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Characteristics and behaviour of the chromosomes of *Leymus mollis* and *L. racemosus* (Triticeae, Poaceae) during mitosis and meiosis

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Abstract

Leymus mollis and *L. racemosus* (Triticeae; Poaceae) are important as genetic resources for wheat improvement, as they carry genes for salt tolerance and disease resistance. Even though these species share common Ns and Xm genomes, the genomic relationship between these two species is not yet clearly understood. In this study, we examined the genomes of the two species by FISH and GISH, using combinations of tandem-repetitive sequences and genomic DNAs. Comparative GISH showed that genomes in the genus *Leymus* were diverse. Nevertheless, chromosomes of these two species were able to undergo complete meiotic pairing in hybrids, suggesting that differences in the subtelomeric heterochromatin and sequences distinguishable by GISH do not affect meiotic pairing.

Introduction

During meiosis, a chromosome searches for its homologous partner, which it can distinguish from non-homologous chromosomes. A picture has emerged of homologous pairing, and how chromosomes find and recognize their homologous partner. In yeast and plants, chromosomes can potentially initiate homologous pairing along their entire length (for review, see Roeder 1997). However, the distribution of initiation sites for pairing is organism-dependent. For example, budding yeast shows multiple pairing initiation sites (Loidl *et al.* 1994), but homologous rye chromosomes in addition lines of wheat start meiotic pairing from the centromeric regions (Martínez-Pérez *et al.* 1999). An important feature in pairing is the clustering of telomeres known as the 'bouquet' structure, which is formed during the early stage of meiosis, and is conserved from yeast to humans and higher plants (for review, see Zickler & Kleckner 1998). This structure is thought to align the chromosomes properly to

help the recognition of homologous pairs. Homologous recognition of chromosomes is believed to be at the DNA level. In yeast and *Drosophila*, a random search model has been proposed, in which homologous segments of chromosomes meet randomly (Scherthan *et al.* 1994, Fung *et al.* 1998). Chromosome structures such as heterochromatin are involved in homologous recognition in *Drosophila* (Hawley *et al.* 1992). Repetitive sequences have also been thought to facilitate recognition.

The genus *Leymus* in the tribe Triticeae consists of about 30 wild species. These species grow mainly along sea coasts and in inland dry areas, being distributed from Iceland and the North Sea across central Asia to East Asia, Alaska, and North America. They are evolutionarily distant from wheat, have strong rhizomes, and are a useful genetic resource for wheat breeding, having characteristics such as salt tolerance (McGuire & Dvorak 1981) and resistance to various diseases including scab (Mujeeb-Kazi *et al.* 1983) and powdery mildew (Faith 1983). Among *Leymus* species, *L. racemosus* ($2n=28$) and *L. mollis* ($2n=28$) have attracted special interest, because they have exceptionally large spikes, strong rhizomes and vigorous growth. *L. racemosus* is distributed along sea coasts or in the dry lands from Eastern Europe to central Asia, whereas *L. mollis* is found from Siberia to Canada and Iceland as well as in the northern parts of Japan. In spite of the potential importance of the genus *Leymus*, the genomic relationships among its species are still largely unknown. All of these species share two common Ns and Xm genomes, where the Ns genome is derived from diploid species of the genus *Psathyrostachys*, and the origin of the Xm genome is unknown (Wang & Jensen 1994). Anamthawat-Jónsson & Bödvarsdóttir (2001) reported on the complexity of the genomes of *Leymus*, in which some *Leymus* species were phylogenetically closer to *Psathyrostachys* species than to other *Leymus* species. During a study of *L. racemosus* chromosomes, we found that the copy number of two tandem repetitive sequences was quite different in *L. racemosus* and *L. mollis* (Kishii *et al.* 1999). These results indicated the need for re-evaluation of the genomes of *Leymus* species.

The aim of the present research was to investigate the genomic differentiation of *L. racemosus* and *L. mollis* by comparing the hybridization patterns of their genomic DNAs.

Materials and methods

Plant materials

Leymus racemosus (Lam.) Tzvelev ($2n=4x=28$, genome NsNsXmXm), a Bulgarian population, were collected (Accession number: HT15405) or donated by Dr. I. Panayotov (Institute of Wheat and Sunflower, Bulgaria) (Accession numbers: MK10101–10105). All plants were collected along the Black Sea coast around Balchik, Bulgaria. *Leymus racemosus* plants from Russia were also used (Accession numbers: MK10201–10206). *L. mollis* (Trin.) Pilger ($2n=4x=28$, NsNsXmXm) was collected at three locations: 10 km north-east of Iwaki, Fukushima, along the Pacific Sea coast of Japan (Accession number: MK10001), 1 km west of Niigata along the Japan Sea coast (MK10020), and 20 km north of Aomori (MK10030) in Japan.

To produce F1 hybrids between *L. racemosus* and *L. mollis*, *L. racemosus* (Accession number: HT15405) was pollinated by *L. mollis* (MK10001).

Cytological analysis

Mitotic and meiotic chromosome slides were prepared by the acetocarmine squash method and were used for FISH and GISH techniques, following Mukai (1996) and Kishii *et al.* (1999). Two kinds of probes were used for FISH: pLrTaiI-1 (TaiI-family sequences from *L. racemosus;* Kishii *et al.* 1999) and pLrPstI-1 (350-bp family sequences from *L. racemosus;* Kishii *et al.* 1999). These clones were labelled with digoxigenin-11-dUTP or biotin-16-dUTP using the PCR method. Probes for the genomic DNAs of *L. mollis* and *L. racemosus* were labelled with biotin-16-dUTP using a BioNick labelling System (Gibco BRL) or with digoxigenin-11-dUTP using a commercial labelling kit (Roche). For blocking DNA in GISH experiments, genomic DNA was sonicated to produce DNA fragments with a mean size of around 500 bp.

Results

Comparison of total genomic sequences in L. mollis *and* L. racemosus

It was reported previously that *L. racemosus* (Accession number: HT15405) carried massive subtelomeric heterochromatic regions in most chromosomes, and that high copy numbers of both TaiI and 350-bp tandem repetitive sequences families were present in those regions (Kishii *et al.* 1999). On the other hand, *L. mollis* did not show subtelomeric heterochromatic blocks containing the 350-bp and TaiI family sequences (Kishii *et al.* 1999). Investigation of additional accessions of *L. racemosus* and *L. mollis* con-

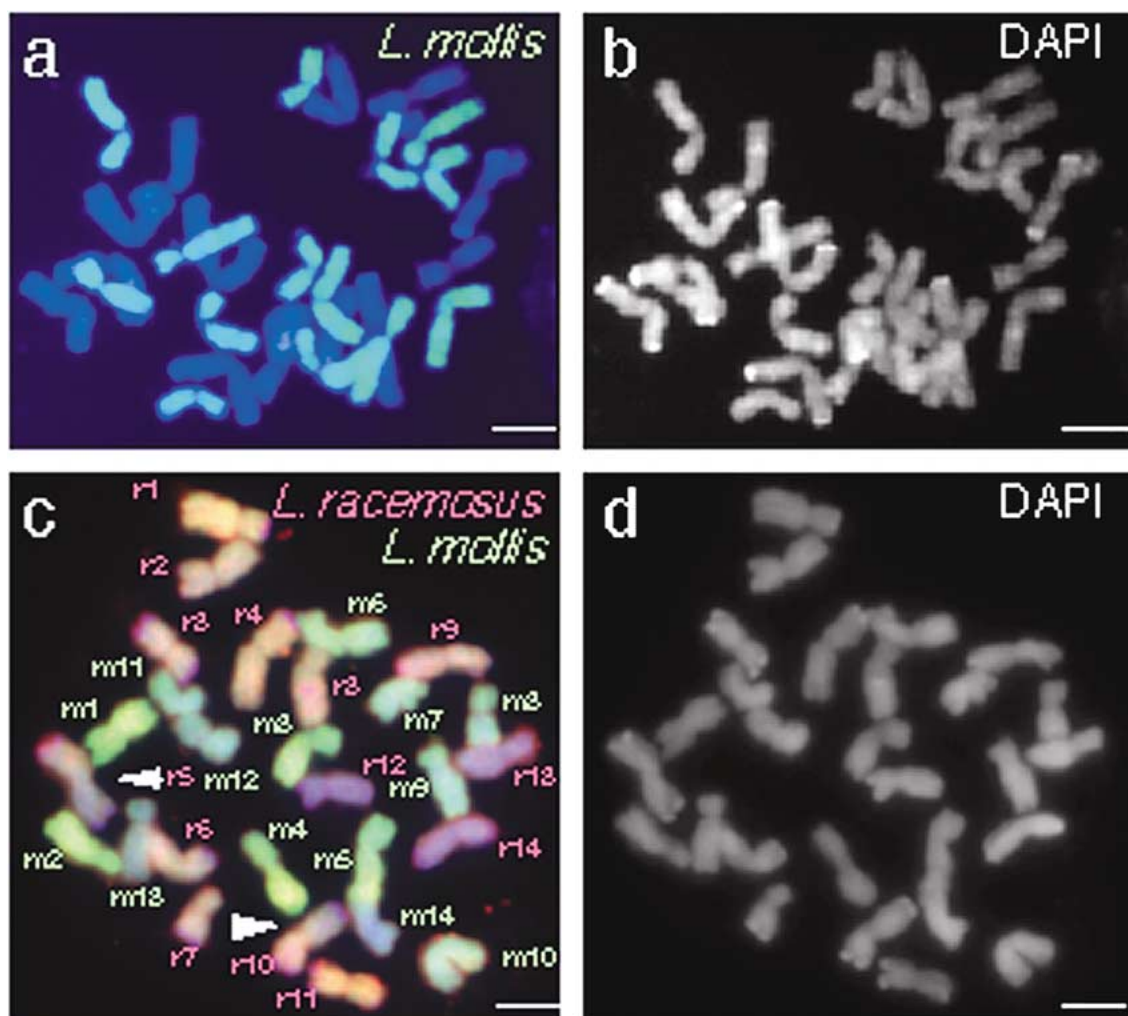


Figure 1. GISH labelling of chromosomes from F1 hybrids of *Leymus mollis* and *L. racemosus*, showing differences in subtelomeric heterochromatin and total genomic DNA between chromosomes of the different species. (**a**) Chromosomes probed with *L. mollis* total genomic DNA (green) with tenfold excess of unlabelled *L. racemosus* total genomic DNA used for blocking. (**b**) DAPI image of **a**. (**c**) Chromosomes probed with *L. mollis* total genomic DNA (green) and *L. racemosus* total genomic DNA (red). The letters 'm' and 'r' indicate *L. mollis* and *L. racemosus* chromosomes, respectively. The arrowheads indicate chromosomes with both red and purple segments. The 14 chromosomes of *L. mollis* (stained green with FITC) appeared bright green to bluish-green in colour (m1—m14 in Figure 1c). Among them, five chromosomes (m1—m5) had an especially bright green colour and four of the others (m11—m14) were bluish-green. Similarly, *L. racemosus* chromosomes (stained red with rhodamine) showed a red to purple colour (r1—r14). (**d**) DAPI image of **c**. All bars equal 10 µm.

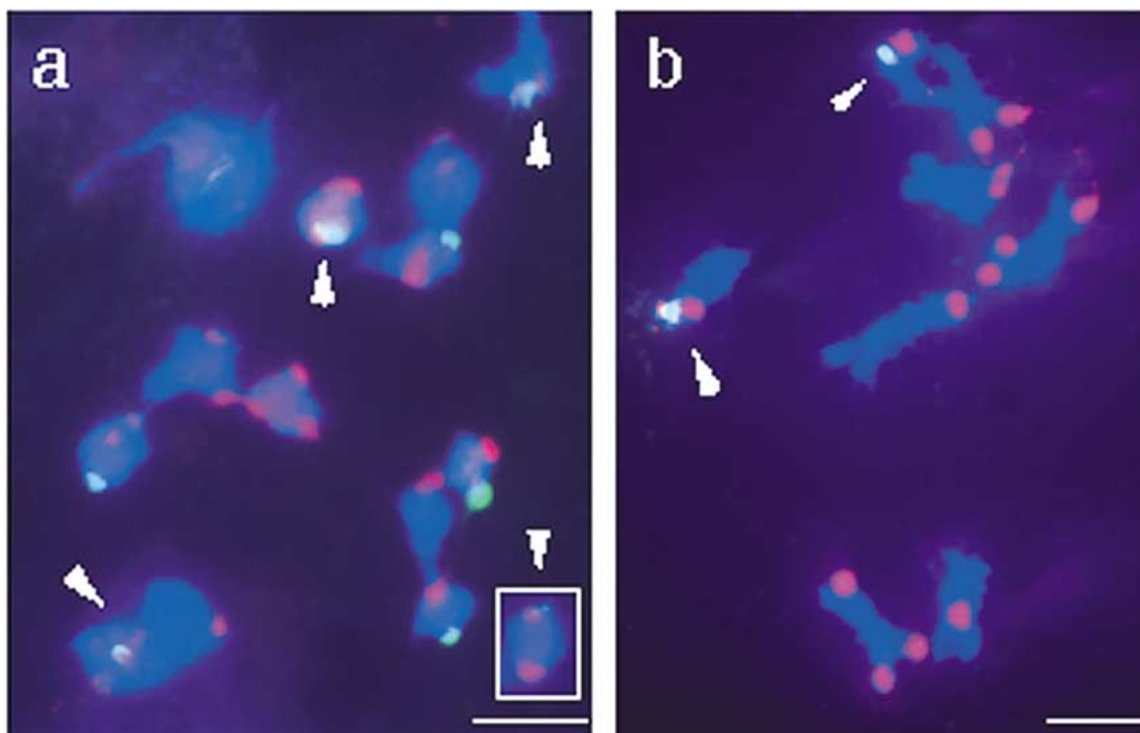
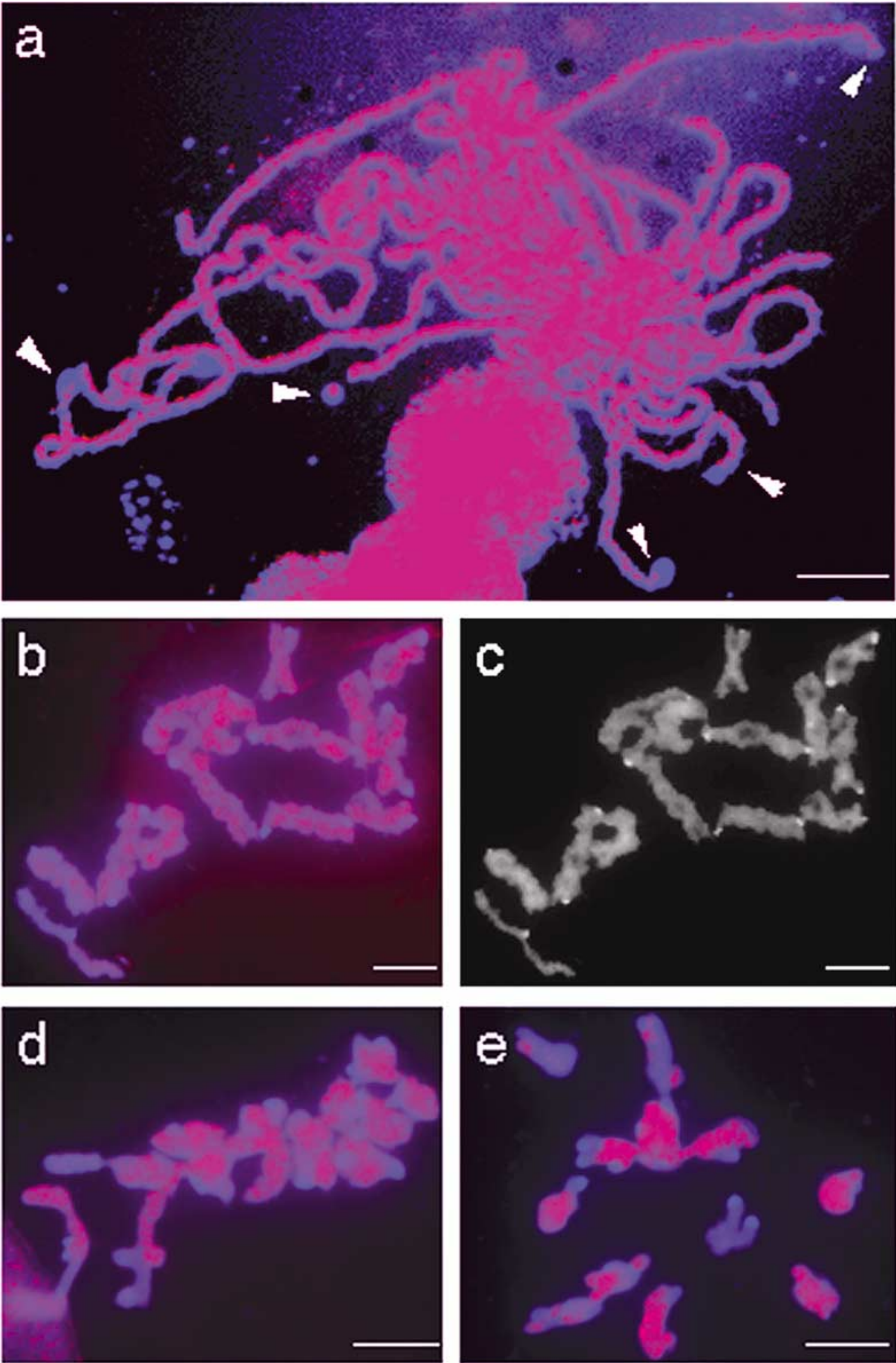


Figure 2. Chromosome pairing of *Leymus racemosus* chromosomes having different subtelomeric repeat sequences. Probes were TaiI-family sequences (red) and 350-bp family sequences (green). Arrowheads indicate chromosome ends with different repeat families in the homologous chromosomes. (**a**) Metaphase stage. Fourteen bivalents can be seen. Homologous chromosomes with different chromosome ends have formed pairs. (**b**) Anaphase stage. Bars equal 10 μm.

firmed that all accessions of *L. racemosus* showed massive signals of these two repeats, while *L. mollis* showed no or small signals of these two repeats (data not shown).

The huge differences between copy numbers of particular tandem repetitive sequences and subtelomeric structures prompted us to investigate differences in the total genomic constitution of the two species. To accomplish this, we firstly made F1 hybrids between them. Analysis of genomic differences was conducted by applying the GISH technique to the F1 plants. Using biotin-labelled genomic DNA of *L. mollis* as a probe, the *L. racemosus* and *L. mollis* genomes were clearly distinguished in the hybrids (Figure 1a, b), staining the 14 chromosomes of *L. mollis* green. This result was confirmed by the fact that subtelomeric heterochromatin was stained darker than other regions by DAPI only in chromosomes of *L. racemosus*. A tenfold excess of blocking DNA over the probe gave good reproducibility of the results, and, even when an amount of blocking DNA equal to that of the probe was used, several chromosomes could be distinguished (data not shown), indicating a very large difference in the genomes of the two species. Application of equal amounts of *L. racemosus* and *L. mollis* genomic probes of different colours

Figure 3. Meiotic pairing of *Leymus mollis* (red) and *L. racemosus* chromosomes in the *L. mollis* × *L. racemosus* hybrid. (**a**) Pachytene stage. *L. mollis* and *L. racemosus* chromosomes are paired with each other. Asymmetrical pairing can be seen in some chromosome pairs (arrowheads). (**b**) Diplotene stage. *L. mollis* chromosomes have paired with *L. racemosus* chromosomes in all cases. (**c**) DAPI image of **b**. (**d**) Metaphase stage. Chromosomes are aligned in the equatorial plane. (**e**) Anaphase stage. Recombination between *L. mollis* and *L. racemosus* chromosomes can be seen. All bars equal 10 μm.


a
b
c
d
e

produced a mosaic-like mixture of the two genomic colours (Figure 1c, d).

Pairing of L. racemosus *chromosomes*

Kishii *et al.* (1999) reported that the signals from the TaiI and 350-bp families were highly polymorphic between homologous chromosomes within *L. racemosus* plants of Bulgarian origin (Accession number; HT15405). We investigated whether this polymorphism would affect meiotic pairing in *L. racemosus*. When we hybridized metaphase chromosomes of *L. racemosus* with TaiI (red) and 350-bp family (green) probes, association of red and green signals was detected (Figure 2a). Since 14 bivalents were formed, the pairings were between homologous chromosomes. Anaphase chromosomes revealed a clearer picture, showing that the signals from the two different repeats were present at the subtelomeric regions of sister chromatid pairs in some chromosomes (Figure 2b).

Pairing between L. racemosus *and* L. mollis *chromosomes*

We next examined the possibility of meiotic pairing between *L. racemosus* and *L. mollis* chromosomes in their hybrids. GISH applied to the pachytene chromosomes clearly showed pairing between *L. mollis* and *L. racemosus* chromosomes (Figure 3a). Some *L. mollis* and *L. racemosus* chromosome pairs made contact at their telomeric ends, but many showed asymmetrical pairing, with one chromosome end extending further than the other.

In the diplotene stage, all 14 bivalents were formed between blue and red chromosomes, showing that the pairing was not intraspecific but interspecific (Figure 3b, c). The presence of a subtelomeric block of heterochromatin on one of the chromosomes in each bivalent confirmed that the bivalents consisted of chromosomes from both species. Chromosomes aligned on the equatorial plane at metaphase (Figure 3d), and recombination between chromosomes of the two species was also detected in anaphase cells (Figure 3e). Multivalents and univalents appeared occasionally at metaphase (data not shown).

Discussion

Divergence of L. mollis *and* L. racemosus *chromosomes*

In this and our previous study, we demonstrated the diversity of subtelomeric regions and total genomic sequences between *L. mollis* and *L. racemosus* (Kishii *et al.* 1999, and Figure 1). Although we discriminated chromosomes of the two species by using a 1 : 10 ratio of the probe and blocking DNA in GISH, a much larger amount of blocking DNA is usually required for separation of species in the same genus. For example, a hundredfold excess of blocking DNA over the probe was employed for separation of *Aegilops* species (Wang *et al.* 2000). From this point of view, the degree of divergence between the genomes of these two *Leymus* species is similar to that seen between genomes from different genera. Perhaps *Leymus* is much older in origin than wheat and *Aegilops*. It is also possible that *Psathyrostachys*, the ancestral species of *Leymus*, had already differentiated before the genus *Leymus* originated, and that different *Leymus* species derived their Ns genomes from different *Psathyrostachys* species.

Comparative GISH using both *L. mollis* and *L. racemosus* genomic DNA as the probes further distinguished *L. mollis* and *L. racemosus* chromosomes with various colours (Figure 1c), indicating that *Leymus* genomes have quite complex constitutions. This mosaic-like pattern would not be produced if the two species had evolved directly from a close common ancestor and accumulated their repetitive sequences evenly throughout their genomes (even though the two species would accumulate different repetitive sequences). The mosaic pattern would be produced, for instance, if several *L. racemosus* chromosomes accumulated certain repetitive sequences that are abundant in the *L. mollis* genome. Alternatively, *Leymus* may have multiple origins. Anamthawat-Jónsson & Bödvarsdóttir (2001) published a phylogenetic tree of *Leymus* and *Psathyrostachys*, the latter being the Ns genome ancestor of *Leymus*, in which *L. mollis* and *L. racemosus* were placed in different groups.

Repetitive sequences do not participate in homologous chromosome recognition

Although 'homologous' chromosomes in *L. mollis* and in *L. racemosus* could be distinguished in their F1 hybrid by GISH (Figure 1a), homoeologous Ns and Xm chromosomes in each species could not be separated by this method. Nevertheless, pairing at meiosis occurred strictly between the 'homologous' chromosomes (Figure 3). This means that chromosomes can recognize their partners when their DNA composition is so different as to be distinguishable by GISH, even though their homoeologous chromosomes could not be distinguished by GISH. Since GISH is a method based mainly on differences in repetitive sequences, it may be that repetitive sequences do not take part in the recognition of homologous chromosome pairs.

Chromosome ends are not significant for homologous chromosome recognition

It is generally accepted that telomere clustering is important for the alignment of homologous chromosomes (for review, see Zickler & Kleckner 1998). Lukaszewski (1997) demonstrated that even a tiny loss from the terminal regions of chromosome arms of wheat led to defective recognition of homologues. However, the present result revealed that homologous chromosomes could form meiotic pairs even when they were different in arm length, subtelomeric structure and subtelomeric sequences (Figures 2 & 3). Homologous chromosomes formed heteromorphic pairs, leaving free subtelomeric and distal regions (Figure 3a). These results imply that differences in chromosomal ends are not significant for pairing in *Leymus* species. The entire length of the chromosome has the potential to undergo homologous pairing (for review, see Roeder 1997). Schwarzacher (2003) argued that several pairing mechanisms could exist in parallel and complement each other to ensure proper pairing. *Leymus* might have adopted some special mechanism to overcome the differences in the terminal regions of the chromosomes.

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References

Anamthawat-Jónsson K, Bödvarsdóttir SK (2001) Genomic and genetic relationships among species of *Leymus* (Poaceae: Triticeae) inferred from 18S-26S ribosomal genes. *Am J Bot* **88**: 553–559.

Faith AMB (1983) Analysis of the breeding potential of wheat–*Agropyron* and wheat–*Elymus* derivatives. I. Agronomic and quality characteristics. *Hereditas* **98**: 287–295.

Fung JC, Marshall WF, Dernburg A, Agard DA, Sedat JW (1998) Homologous chromosome pairing in *Drosophila melanogaster* proceeds through multiple independent initiations. *J Cell Biol* **141**: 5–20.

Hawley RS, Irick H, Zitron AE *et al.* (1992) There are two mechanisms of achiasmate segregation in *Drosophila* females, one of which requires heterochromatic homology. *Dev Genet* **13**: 440–467.

Kishii M, Nagaki K, Tsujimoto H, Sasakuma T (1999) Exclusive localization of tandem repetitive sequences in subtelomeric heterochromatin regions of *Leymus racemosus* (Poaceae, Triticeae). *Chromosome Res* **7**: 519–529.

Loidl J, Klein F, Scherthan H (1994) Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. *J Cell Biol* **125**: 1191–1200.

Lukaszewski AJ (1997) The development and meiotic behavior of asymmetrical isochromosomes in wheat. *Genetics* **145**: 1155–1160.

Martínez-Pérez E, Shaw P, Reader S, Aragon-Alcaide L, Miller T, Moore G (1999) Homologous chromosome pairing in wheat. *J Cell Sci* **112**: 1761–1769.

McGuire PE, Dvorak J (1981) High salt-tolerance potential in wheatgrasses.*Crop Sci* **21**: 702–705.

Mujeeb-Kazi A, Bernard M, Bekele GT, Mirand JL (1983) Incorporation of alien genetic information from *Elymus giganteus* into *Triticum aestivum*. In: S. Sakamoto ed. *Proceedings of the 6th International Wheat Genetic Symposium*. Maruzen, Kyoto, Japan pp 223–231.

Mukai Y (1996) *In-situ* hybridization. In: K. Fukui and S. Nakayama, eds. *Plant Chromosomes: Laboratory Methods*. Boca Raton, Florida: CRC Press, pp 155–170.

Roeder GS (1997) Meiotic chromosomes: it takes two to tango. *Genes Dev* **11**: 2600–2621.

Scherthan H, Bahler J, Kohli J (1994) Dynamics of chromosome organization and pairing during meiotic prophase in fission yeast. *J Cell Biol* **127**: 273–285.

Schwarzacher T (2003) Meiosis, recombination and chromosomes: a review of gene isolation and fluorescent *in situ* hybridization data in plants. *J Exp Bot* **54**: 11–23.

Wang RR-C, Jensen KB (1994) Absence of the J genome in *Leymus* species (Poaceae: Triticeae): evidence from DNA hybridization and meiotic pairing. *Genome* **37**: 231–235.

Wang ZN, Hang A, Hansen J, Burton C, Mallory-Smith CA, Zemetra RS, (2000) Visualization of A- and B-genome chromosomes in wheat (*Triticum aestivum* L.) × jointed goatgrass (*Aegilops cylindrica* Host) backcross progenies. *Genome* **43**: 1038–1044.

Zickler D, Kleckner N (1998) The leptotene–zygotene transition of meiosis. *Annu Rev Genet* **32**: 619–697.