D.Y., Pray-Grant, M.G., Qiu, Q.F., Harmon, K.E., Megee, P.C., Grant, P.A., Smith, M.M., and Christman, M.F. (2002). Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. Nature **419,** 411-415.

Blunden, R., Wilkes, T.J., Forster, J.W., Jimenez, M.M., Sandery, M.J., Karp, A., and Jones, R.N. (1993). Identification of the E3900 family, a 2nd family of rye B chromosome specific repeated sequences. Genome **36,** 706-711.

Bosemark, N.O. (1957a). Further studies on accessory chromosomes in grasses. Hereditas **43,** 236-297.

Bosemark, N.O. (1957b). On accessory chromosomes in *Festuca pratensis* .5. Influence of accessory chromosomes on fertility and vegetative development. Hereditas **43,** 211-235.

Bougourd, S.M., and Parker, J.S. (1979). B chromosome system of *Allium schoenoprasum*. 2. Stability, inheritance and phenotypic effects. Chromosoma **75,** 369-383.

Bouzinba-Segard, H., Guais, A., and Francastel, C. (2006). Accumulation of small murine minor satellite transcripts leads to impaired centromeric architecture and function. Proc Natl Acad Sci USA **103,** 8709-8714.

Brandes, A., HeslopHarrison, J.S., Kamm, A., Kubis, S., Doudrick, R.L., and Schmidt, T. (1997). Comparative analysis of the chromosomal and genomic organization of Ty1-copia-like retrotransposons in pteridophytes, gymnosperms and angiosperms. Plant Molecular Biology **33,** 11-21.

Breyne, P., Dreesen, R., Vandepoele, K., De Veylder, L., Van Breusegem, F., Callewaert, L., Rombauts, S., Raes, J., Cannoot, B., Engler, G., Inze, D., and Zabeau, M. (2002). Transcriptome analysis during cell division in plants. Proc Natl Acad Sci USA **99,** 14825-14830.

Brockhouse, C., Bass, J.A.B., Feraday, R.M., and Straus, N.A. (1989). Supernumerary chromosome evolution in the *Simulium vernum* group (Diptera, Simuliidae). Genome **32,** 516-521.

Bugrov, A.G., Karamysheva, T.V., Perepelov, E.A., Elisaphenko, E.A., Rubtsov, D.N., Warchalowska-Sliwa, E., Tatsuta, H., and Rubtsov, N.B. (2007). DNA content of the B chromosomes in grasshopper *Podisma kanoi* Storozh. (Orthoptera, Acrididae). Chromosome Research **15,** 315-326.

Burt, A., and Trivers, R. (1998). Selfish DNA and breeding system in flowering plants. Proceedings of the Royal Society of London Series B-Biological Sciences **265,** 141-146.

Burt, A. and Trivers, R. L. (2006) Genes in Conflict : The Biology of Selfish Genetic Elements. Belknap Press, Harvard.

Camacho, J.P. (2005). B chromosomes. Chapter in: The evolution of the Genome**,** Edited by T. Ryan Gregory, Elsevier, 223 - 286.

Camacho, J.P.M., Sharbel, T.F., and Beukeboom, L.W. (2000). B chromosome evolution. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **355,** 163-178.

Carchilan, M., Delgado, M., Ribeiro, T., Costa-Nunes, P., Caperta, A., Morais-Cecilio, L., Jones, R.N., Viegas, W., and Houben, A. (2007). Transcriptionally active heterochromatin in rye B chromosomes. The Plant Cell **19,** 1738-1749.

Carlson, W.R. (1969). Factors affecting preferential fertilization in maize. Genetics **62,** 543-&.

Carlson, W.R. (1978). B chromosome of corn. Annual Review of Genetics **12,** 5-23.

Carlson, W.R. (1986). The B chromosome of maize. Critical Reviews in Plant Sciences **3,** 201-226.

Carlson, W.R. (1994). Crossover effects of B chromosomes may be selfish. Heredity **72,** 636-638.

Carlson, W.R., and Roseman, R.R. (1992). A new property of the maize B chromosome. Genetics **131,** 211-223.

Carter, C.R., and Smith-White, S. (1972). Cytology of *Brachycome lineariloba* .3. Accessory chromosomes. Chromosoma **39,** 361-&.

Chaumeil, J., Le Baccon, P., Wutz, A., and Heard, E. (2006). A novel role for Xist RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced. Genes & Development **20,** 2223-2237.

Cheng, Y.M., and Lin, B.Y. (2003). Cloning and characterization of maize B chromosome sequences derived from microdissection. Genetics **164,** 299-310.

Cheng, Z.K., Yu, H.X., Yan, H.H., Gu, M.H., and Zhu, L.H. (2000). B chromosome in a rice aneuploid variation. Theoretical and Applied Genetics **101,** 564-568.

Chilton, M.D., and Mccarthy, B.J. (1973). DNA from maize with and without B chromosomes - comparative study. Genetics **74,** 605-614.

Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Analytical Biochemistry **162,** 156-159.

Cnudde, F., Hedatale, V., de Jong, H., Pierson, E.S., Rainey, D.Y., Zabeau, M., Weterings, K., Gerats, T., and Peters, J.L. (2006). Changes in gene expression during male meiosis in *Petunia hybrida*. Chromosome Research **14,** 919-932.

Covert, S.F. (1998). Supernumerary chromosomes in filamentous fungi. Current Genetics **33,** 311-319.

Craig, J.M. (2005). Heterochromatin-many flavours, common themes. Bioessays **27,** 17-28.

Cuadrado, A., and Jouve, N. (1994). Highly repetitive sequences in B chromosomes of *Secale cereale* revealed by fluorescence in-situ hybridization. Genome **37,** 709-712.

Cunado, N., Barrios, J., and Santos, J.L. (2000). Organization of highly repeated sequences in surface-spread pachytene chromosomes of rye. Genome **43,** 945-948.

de Jesus, C.M., Galetti, P.M., Valentini, S.R., and Moreira, O. (2003). Molecular characterization and chromosomal localization of two families of satellite DNA in *Prochilodus lineatus* (Pisces, Prochilodontidae), a species with B chromosomes. Genetica **118,** 25-32.

Delgado, M., Moraiscecilio, L., Neves, N., Jones, R.N., and Viegas, W. (1995). The influence of B chromosomes on rDNA organization in rye interphase nuclei. Chromosome Research **3,** 487-491.

Delgado, M., Caperta, A., Ribeiro, T., Viegas, W., Jones, R.N., and Morais-Cecilio, L. (2004). Different numbers of rye B chromosomes induce identical compaction changes in distinct A chromosome domains. Cytogenetic and genome research **106,** 320-324.

Dhar, M.K., Friebe, B., Koul, A.K., and Gill, B.S. (2002). Origin of an apparent B chromosome by mutation, chromosome fragmentation and specific DNA sequence amplification. Chromosoma **111,** 332-340.

Diatchenko, L., Lau, Y.F.C., Campbell, A.P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E.D., and Siebert, P.D. (1996). Suppression subtractive hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA **93,** 6025-6030.

Dimitri, P., Corradini, N., Rossi, F., and Verni, F. (2005). The paradox of functional heterochromatin. Bioessays **27,** 29-41.

Donald, T.M., Leach, C.R., Clough, A., and Timmis, J.N. (1995). Ribosomal RNA genes and the B chromosome of *Brachycome dichromosomatica*. Heredity **74,** 556-561.

Dorer, D.R., and Henikoff, S. (1994). Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*. Cell **77,** 993-1002.

Dover, G.A., and Riley, R. (1972). Prevention of pairing of homoeologous meiotic chromosomes of wheat by an activity of supernumerary chromosomes of *Aegilops*. Nature **240,** 159-&.

Ebert, A., Lein, S., Schotta, G., and Reuter, G. (2006). Histone modification and the control of heterochromatic gene silencing in Drosophila. Chromosome Research **14,** 377-392.

Endo, T., Nasuda, S, Jones, N, Dou, Q, Akahori, A, Wakimoto, M, Tanaka, H, Niwa, K and Tsujimoto H. (2008). Dissection of rye B chromosomes and nondisjunction of the dissected segments in common wheat. Genes & Genetic Systems. **83**.

Enkerli, J., Bhatt, G., and Covert, S.F. (1997). Nht1, a transposable element cloned from a dispensable chromosome in *Nectria haematococca*. Molecular Plant-Microbe Interactions **10,** 742-749.

Evans, G.M. (1960). Supernumerary chromosomes in wild populations of the snail *Helix pomatia* L. . Heredity **15,** 129 -138.

Evans, G.M., and Davies, E.W. (1985). The genetics of meiotic chromosome pairing in *Lolium temulentum* X *Lolium perenne* tetraploids. Theoretical and Applied Genetics **71,** 185-192.

Felsenfeld, G., and Groudine, M. (2003). Controlling the double helix. Nature **421,** 448-453.

Fox, D.P., Hewitt, G.M., and Hall, D.J. (1974). DNA replication and RNA transcription of euchromatic and heterochromatic chromosome regions during grasshopper meiosis. Chromosoma **45,** 43-62.

Franks, T.K., Houben, A., Leach, C.R., and Timmis, J.N. (1996). The molecular organisation of a B chromosome tandem repeat sequence from *Brachycome dichromosomatica*. Chromosoma **105,** 223-230.

Fregonezi, J.N., Vilas-Boas, L.A., Fungaro, M.H.P., Gaeta, M.L., and Vanzela, A.L. (2007). Distribution of a Ty3/gypsy-like retroelement on the a and B-chromosomes of *Cestrum strigilatum* Ruiz & Pav. and *Cestrum intermedium* sendtn. (Solanaceae). Genetics and Molecular Biology **30,** 599-604.

Friebe, B. (1980). Lack of effect of B chromosomes on sister chromatid exchanges in *Secale cereale*. Environmental and Experimental Botany **20,** 21-26.

Frost, S. (1956). The cytological behaviour of accessory chromosomes in *Centaurea scabiosa*. Hereditas **42,** 415-431.

Frost, S. (1958). Studies of the genetical effects of accessory chromosomes in *Centaurea scabiosa*. Hereditas **44,** 112-122.

Frost, S. (1959). The cytological behaviour and mode of transmission of accessory chromosomes in *Plantago serraria*. Hereditas **45,** 191-210.

Fuchs, J., Demidov, D., Houben, A., and Schubert, I. (2006). Chromosomal histone modification patterns - from conservation to diversity (vol 11, pg 199, 2006). Trends in Plant Science **11,** 212-212.

Gehring, M., and Henikoff, S. (2007). DNA methylation dynamics in plant genomes. Biochimica Et Biophysica Acta-Gene Structure and Expression **1769,** 276-286.

Gibson, I., and Hewitt, G. (1970). Isolation of DNA from B chromosomes in grasshoppers. Nature **225,** 67-&.

Gillies, C.B. (1983). Ultrastructural studies of the association of homologous and non homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Maydica **28,** 265-287.

Gonzalez-Sanchez, M., Gonzalez-Gonzalez, E., Molina, F., Chiavarino, A.M., Rosato, M., and Puertas, M.J. (2003). One gene determines maize B chromosome accumulation by preferential fertilisation; another gene(s) determines their meiotic loss. Heredity **90,** 122-129.

Gonzalez-Sanchez, M., Chiavarino, M., Jimenez, G., Manzanero, S., Rosato, M., and Puertas, M.J. (2004). The parasitic effects of rye B chromosomes might be beneficial in the long term. Cytogenetic and genome research **106,** 386-393.

Gorlov, I.P., and Tsurusaki, N. (2000). Morphology and meiotic/mitotic behavior of B cromosomes in a Japanese harvestman, *Metagagrella tenuipes* (Arachnida: Opiliones): No evidence for B accumulation mechanisms. Zoological Science **17,** 349-355.

Gotoh, K. (1924). Über die Chromosomenzahl von *Secale cereale* L.. Bot. Mag.Tokyo **38,** 135-152.

Grandbastien, M.A. (1998). Activation of plant retrotransposons under stress conditions. Trends in Plant Science **3,** 181-187.

Graphodatsky, A.S., Kukekova, A.V., Yudkin, D.V., Trifonov, V.A., Vorobieva, N.V., Beklemisheva, V.R., Perelman, P.L., Graphodatskaya, D.A., Trut, L.N., Yang, F.T., Ferguson-Smith, M.A., Acland, G.M., and Aguirre, G.D. (2005). The proto-oncogene C-KIT maps to canid B chromosomes. Chromosome Research **13,** 113-122.

Gray, C.T., and Thomas, S.M. (1985). Germination and B chromosomes in *Allium porrum* L. Journal of Plant Physiology **121,** 281-285.

Green, D.M. (1988). Cytogenetics of the endemic New Zealand frog, *Leiopelma hochstetteri* - extraordinary supernumerary chromosome variation and a unique sex chromosome system. Chromosoma **97,** 55-70.

Green, D.M. (1990). Muller Ratchet and the evolution of supernumerary chromosomes. Genome **33,** 818-824.

Green, D.M., Zeyl, C.W., and Sharbel, T.F. (1993). The evolution of hypervariable sex and supernumerary (B) chromosomes in the relict New Zealand frog, *Leiopelma hochstetteri*. Journal of evolutionary biology **6,** 417-441.

Greilhuber, J., and Speta, F. (1976). C-Banded Karyotypes in Scilla-Hohenackeri Group, Scilla-Persica, and Puschkinia (Liliaceae). Plant Systematics and Evolution **126,** 149-188.

Gruenbaum, Y., Navehmany, T., Cedar, H., and Razin, A. (1981). Sequence specificity of methylation in higher-plant DNA. Nature **292,** 860-862.

Grun, P. (1959). Variability of accessory chromosomes in native populations of *Allium cernuum*. American Journal of Botany **46,** 218-224.

Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. Nature **389,** 349-352.

Gutknecht, J., Sperlich, D., and Bachmann, L. (1995). A species specific satellite DNA family of *Drosophila subsilvestris* appearing predominantly in B chromosomes. Chromosoma **103,** 539-544.

Hakansson, A. (1948). Behaviour of accessory rye chromosomes in the embryo-sac. Hereditas **34,** 35-59.

Hakansson, A. (1957). Meiosis and pollen mitosis in rye plants with many accessory chromosomes. Hereditas **43,** 603-620.

Han, Y.N., Liu, X.G., Benny, U., Kistler, H.C., and VanEtten, H.D. (2001). Genes determining pathogenicity to pea are clustered on a supernumerary chromosome in the fungal plant pathogen *Nectria haematococca*. Plant Journal **25,** 305-314.

Hansen, J.C. (2002). Conformational dynamics of the chromatin fiber in solution: Determinants, mechanisms, and functions. Annual Review of Biophysics and Biomolecular Structure **31,** 361-392.

Harvey, A.W., and Hewitt, G.M. (1979). B chromosomes slow development in a grasshopper. Heredity **42,** 397-401.

Hatta, R., Ito, K., Hosaki, Y., Tanaka, T., Tanaka, A., Yamamoto, M., Akimitsu, K., and Tsuge, T. (2002). A conditionally dispensable chromosome controls host-specific pathogenicity in the fungal plant pathogen Alternaria alternata. Genetics **161,** 59-70.

Heitz, E. (1928). Das Heterochromatin der Moose. Jahrbücher Wiss. Bot. **69,** 762-818.

Heslop-Harrison, J.S., Brandes, A., Taketa, S., Schmidt, T., Vershinin, A.V., Alkhimova, E.G., Kamm, A., Doudrick, R.L., Schwarzacher, T., Katsiotis, A., Kubis, S., Kumar, A., Pearce, S.R., Flavell, A.J., and Harrison, G.E. (1997). The chromosomal distributions of Ty1-copia group retrotransposable elements in higher plants and their implications for genome evolution. Genetica **100,** 197-204.

Hewitt, G. (1973). Variable transmission rates of a B chromosome in *Myrmeleotettix maculatus* (Thunb) (Acridiae-Orthoptera). Chromosoma **40,** 83-106.

Hewitt, G.M. (1976). Meiotic drive for B chromosomes in primary oocytes of *Myrmeleotettix maculatus* (Orthoptera-Acrididae). Chromosoma **56,** 381-391.

Hewitt, G.M., East, T.M., and Shaw, M.W. (1987). Sperm dysfunction produced by B chromosomes in the grasshopper *Myrmeleotettix maculatus*. Heredity **58,** 59-68.

Hiremath, S.C., and Murthy, H.N. (1986). The structure, stability and meiotic behavior of B chromosome in *Guizotia scabra* (Vis) Chiov Ssp Scabra (Compositae). Caryologia **39,** 397-402.

Hirochika, H. (1993). Activation of tobacco retrotransposons during tissue culture. Embo J **12,** 2521-2528.

Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H., and Kanda, M. (1996a). Retrotransposons of rice involved in mutations induced by tissue culture. Proc Natl Acad Sci USA **93,** 7783-7788.

Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H., and Kanda, M. (1996b). Retrotransposons of rice involved in mutations induced by tissue culture. Proc Natl Acad Sci U S A **93,** 7783-7788.

Hizume, M., Sato, S., and Tanaka, A. (1980). A highly reproducible method of nucleolus organizing regions staining in plants. Stain technology **55,** 87-90.

Hobza, R., Lengerova, M., Svoboda, J., Kubekova, H., Kejnovsky, E., and Vyskot, B. (2006). An accumulation of tandem DNA repeats on the Y chromosome in *Silene latifolia* during early stages of sex chromosome evolution. Chromosoma **115,** 376-382.

Holmes, D.S., and Bougourd, S.M. (1989). B chromosome selection in *Allium schoenoprasum*.1. Natural populations. Heredity **63,** 83-87.

Holmes, D.S., and Bougourd, S.M. (1991). B chromosome selection in *Allium schoenoprasum*. 2. Experimental populations. Heredity **67,** 117-122.

Houben, A., Orford, S.J., and Timmis, J.N. (2006). In situ hybridization to plant tissues and chromosomes. Methods Mol Biol **326,** 203-218.

Houben, A., Belyaev, N.D., Leach, C.R., and Timmis, J.N. (1997). Differences of histone H4 acetylation and replication timing between A and B chromosomes of *Brachycome dichromosomatica*. Chromosome Research **5,** 233-237.

Houben, A., Verlin, D., Leach, C.R., and Timmis, J.N. (2001). The genomic complexity of micro B chromosomes of Brachycome dichromosomatica. Chromosoma **110,** 451-459.

Houben, A., Kynast, R.G., Heim, U., Hermann, H., Jones, R.N., and Forster, J.W. (1996). Molecular cytogenetic characterisation of the terminal heterochromatic segment of the B-chromosome of rye (*Secale cereale*). Chromosoma **105,** 97-103.

Houben, A., Demidov, D., Gernand, D., Meister, A., Leach, C.R., and Schubert, I. (2003). Methylation of histone H3 in euchromatin of plant chromosomes depends on basic nuclear DNA content. Plant Journal **33,** 967-973.

Houben, A., Demidov, D., Caperta, A.D., Karimi, R., Agueci, F., and Vlasenko, L. (2007). Phosphorylation of histone H3 in plants-A dynamic affair. Biochimica et biophysica acta **1769,** 308-315.

Ikura, T., Ogryzko, V.V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000). Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell **102,** 463-473.

Ishak, B., Jaafar, H., Maetz, J.L., and Rumpler, Y. (1991). Absence of transcriptional activity of the B chromosomes of *Apodemus peninsulae* during pachytene. Chromosoma **100,** 278-281.

Jackson, J.P., Johnson, L., Jasencakova, Z., Zhang, X., PerezBurgos, L., Singh, P.B., Cheng, X.D., Schubert, I., Jenuwein, T., and Jacobsen, S.E. (2004). Dimethylation of histone H3 lysine 9 is a critical mark for DNA methylation and gene silencing in Arabidopsis thaliana. Chromosoma **112,** 308-315.

Jackson, R.C., and Newmark, P. (1960). Effects of supernumerary chromosomes on production of pigment in *Haplopappus gracilis*. Science **132,** 1316-1317.

Jamilena, M., Rejon, C.R., and Rejon, M.R. (1994). A molecular analysis of the origin of the *Crepis capillaris* B chromosome. Journal of Cell Sciences **107,** 703-708.

Jamilena, M., Garridoramos, M., Rejon, M.R., Rejon, C.R., and Parker, J.S. (1995). Characterization of repeated sequences from microdissected B chromosomes of *Crepis capillaris*. Chromosoma **104,** 113-120.

Jasencakova, Z., Soppe, W.J.J., Meister, A., Gernand, D., Turner, B.M., and Schubert, I. (2003). Histone modifications in Arabidopsis - high methylation of H3 lysine 9 is dispensable for constitutive heterochromatin. Plant Journal **33,** 471-480.

Jenkins, G. (1986). Synaptonemal complex formation in hybrids of *Lolium temulentum* X *Lolium perenne* (L) .3. Tetraploid. Chromosoma **93,** 413-419.

Jenkins, G., and Jones, R.N. (2004). B chromosomes in hybrids of temperate cereals and grasses. Cytogenetic and genome research **106,** 314-319.

Jenuwein, T., and Allis, C.D. (2001). Translating the histone code. Science **293,** 1074-1080.

Jimenez, G., Manzanero, S., and Puertas, M. (2000). Relationship between pachytene synapsis, metaphase I associations, and transmission of 2B and 4B chromosomes in rye. Genome **43,** 232-239.

Jimenez, M.M., Romera, F., Puertas, M.J., and Jones, R.N. (1994). B chromosomes in inbred lines of rye (*Secale cereale* L) .1. Vigor and fertility. Genetica **92,** 149-154.

Jimenez, M.M., Romera, F., GonzalezSanchez, M., and Puertas, M.J. (1997). Genetic control of the rate of transmission of rye B chromosomes .3. Male meiosis and gametogenesis. Heredity **78,** 636-644.

Jojic, V., Blagojevic, J., Ivanovic, A., Bugarski-Stanojevic, V., and Vujosevic, M. (2007). Morphological integration of the mandible in yellow-necked field mice: The effects of B chromosomes. Journal of Mammalogy **88,** 689-695.

Jones, G.H., Whitehorn, J.A.F., and Albini, S.M. (1989). Ultrastructure of meiotic pairing in B chromosomes of *Crepis capillaris* .1. One-B and 2-B pollen mother cells. Genome **32,** 611-621.

Jones, G.H., Albini, S.M., and Whitehorn, J.A.F. (1991). Ultrastructure of Meiotic Pairing in B-Chromosomes of Crepis-Capillaris .2. 4 B Pollen Mother Cells. Chromosoma **100,** 193-202.

Jones, J.D.G., and Flavell, R.B. (1982). The structure, amount and chromosomal localization of defined repeated DNA sequences in species of the genus *Secale*. Chromosoma **86,** 613-641.

Jones, N., and Houben, A. (2003). B chromosomes in plants: escapees from the A chromosome genome? Trends in Plant Science **8,** 417-423.

Jones, R.N. (1975). B chromosome systems in flowering plants and animal species. International Revew of Cytology. **40,** 1-100.

Jones, R.N. (1991). B-Chromosome drive. American Naturalist **137,** 430-442.

Jones, R.N. (1995). Tansley review no 85 - B chromosomes in plants. New Phytologist **131,** 411-434.

Jones, R.N., and Rees, H. (1967). Genotypic control of chromosome behaviour in rye .11. Influence of B chromosomes on meiosis. Heredity **22,** 333-&.

Jones, R.N., and Diez, M. (2004). The B chromosome database. Cytogenetic and genome research **106,** 149-150.

Jones, R.N., Puertas, M.J. (1993). The B chromosomes of rye (*Secale cereale* L.). In Dhir KK, Sareen TS, eds. Frontiers in Plant Science Research. (Dehli (India): Bhagwati Enterprises), pp. 81-112.

Jones, R.N., Rees., H. (1982). B chromosomes. (London: Academic Press).

Kaszas, E., and Cande, W.Z. (2000). Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin. Jounal of Cell Sciences **113,** 3217-3226.

Kayano, H. (1957). Cytogenetic studies in *Lilium callosum.* III Preferential segregationof a supernumerary chromosome in EMCs. Proccedings of the Japanese Academy **33,** 553-558.

Kayano, H. (1971). Accumulation of B chromosomes in germ line of *Locusta migratoria*. Heredity **27,** 119-123.

Kimura, M., and Kayano, H. (1961). Maintenance of supernumerary chromosomes in wild populations of *Lilium callosum* by preferential segregation. Genetics **46,** 1699-&.

Kirk, D., and Jones, R.N. (1970). Nuclear genetic activity in B chromosome rye, in terms of quantitative interrelationships between nuclear protein, nuclear RNA and histone. Chromosoma **31,** 241-&.

Kishikawa.H. (1968). Cytological studies on rye plants with 7 and 8 accessory chromosomes. Japanese Journal of Genetics **43,** 33-&.

Kistler, H.C., and Miao, V.P.W. (1992). New modes of genetic change in filamentous fungi. Annual Review of Phytopathology **30,** 131-152.

Klein, A.S., and Eckhardt, R.A. (1976). DNAs of A and B chromosomes of Mealy Bug, *Pseudococcus obscurus*. Chromosoma **57,** 333-340.

Kornberg, R.D., and Lorch, Y.L. (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell **98,** 285-294.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell **128,** 693-705.

Krishnamoorthy, T., Chen, X., Govin, J., Cheung, W.L., Dorsey, J., Schindler, K., Winter, E., Allis, C.D., Guacci, V., Khochbin, S., Fuller, M.T., and Berger, S.L. (2006). Phosphorylation of histone H4 Ser1 regulates sporulation in yeast and is conserved in fly and mouse spermatogenesis. Gene & Development **20,** 2580-2592.

Kumar, A., and Bennetzen, J.L. (1999). Plant retrotransposons. Annual Review of Genetics **33,** 479-532.

Lamb, J.C., Riddle, N.C., Cheng, Y.M., Theuri, J., and Birchler, J.A. (2007a). Localization and transcription of a retrotransposon-derived element on the maize B chromosome. Chromosome Research**15,** 383-398.

Lamb, J.C., Meyer, J.M., Corcoran, B., Kato, A., Han, F., and Birchler, J.A. (2007b). Distinct chromosomal distributions of highly repetitive sequences in maize. Chromosome Research **15,** 33-49.

Langdon, T., Seago, C., Jones, R.N., Ougham, H., Thomas, H., Forster, J.W., and Jenkins, G. (2000). De novo evolution of satellite DNA on the rye B chromosome. Genetics **154,** 869-884.

Le Thi, K., Lespinasse, R., Siljakyakovlev, S., Robert, T., Khalfallah, N., and Sarr, A. (1994). Karyotypic modifications in androgenetic plantlets of *Pearl millet*, *Pennisetum glaucum* (L) R-Brunken - occurrence of B chromosomes. Caryologia **47,** 1-10.

Leach, C.R., Donald, T.M., Franks, T.K., Spiniello, S.S., Hanrahan, C.F., and Timmis, J.N. (1995). Organisation and origin of a B chromosome centromeric sequence from *Brachycome dichromosomatica*. Chromosoma **103,** 708-714.

Leach, C.R., Houben, A., Field, B., Pistrick, K., Demidov, D., and Timmis, J.N. (2005). Molecular evidence for transcription of genes on a B chromosome in *Crepis capillaris*. Genetics **171,** 269-278.

Levin, D.A., Palestis, B.G., Jones, R.N., and Trivers, R. (2005). Phyletic hot spots for B chromosomes in angiosperms. Evolution **59,** 962-969.

Lia, V.V., Confalonieri, V.A., and Poggio, L. (2007). B chromosome polymorphism in maize landraces: Adaptive vs. demographic hypothesis of clinal variation. Genetics **177,** 895-904.

Liang, P., and Pardee, A.B. (1992). Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science **257,** 967-971.

Lima-De-Faria, A. (1962). Genetic Interaction in Rye Expressed at the Chromosome Phenotype. Genetics **47,** 1455-1462.

Linacero, R., Alves, E.F., and Vazquez, A.M. (2000). Hot spots of DNA instability revealed through the study of somaclonal variation in rye. Theoretical and Applied Genetics **100,** 506-511.

Lindroth, A.M., Shultis, D., Jasencakova, Z., Fuchs, J., Johnson, L., Schubert, D., Patnaik, D., Pradhan, S., Goodrich, J., Schubert, I., Jenuwein, T., Khorasanizadeh, S., and Jacobsen, S.E. (2004). Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with CHROMOMETHYLASE3. Embo J **23,** 4146-4155.

Lopez-Leon, M.D., Neves, N., Schwarzacher, T., Heslop-Harrison, J.S., Hewitt, G.M., and Camacho, J.P. (1994). Possible origin of a B chromosome deduced from its DNA composition using double FISH technique. Chromosome Research **2,** 87-92.

Lopezleon, M.D., Cabrero, J., Pardo, M.C., Viseras, E., Camacho, J.P.M., and Santos, J.L. (1993). Generating high variability of B chromosomes in *Eyprepocnemis plorans* (grasshopper). Heredity **71,** 352-362.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 angstrom resolution. Nature **389,** 251-260.

Lyon, M.F. (1998). X-chromosome inactivation: a repeat hypothesis. Cytogenetics and cell genetics **80,** 133-137.

Maistro, E.L., Foresti, F., Oliveira, C., and Toledo, L.F.D. (1992). Occurrence of macro-B chromosomes in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). Genetica **87,** 101-106.

Maistro, E.L.F., F. Oliveira, C. Almeida-Toledo, L.F. (1992). Occurrence of macro B chromosomes in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae) Genetica **87,** 101-106.

Maluszynska, J., and Schweizer, D. (1989). Ribosomal RNA genes in B chromosomes of *Crepis capillaris* detected by non-radioactive in situ hybridization. Heredity **62,** 59-65.

Marschner, S., Kumke, K., and Houben, A. (2007a). B chromosomes of *B. dichromosomatica* show a reduced level of euchromatic histone H3 methylation marks. Chromosome Research **15,** 215-222.

Marschner, S., Meister, A., Blattner, F.R., and Houben, A. (2007b). Evolution and function of B chromosome 45S rDNA sequences in *Brachycome dichromosomatica*. Genome **50,** 638-644.

Martin, C., and Zhang, Y. (2005). The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol **6,** 838-849.

Mathieu, O., Probst, A.V., and Paszkowski, J. (2005). Distinct regulation of histone H3 methylation at lysines 27 and 9 by CpG methylation in Arabidopsis. Embo J **24,** 2783-2791.

May, B.P., Lippman, Z.B., Fang, Y., Spector, D.L., and Martienssen, R.A. (2005). Differential regulation of strand-specific transcripts from *Arabidopsis* centromeric satellite repeats. PLoS Genet **1,** e79.

McAllister, B.F., and Werren, J.H. (1997). Hybrid origin of a B chromosome (PSR) in the parasitic wasp *Nasonia vitripennis*. Chromosoma **106,** 243-253.

McIntyre, C.L., Pereira, S., Moran, L.B., and Appels, R. (1990). New Secale-Cereale (Rye) DNA Derivatives for the Detection of Rye Chromosome Segments in Wheat. Genome **33,** 635-640.

McVean, G.T. (1995). Fractious chromosomes - hybrid disruption and the origin of selfish genetic elements. Bioessays **17,** 579-582.

Mendelson, D., and Zohary, D. (1972). Behavior and transmission of supernumerary chromosomes in *Aegilops speltoides*. Heredity **29,** 329-&.

Mette, M.F., Aufsatz, W., Kanno, T., Daxinger, L., Rovina, P., Matzke, M., and Matzke, A. J. . (2005). Analysis of double-stranded RNA and small RNAs involved in RNA-mediated transcriptional gene silencing. Methods Mol Biol **309,** 61-82.

Miao, F., and Natarajan, R. (2005). Mapping global histone methylation patterns in the coding regions of human genes. Mol Cell Biol **25,** 4650-4661.

Miao, V.P., Covert, S.F., and Vanetten, H.D. (1991). A fungal gene for antibiotic resistance on a dispensable (B) chromosome. Science **254,** 1773-1776.

Mizoguchi, S.M.H.N., and Martins-Santos, I.C. (1997). Macro- and microchromosomes B in females of *Astyanax scabripinnis* (Pisces, Characidae). Hereditas **127,** 249-253.

Morais-Cecilio, L., Delgado, M., Jones, R.N., and Viegas, W. (1996). Painting rye B chromosomes in wheat: interphase chromatin organization, nuclear disposition and association in plants with two, three or four Bs. Chromosome Research **4,** 195-200.

Müntzing, A. (1943). Genetical effects of duplicated fragment chromosomes in rye. Hereditas **29** 91-112.

Müntzing, A. (1946). Cytological studies of extra fragment chromosomes in rye .3. The mechanism of non-disjunction at the pollen mitosis. Hereditas **32,** 97-119.

Müntzing, A. (1954). An analysis of hybrid vigour in tetraploid rye. Hereditas **40,** 265-277.

Müntzing, A. (1963). Effects of accessory chromosomes in diploid and tetraploid rye. Hereditas **49,** 371-&.

Müntzing, A. (1967). Some main results from investigations of accessory chromosomes. Hereditas-Genetiskt Arkiv **57,** 432-438.

Müntzing, A. (1970). Chromosomal variation in Lindstrom strain of wheat carrying accessory chromosomes of rye. Hereditas-Genetiskt Arkiv **66,** 279-&.

Müntzing, A. (1973). Effects of accessory chromosomes of rye in gene environment of hexaploid wheat. Hereditas **74,** 41-55.

Müntzing, A., and Lima-de-Faria, A. (1952). Pachytene analysis of a deficient accessory chromosome in rye. Hereditas **38,** 1-10.

Müntzing, A., and Nygren, A. (1955). A new diploid variety of *Poa alpina* with 2 accessory chromosomes at meiosis. Hereditas **41,** 405-&.

Naumann, K., Fischer, A., Hofmann, I., Krauss, V., Phalke, S., Irmler, K., Hause, G., Aurich, A.C., Dorn, R., Jenuwein, T., and Reuter, G. (2005). Pivotal role of AtSUVH2 in heterochromatic histone methylation and gene silencing in Arabidopsis. Embo J **24,** 1418-1429.

Neo, D.M., Filho, O.M., and Camacho, J.P. (2000). Altitudinal variation for B chromosome frequency in the characid fish *Astyanax scabripinnis*. Heredity **85 (Pt 2),** 136-141.

Nielsen, P.S., Kleinhofs, A., and Olsen, O.A. (1997). Barley elongation factor 1 alpha: genomic organization, DNA sequence, and phylogenetic implications. Genome **40,** 559-565.

Niwa, K., and Tsujimoto, H. (1992). 18s.26s Ribosomal-Rna Genes Are Not in Every Case Located on the B-Chromosomes in the Rye Genome. Plant Breeding **109,** 78-81.

Niwa, K., and Sakamoto, S. (1995). Origin of B-Chromosomes in Cultivated Rye. Genome **38,** 307-312.

Niwa, K., and Sakamoto, S. (1996). Detection of B chromosomes in rye collected from Pakistan and China. Hereditas **124,** 211-215.

Nur, U. (1962). A supernumerary chromosome with an accumulation mechanism in lecanoid genetic system. Chromosoma **13,** 249-&.

Nur, U. (1963). A mitotically unstable supernumerary chromosome with an accumulation mechanism in a grasshopper. Chromosoma **14,** 407-&.

Nur, U. (1969). Mitotic instability leading to an accumulation of B chromosomes in grasshoppers. Chromosoma **27,** 1-&.

Nur, U., and Nevo, E. (1969). Supernumerary chromosomes in cricket frog *Acris crepitans*. Caryologia **22,** 97-&.

Ortiz, M., Puertas, M.J., Jimenez, M.M., Romera, F., and Jones, R.N. (1996). B-chromosomes in inbred lines of rye (*Secale cereale* L) .2. Effects on metaphase I and first pollen mitosis. Genetica **97,** 65-72.

Ostergren, G. (1947). Heterochromatic B chromosomes in *Anthoxanthum*. Hereditas **33,** 261-296.

Page, B.T., Wanous, M.K., and Birchler, J.A. (2001). Characterization of a maize chromosome 4 centromeric sequence: Evidence for an evolutionary relationship with the B chromosome centromere. Genetics **159,** 291-302.

Palestis, B.G., Trivers, R., Burt, A., and Jones, R.N. (2004a). The distribution of B chromosomes across species. Cytogenetic and genome research **106,** 151-158.

Palestis, B.G., Burt, A., Jones, R.N., and Trivers, R. (2004b). B chromosomes are more frequent in mammals with acrocentric karyotypes: support for the theory of centromeric drive. Proceedings of the Royal Society of London Series B-Biological Sciences **271,** S22-S24.

Paliwal, R.L., and Hyde, B.B. (1959). The association of a single B chromosome with male sterility in *Plantago coronopus*. American Journal of Botany **46,** 460-466.

Parker, J.S., Jones, G.H., Edgar, L., and Whitehouse, C. (1989). The population cytogenetics of *Crepis capillaris* .2. The sability and inheritance of B chromosomes. Heredity **63,** 19-27.

Pearce, S.R., Harrison, G., HeslopHarrison, P.J.S., Flavell, A.J., and Kumar, A. (1997). Characterization and genomic organization of Ty1-copia group retrotransposons in rye (Secale cereale). Genome **40,** 617-625.

Pearce, S.R., Harrison, G., Li, D.T., HeslopHarrison, J.S., Kumar, A., and Flavell, A.J. (1996). The Ty1-copia group retrotransposons in *Vicia* species: Copy number, sequence heterogeneity and chromosomal localisation. Molecular & General Genetics **250,** 305-315.

Peters, A., Kubicek, S., Mechtler, K., O'Sullivan, R.J., Derijck, A., Perez-Burgos, L., Kohimaier, A., Opravil, S., Tachibana, M., Shinkai, Y., Martens, J.H.A., and Jenuwein, T. (2003). Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell **12,** 1577-1589.

Plowman, A.B., and Bougourd, S.M. (1994). Selectively advantageous effects of B chromosomes on germination behavior in *Allium schoenoprasum* L. Heredity **72,** 587-593.

Pohler, W., and Schlegel, R. (1990). A rye plant with frequent A-B chromosome pairing. Hereditas **112,** 217-220.

Portela-Castro, A.L.D., Juilo, H.F., and Nishiyama, P.B. (2000). New occurrence of microchromosomes B in *Moenkhausia sanctaefilomenae* (Pisces, Characidae) from the Parana River of Brazil: analysis of the synaptonemal complex. Genetica **110,** 277-283.

Price, L., Short, K.C., and Roberts, A.V. (1981). Poor resolution of C bands and the presence of B chromosomes in *Rosa rugosa scabrosa*. Caryologia **34,** 69-72.

Prigent, C., and Dimitrov, S. (2003). Phosphorylation of serine 10 in histone H3, what for? J Cell Sci **116,** 3677-3685.

Probst, A.V., Fagard, M., Proux, F., Mourrain, P., Boutet, S., Earley, K., Lawrence, R.J., Pikaard, C.S., Murfett, J., Furner, I., Vaucheret, H., and Scheid, O.M. (2004). Arabidopsis histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. The Plant Cell **16,** 1021-1034.

Pryor, A., Faulkner, K., Rhoades, M.M., and Peacock, W.J. (1980). Asynchronous replication of heterochromatin in maize. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences **77,** 6705-6709.

Puertas, M.J., and Lacadena, J.R. (1974). Transmission of rye B chromosomes in natural pollination. Theoretical and Applied Genetics **45,** 197-204.

Puertas, M.J., Romera, F., and Delapena, A. (1985). Comparison of B chromosome effects on *Secale cereale* and *Secale vavilovii*. Heredity **55,** 229-234.

Puertas, M.J., Ramirez, A., and Baeza, F. (1987). The transmission of B chromosomes in *Secale cereale* and *Secale vavilovii* populations .2. Dynamics of populations. Heredity **58,** 81-85.

Puertas, M.J., Jimenez, M.M., Romera, F., Vega, J.M., and Diez, M. (1990). Maternal imprinting effect on B chromosome transmission in rye. Heredity **64,** 197-204.

Raghuvanshi, S.S., and Kumar, G. (1983). No male sterility gene on B chromosomes in Plantago coronopus. Heredity **51,** 429-&.

Raman, R., and Sharma, T. (1974). DNA replication, G-bands and C-bands and meiotic behavior of supernumerary chromosomes of *Rattus rattus* (Linn). Chromosoma **45,** 111-119.

Richards, E.J., and Elgin, S.C.R. (2002). Epigenetic codes for heterochromatin formation and silencing: Rounding up the usual suspects. Cell **108,** 489-500.

Rimpau, J., and Flavell, R.B. (1975). Characterization of rye B chromosome DNA by DNA-DNA hybridization. Chromosoma **52,** 207-217.

Rizzi, N., Denegri, M., Chiodi, I., Corioni, M., Valgardsdottir, R., Cobianchi, F., Riva, S., and Biamonti, G. (2004). Transcriptional activation of a constitutive heterochromatic domain of the human genome in response to heat shock. Molecular Biology of the Cell **15,** 543-551.

Roman, H. (1947). Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. Genetics **32,** 391-409.

Roman, H. (1948a). Directed Fertilization in Maize. Proc Natl Acad Sci USA **34,** 36-42.

Roman, H. (1948b). Selective Fertilization in Maize. Genetics **33,** 122-122.

Romera, F., Jimenez, M.M., and Puertas, M.J. (1991). Genetic control of the rate of transmission of rye B chromosomes .1. Effects in 2Bx0B crosses. Heredity **66,** 61-65.

Rosato, M., Chiavarino, A.M., Naranjo, C.A., Hernandez, J.C., and Poggio, L. (1998). Genome size and numerical polymorphism for the B chromosome in races of maize (*Zea mays* ssp. *Mays*, Poaceae). American Journal of Botany **85,** 168-174.

Rothfels, K.H. (1950). Chromosome complement, polyploidy and supernumeraries in *Neopodismopsis abdominalis* (Acrididae). Journal of Morphology **87,** 287-315.

Ruizrejon, M., Posse, F., and Oliver, J.L. (1980). The B chromosome system of *Scilla autumnalis* (Liliaceae) - effects at the isoenzyme level. Chromosoma **79,** 341-348.

Sakamoto, K., Ohmido, N., Fukui, K., Kamada, H., and Satoh, S. (2000). Site-specific accumulation of a LINE-like retrotransposon in a sex chromosome of the dioecious plant *Cannabis sativa*. Plant Molecular Biology **44,** 723-732.

Sambrook, J, R.D., Fritsch, E.F., Maniatis, T., (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press.

Sandery, M.J., Forster, J.W., Blunden, R., and Jones, R.N. (1990). Identification of a family of repeated sequences on the rye B chromosome. Genome **33,** 908-913.

Sanguinetti, C.J., Neto, E.D., and Simpson, A.J.G. (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques **17,** 914-&.

Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C.T., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. Nature **419,** 407-411.

Sapre, A.B., and Deshpande, D.S. (1987). Origin of B chromosomes in *Coix* L through spontaneous interspecific hybridization. Journal of Heredity **78,** 191-196.

Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J.T., Schmid, M., and Parzefall, J. (1995). Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish (Vol 373, Pg 68, 1995). Nature **374,** 196-196.

Schmid, M., Ziegler, C.G., Steinlein, C., Nanda, I., and Haaf, T. (2002). Chromosome banding in amphibia - XXIV. The B chromosomes of *Gastrotheca espeletia* (Anura, Hylidae). Cytogenetic and genome research **97,** 205-218.

Schmid, M., Ziegler, C.G., Steinlein, C., Nanda, I., and Schartl, M. (2006). Cytogenetics of the bleak (*Alburnus alburnus*), with special emphasis on the B chromosomes. Chromosome Research **14,** 231-242.

Schmidt, T. (1999). LINEs, SINEs and repetitive DNA: non-LTR retrotransposons in plant genomes. Plant Molecular Biology **40,** 903-910.

Schneider, R., Bannister, A.J., Myers, F.A., Thorne, A.W., Crane-Robinson, C., and Kouzarides, T. (2004). Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. Nat Cell Biol **6,** 73-77.

Schotta, G., Lachner, M., Sarma, K., Ebert, A., Sengupta, R., Reuter, G., Reinberg, D., and Jenuwein, T. (2004). A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Gene Dev **18,** 1251-1262.

Schubeler, D., MacAlpine, D.M., Scalzo, D., Wirbelauer, C., Kooperberg, C., van Leeuwen, F., Gottschling, D.E., O'Neill, L.P., Turner, B.M., Delrow, J., Bell, S.P., and Groudine, M. (2004). The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Gene Dev **18,** 1263-1271.

Schwarzacher, T. (2003). DNA, chromosomes, and in situ hybridization. Genome **46,** 953-962.

Shambulingappa, K.G. (1965). Occurrence of B chromosomes in genus *Clematis*. Current Science **34,** 670-&.

Sharbel, T.F., Green, D.M., and Houben, A. (1998). B-chromosome origin in the endemic New Zealand frog *Leiopelma hochstetteri* through sex chromosome devolution. Genome **41,** 14-22.

Shi, J.H., and Dawe, R.K. (2006). Partitioning of the maize epigenome by the number of methyl groups on histone H3 lysines 9 and 27. Genetics **173,** 1571-1583.

Solovei, I.V., Joffe, B.I., Gaginskaya, E.R., and Macgregor, H.C. (1996). Transcription on lampbrush chromosomes of a centromerically localized highly repeated DNA in pigeon (*Columba*) relates to sequence arrangement. Chromosome Research **4,** 588-603.

Soppe, W.J.J., Jasencakova, Z., Houben, A., Kakutani, T., Meister, A., Huang, M.S., Jacobsen, S.E., Schubert, I., and Fransz, P.F. (2002). DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis. Embo J **21,** 6549-6559.

Souza, E.D. (2006). Eigenes Protokoll statt DNA-Kit. Protokoll für die Extraktion von DNA aus Blättern. Laborjournal **7,** 58.

Spada, F., Vincent, M., and Thompson, E.M. (2005). Plasticity of histone modifications across the invertebrate to vertebrate transition: histone H3 lysine 4 trimethylation in heterochromatin. Chromosome Research **13,** 57-72.

Sreenivasan, T.V. (1981). Cytogenetical studies in Erianthus: Meiosis and behavior of B chromosomes in 2n=20 forms. Genetica **55,** 129-132.

Stark, E.A., Connerton, I., Bennett, S.T., Barnes, S.R., Parker, J.S., and Forster, J.W. (1996). Molecular analysis of the structure of the maize B-chromosome. Chromosome Research **4,** 15-23.

Staub, R.W. (1987). Leaf striping correlated with the presence of B chromosomes in maize. Journal of Heredity **78,** 71-74.

Steinemann, M., and Steinemann, S. (1992). Degenerating Y chromosome of *Drosophila miranda*: a trap for retrotransposons. Proc Natl Acad Sci U S A **89,** 7591-7595.

Steinemann, S., and Steinemann, M. (2005). Retroelements: tools for sex chromosome evolution. Cytogenetic and genome research **110,** 134-143.

Stitou, S., de la Guardia, R.D., Jimenez, R., and Burgos, M. (2000). Inactive ribosomal cistrons are spread throughout the B chromosomes of *Rattus rattus* (Rodentia, Muridae). Implications for their origin and evolution. Chromosome Research **8,** 305-311.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature **403,** 41-45.

Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. Gene Dev **12,** 599-606.

Switonski, M., Gustavsson, I., Hojer, K., and Ploen, L. (1987). Synaptonemal complex analysis of the B chromosomes in spermatocytes of the silver fox (*Vulpes-Fulvus* Desm). Cytogenetics and cell genetics **45,** 84-92.

Tanaka, R., and Matsuda, T. (1972). High occurrence of accessory chromosomal type in *Tainia laxiflora*, Orchidaceae. Botanical Magazine-Tokyo **85,** 43-&.

Tanic, N., Vujosevic, M., Dedovi-Tanic, N., and Dimitrijevic, B. (2005). Differential gene expression in yellow-necked mice *Apodemus flavicollis* (Rodentia, Mammalia) with and without B chromosomes. Chromosoma **113,** 418-427.

Teoh, S.B., and Rees, H. (1977). B-Chromosomes in white spruce. Proceedings of the Royal Society of London Series B-Biological Sciences **198,** 325-344.

Theuri, J., Phelps-Durr, T., Mathews, S., and Birchler, J. (2005). A comparative study of retrotransposons in the centromeric regions of A and B chromosomes of maize. Cytogenetic and genome research **110,** 203-208.

Tian, L., Fong, M.P., Wang, J.Y.J., Wei, N.E., Jiang, H.M., Doerge, R.W., and Chen, Z.J. (2005). Reversible histone acetylation and deacetylation mediate genome-wide, promoter-dependent and locus-specific changes in gene expression during plant development. Genetics **169,** 337-345.

Timmis, J.N., Ingle, J., Sinclair, J., and Jones, R.N. (1975). Genomic quality of rye B chromosomes. J Exp Bot **26,** 367-&.

Tomita, M. (1998). Cloning and chromosomal localization of a new mobile DNA element distribute in the rye genome. Proceedings of the 9th International Whet Genetics Symposium, section 1 - Cytogenetics and Evolution, 24-28.

Tomita, M., Shinohara, K., Morimoto, M. (2003). Amplification and elimination of a new class of transposon-like element through the evolution of *Triticea* genome. . Proceedings of the 10th International Whet Genetics Symposium, Paestum, Italy**,** Section 1 - Evolution and Genetic Diversity, 46-50.

Topp, C.N., Zhong, C.X., and Dawe, R.K. (2004). Centromere-encoded RNAs are integral components of the maize kinetochore. Proc Natl Acad Sci USA **101,** 15986-15991.

Trivers, R., Burt, A., and Palestis, B.G. (2004). B chromosomes and genome size in flowering plants. Genome **47,** 1-8.

Tsujimoto, H., and Niwa, K. (1992a). DNA structure of the B chromosome of rye revealed by in situ hybridization using repetitive sequences. Jpn J Genet **67,** 233-241.

Tsujimoto, H., and Niwa, K. (1992b). DNA-Structure of the B-Chromosome of Rye Revealed by Insitu Hybridization Using Repetitive Sequences. Jpn J Genet **67,** 233-241.

Ugarkovic, D. (2005). Functional elements residing within satellite DNAs. Embo Rep **6,** 1035-1039.

van Holde, K.E. (1988). Chromatin. Springer-Verlag Berlin Heidelberg.

van Vugt, J.J., de Nooijer, S., Stouthamer, R., and de Jong, H. (2005). NOR activity and repeat sequences of the paternal sex ratio chromosome of the parasitoid wasp *Trichogramma kaykai*. Chromosoma **114,** 410-419.

Vicente, V.E., Moreira-Filho, O., and Camacho, J.P. (1996). Sex-ratio distortion associated with the presence of a B chromosome in *Astyanax scabripinnis* (Teleostei, Characidae). Cytogenetics and cell genetics **74,** 70-75.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Vandelee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP - a new technique for DNA fingerprinting. Nucleic Acids Research **23,** 4407-4414.

Vosa, C.G. (1973). The enhanced and reduced quinacrine fluorescence bands and their relationship to the Giemsa patterns in *Allium flavum*. "Nobel Symp. 23 (Med. Nat. Sci.), Chromosome identification" (T. Casperson and L. Zech eds.)**,** 156-158.

Vujosevic, M., and Blagojevic, J. (2004). B chromosomes in populations of mammals. Cytogenetic and genome research **106,** 247-256.

Werren, J.H. (1991). The Paternal sex ratio chromosome of *Nasonia*. American Naturalist **137,** 392-402.

Werren, J.H., and Stouthamer, R. (2003). PSR (paternal sex ratio) chromosomes: the ultimate selfish genetic elements. Genetica **117,** 85-101.

Wicker, T., Stein, N., Albar, L., Feuillet, C., Schlagenhauf, E., and Keller, B. (2001). Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L.) reveals multiple mechanisms of genome evolution. Plant J **26,** 307-316.

Wilkes, T.M., Francki, M.G., Langridge, P., Karp, A., Jones, R.N., and Forster, J.W. (1995). Analysis of rye B chromosome structure using fluorescence in situ hybridization (Fish). Chromosome Research **3,** 466-472.

Wilson, E.B. (1907). The supernumerary chromosomes of *Hemiptera*. Science **26,** 870-871.

Wu, T.P. (1992). B-Chromsomes in *Sorghum stipoideum*. Heredity **68,** 457-463.

Xiong, Y., and Eickbush, T.H. (1990). Origin and evolution of retroelements based upon their reverse transcriptase sequences. Embo J **9,** 3353-3362.

Zhang, W., Yi, C., Bao, W., Liu, B., Cui, J., Yu, H., Cao, X., Gu, M., Liu, M., and Cheng, Z. (2005). The transcribed 165-bp CentO satellite is the major functional centromeric element in the wild rice species Oryza punctata. Plant Physiol **139,** 306-315.

Zhang, X.Y., Clarenz, O., Cokus, S., Bernatavichute, Y.V., Pellegrini, M., Goodrich, J., and Jacobsen, S.E. (2007). Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis. Plos Biology **5,** 1026-1035.

Zhang, Y., and Reinberg, D. (2001). Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev **15,** 2343-2360.

Ziegler, C.G., Lamatsch, D.K., Steinlein, C., Engel, W., Schartl, M., and Schmid, M. (2003). The giant B chromosome of the cyprinid fish *Alburnus alburnus* harbours a retrotransposon-derived repetitive DNA sequence. Chromosome Research **11,** 23-35.

Zima, J., Pialek, J., and Macholan, M. (2003). Possible heterotic effects of B chromosomes on body mass in a population of *Apodemus flavicollis*. Canadian Journal of Zoology-Revue Canadienne De Zoologie **81,** 1312-1317.

Publications in connection with the submitted thesis

Carchilan, M., Delgado, M., Ribeiro, T., Costa-Nunes, P., Caperta, A., Morais-Cecilio, L., Jones, R.N., Viegas, W., and Houben, A. (2007). Transcriptionally active heterochromatin in rye B chromosomes. The Plant Cell **19,** 1738-1749.

Carchilan, M., and Houben, A. (2007). Transcription analysis of rye B chromosomes. Chromosome Research **15,** 72-72.

Houben, A., and Carchilan, M. Plant B chromosomes – What makes them different? Chapter in book Plant cytogenetics, edited by Bass H.W. & Birchler J.A., Springer Press eds. (submitted).

Eidesstattliche Erklärung

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder der Mathematisch-Naturwissenschaftlich-Technischen Fakultät der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.
Ich erkläre ferner, dass ich diese Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Gatersleben, den .....

M. Carchilan

CURRICULUM VITAE

Name	Mariana CARCHILAN
Birth	23 / 10 / 1977 in Nisporeni, Moldova
Nationality	Republic of Moldova
Marital Status	married
Contact information	"Chromosome structure and function" group Institute of Plant Genetics and Crop Plant Research (IPK) Corrensstraße 3 06466 Gatersleben, Germany phone: 0049 (0) 39482 5734 FAX: 0049 (0) 39482 5137 e-mail: carchila@ipk-gatersleben.de

ACADEMIC QUALIFICATIONS

1999 – 2000	Master in Chemistry (Diploma de Magistru), Moldova State University
1994 – 1999	Bachelor in Chemical Technology (Diploma de Licenta), Moldova State University
1984 – 1994	Diploma of secondary education, Nisporeni, Republic of Moldova

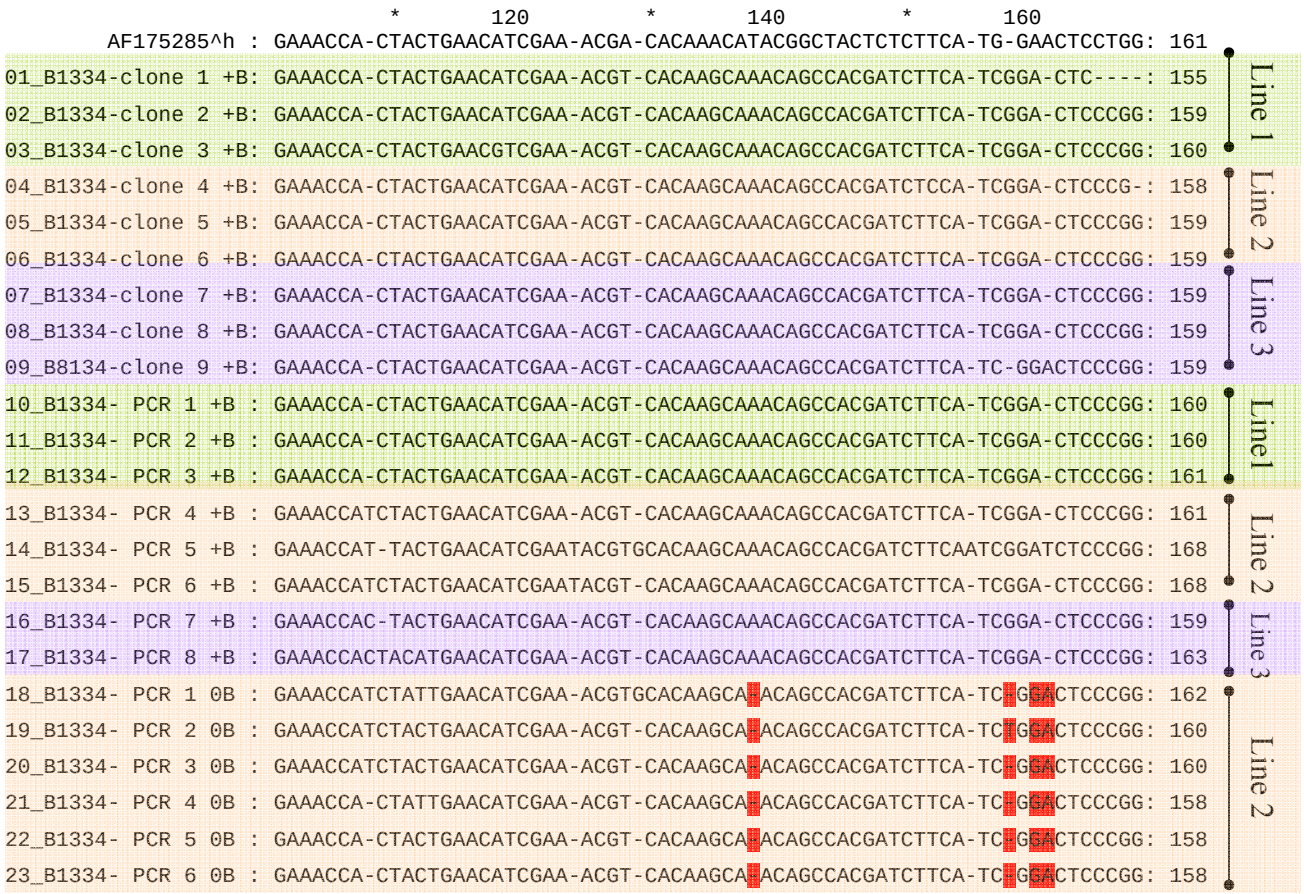
EMPLOYMENT HISTORY / RESEARCH EXPERIENCE

Since October 2004	PhD. Student, Chromosome Structure and Function group, Department of Cytogenetics, Institute of Plant Genetics and Crop Plant Research (IPK), Germany, (DAAD Scholarship, IPK funding), Supervisor Dr. A. Houben
2000 –2004	Junior Scientist, Laboratory of Plant Biochemistry, Moldova State University. Research topic: purification of cysteine proteases from kidney bean seedlings and their action on storage proteins (in the frame of the joint moldo-american project "Storage Protein Mobilization in Germinating Legume Seeds: Enzyme Machinery and Regulation", grant CRDF MBI 3000). Supervisor Prof. I.Vaintraub.
1999 – 2000	M. Sc. research work in the Lab of Plant Biochemistry "Usage of protein substrates and phytate for determination of reversible inhibition constants of proteinases". Supervisor Prof. I.Vaintraub.
1998 – 1999	Diploma work in the Lab of Protein Chemistry "Kinetics of proteolysis of 11S protein from vetch". Supervisor Prof. I.Vaintraub.

Appendix

Appendix Fig. 1. Alighnment of B 1334 sequences amplified from cDNA of plants with (+B) and without (0B) B chromosomes. The deletions and point mutations are highlighted in red.

Appendix Fig. 1. continued



Appendix Fig. 2. Alignment of B 8149 sequences amplified from cDNA of plants with (+B) and without (0B) B chromosomes. The deletions and point mutations are highlighted in red.

Appendix Fig. 2. continued

Appendix Fig. 2. continued

Appendix Fig. 2. continued