Homology of two alien chromosomes during meiosis in wheat

Seong-Woo Cho, Yosuke Moritama, Takayoshi Ishii, Masahiro Kishii, Hiroyuki Tanaka, Amin Elsadig Eltayeb and Hisashi Tsujimoto

Received: September 26, 2011 / Accepted: November 22, 2011
© 2011 by the Society of Chromosome Research

Abstract
Chromosome relationships in allopolyploid species are either homologous, homoeologous, or non-homologous. To discern the behavior of chromosomes in each of these relationships, we produced double monosomic addition lines (DMAs) of wheat carrying one *Leymus racemosus* chromosome and one *L. mollis* chromosome in different combinations, and observed the *Leymus* chromosomes in meiosis by differential genomic in situ hybridization. First we observed the distribution of these alien chromosomes in tetrad cells and measured their homology by an index of their interaction. Values differed greatly among DMAs. We observed prophase to anaphase cells of meiotic division I and elucidated the differences in homology through chromosome behavior in meiosis. A line carrying chromosomes [l] of *L. racemosus* and [M] of *L. mollis* formed bivalents in about half of the prophase cells, but these chromosomes became univalent in metaphase because of a lack of chiasmata, and segregated normally to each pole. The chromosomes in the other half of the prophase cells did not associate and behaved randomly from anaphase to tetrads. The DMAs proved useful in studying the homology of chromosomes.

Keywords: Chromosome association, Chiasma, Double monosomic addition line, GISH, Homology

Introduction
Chromosome relationships in allopolyploid species are classified into homologous, homoeologous, and non-homologous. Normally, chromosomes in a homologous relationship pair up and form a bivalent during meiosis. Chromosomes in a homoeologous relationship do not form a bivalent at the first meiotic metaphase (MI), despite having most of their genes in common. Chromosomes in a non-homologous relationship do not interact during meiosis. We investigated the meiotic behavior of chromosomes in these different relationships by using unique materials.

*Leymus racemosus* and *Leymus mollis* are species in the tribe Triticeae (Poaceae). Both are tetraploid and carry the same genome structure (2n=4x=28, 14", NsNsXmXm). Their F1 hybrid forms 14 bivalents at MI (Kishii et al. 2003). Because the chromosomes of each species can be distinguished by genomic in situ hybridization (GISH) and thus stained with different colors, pairing of homologous chromosomes derived from each species can be made visible (Kishii et al. 2003).

Kishii et al. (2004) produced eight disomic addition lines (DAs) of wheat carrying *L. racemosus* chromosomes (2n=44, 22", AABBDD + xx, where x means a *L. racemosus* chromosome). In these lines, a homologous pair of *L. racemosus* chromosomes is added to the genome of common wheat (*Triticum aestivum*, 2n=6x=42, 21", AABBDD). Similar lines were also produced with *L. mollis* chromosomes (2n=44, 22", AABBDD + yy, where y means a *L. mollis* chromosome; unpublished). By crossing these DAs, it was possible to produce double monosomic addition lines (DMAs) carrying one *L. racemosus* chromosome and one *L. mollis* chromosome (2n=44, AABBDD + xy). The two alien chromosomes (x and y) within each DMA must be in a homologous, a homoeologous, or a non-homologous relationship.

Addition lines allow the investigation of chromosome behavior during meiosis, because GISH reveals a single target chromosome within a tangled mass of chromosomes in prophase and even in the interphase nuclei of tetrads. Here we observed the meiotic behavior of the two alien chromosomes in detail by GISH.
Materials and methods

Plant materials

We used three DAs of *L. racemosus* (chromosomes [A’, [A]], and [F’]) and two of *L. mollis* ([M’] and [A’’]). Plant morphology and DNA markers indicate that [I’], [A’], [M’], and [A’’] belong to homoeologous group 2 (Kishii et al. 2004; Larson et al. 2011; unpublished results), although their genomes are unknown (Ns or Xm). [F’] belongs to homoeologous group 4 (Kishii et al. 2004; Larson et al. 2011). All of these addition lines have the same genetic background of the common wheat cultivar ‘Chinese Spring’ (CS).

Observation of meiosis by GISH

Fresh anthers at different stages of meiosis were collected from young spikes, fixed in ethanol : acetic acid (3:1, v/v) at room temperature for 5 days, and then kept at 4°C until use. A tiny part of an anther was removed and then the pollen mother cells (PMCs) were squeezed out. PMCs were squashed in 45% acetic acid on microscope slides. After observation of the cells by differential interference microscope, the slides were frozen at –80°C to remove the cover slips and air-dried for GISH.

To make probes for GISH, we labeled genomic DNAs of *L. mollis* with tetramethyl-rhodamine-5-dUTP (Roche) and those of *L. racemosus* with fluorescein-12-dUTP (Roche) by a random-primer labeling method. PMCs on slides were denatured in 0.2 M NaOH in 70% ethanol at room temperature for 5 min. After denaturation, the slides were dehydrated in a cold ethanol series (70%, 90%, and 99.5% ethanol for 5 min at each concentration). For GISH, hybridization solution (50% formamide, 10% dextran sulfate, 50–500 ng labeled probe, 2× saline sodium citrate (SSC), 11.5 µg/µL salmon sperm DNA) was applied to the slides. The slides were incubated for at least 20 h at 37°C in a humidity box. After hybridization, the slides were washed for 5 min in 2× SSC plus 0.1% Triton X-100, followed by 5 min in 2× SSC. The slides were briefly air-dried and then covered with a drop of Vectashield mounting medium (Vector) containing 1 ng/µL 4’,6-diamidino-2-phenylindole (DAPI). Images were captured under a fluorescence microscope (Olympus BX61) with a cooled CCD camera (Photometrics CoolSNAP fx; Roper Scientific) and processed with Meta Imaging Series 3.0 software (Universal Imaging Corporation).

Coefficient of homology – an index of chromosome relatedness in meiosis

If the two alien chromosomes added into wheat are completely homologous, they will form a bivalent in MI and segregate normally to all four cells in the tetrad. If the chromosomes are non-homologous, each will form a univalent and behave independently. In theory, the tetrad spores will segregate in a 1:1:1:1 ratio of both alien chromosomes: one alien chromosome: the other alien chromosome: no alien chromosomes. To indicate chromosome relatedness between the *L. racemosus* and *L. mollis* chromosomes in the DMAs, we defined a ‘coefficient of homology’ (h):

\[
h = \frac{(n_1 + n_2) - 0.5}{n} / 0.5
\]

where \(n_1\) and \(n_2\) is the total numbers of spores with *L. racemosus* (*L. mollis*) chromosomes, and \(n\) is the total number of cells observed. When the two chromosomes are completely homologous, \(h = 1\); when they are non-homologous, \(h = 0\). When recombination occurs, the major chromosome segment is considered as a whole.

Results

Production of double monosomic addition lines

We produced four DMAs, each carrying one *L. racemosus* chromosome and one *L. mollis* chromosome in addition to the 42 wheat chromosomes (Table 1, Fig. 1). Because chromosomes [I’], [A’], [A’’], and [M’] belong to homoeologous group 2 (Kishii et al. 2004; Larson et al. 2011; unpublished results), the two alien chromosomes in DMA[I’ M’], DMA[I’ A’’], and DMA[A’ M’] must be either homologous or homoeologous. Because chromosome [F’] belongs to group 4 (Kishii et al. 2004; Larson et al. 2011), the alien chromosomes in DMA [F’ M’] are non-homologous.

Coefficient of homology of DMAs

The *L. racemosus* and *L. mollis* chromosomes segregated in the tetrad spores (Fig. 2). DMA[I’ M’] and DMA[I’ A’’] showed significantly different values from the 1:1:1:1 segregation ratio that is expected in the case of non-homologous combination (Table 2). On the other hand, DMA[A’ M’] and DMA[F’ M’] fitted the ratio, although in DMA[A’ M’] more spores carried one chromosome than others.

<table>
<thead>
<tr>
<th>DMA Line</th>
<th>Chromosome constitution</th>
<th>No. chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMA[I’ M’]</td>
<td>AABBDD + [I’] + [M’]</td>
<td>44</td>
</tr>
<tr>
<td>DMA[I’ A’]</td>
<td>AABBDD + [I’] + [A’]</td>
<td>44</td>
</tr>
<tr>
<td>DMA[A’ M’]</td>
<td>AABBDD + [A’] + [M’]</td>
<td>44</td>
</tr>
<tr>
<td>DMA[F’ M’]</td>
<td>AABBDD + [F’] + [M’]</td>
<td>44</td>
</tr>
<tr>
<td>DMA[M’ M’]</td>
<td>AABBDD + [M’] + [M’]</td>
<td>44</td>
</tr>
</tbody>
</table>

(A’), [I’], and [F’] indicate *L. racemosus* chromosomes. [A’] and [M’] indicate *L. mollis* chromosomes.

<table>
<thead>
<tr>
<th>DMA Line</th>
<th>No. cells in tetrads</th>
<th>Presence of alien chromosomes</th>
<th>Others (1:1:1:1)</th>
<th>(\chi^2)</th>
<th>(h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMA[I’ M’]</td>
<td>116</td>
<td>11</td>
<td>47</td>
<td>47</td>
<td>11</td>
</tr>
<tr>
<td>DMA[I’ A’]</td>
<td>120</td>
<td>20</td>
<td>42</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>DMA[A’ M’]</td>
<td>120</td>
<td>25</td>
<td>35</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>DMA[F’ M’]</td>
<td>120</td>
<td>28</td>
<td>34</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>DMA[M’ M’]</td>
<td>120</td>
<td>0</td>
<td>119</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Significant at 5%. † Lr, L. racemosus; Lm, L. mollis. Others are not included in the calculations.

Table 2. Coefficients of homology (h) of the four double monosomic addition (DMA) lines calculated from the distribution of signals in tetrads.
Figure 1. Mitotic metaphase cells of disomic addition lines (DA) and double monosomic addition lines (DMA) produced by crossing of different DAs. a: DA[rl]; L. racemosus chromosomes are red. b: DA[Mm]; L. mollis chromosomes are green. c, d: DMA[lM] showing L. racemosus (c) and L. mollis chromosomes (d). Both of the alien chromosomes show signals. e: Merged image of c and d. L. racemosus chromosome [rl] is red and L. mollis [Mm] is green. Wheat chromosomes are blue. Scale bar, 10 µm.

Figure 2. Signal distribution in tetrads of DMAs. GISH shows L. racemosus chromosomes in red and L. mollis in green. a: All four spores carry a red or green signal. b: Two spores carry both red and green signals, and the other two carry none. c: One spore shows red and green signals, one shows red, one shows green, and one shows none. d–g: Enlarged spores carrying no signal, one red, one green, and both red and green, respectively. Wheat chromosomes are blue. Scale bar, 10 µm.
carried no or both chromosomes. These results indicate that the two alien chromosomes of DMA[1\textsuperscript{r} M\textsuperscript{m}] and DMA[1\textsuperscript{r} A\textsuperscript{m}] interacted with each other, but they behaved independently in DMA[1\textsuperscript{r} M\textsuperscript{m}]. They may have interacted to some extent in DMA[A\textsuperscript{r} M\textsuperscript{m}]. We had expected that the two alien chromosomes would have been in a homologous relationship in at least one of DMA[1\textsuperscript{r} M\textsuperscript{m}], DMA[1\textsuperscript{r} A\textsuperscript{m}] and DMA[A\textsuperscript{r} M\textsuperscript{m}], but no lines had an \( h \)-value close to 1.

**Behavior of alien chromosomes during meiotic metaphase I and anaphase**

Since \( h \) of DMA[1\textsuperscript{r} M\textsuperscript{m}] was 0.62—that is, intermediate between homologous and non-homologous—we additionally examined the non-homologous DMA[1\textsuperscript{r} M\textsuperscript{m}] and the homologous DA[1\textsuperscript{r} M\textsuperscript{m}]. Most MI cells of DA[1\textsuperscript{r} M\textsuperscript{m}] showed a clear bivalent between the alien chromosomes (Table 3, Fig. 3a, b). In contrast, most MI
cells of DMA[l Mm] showed two univalents (Table 3, Fig. 3c, d). Although some of the alien chromosomes seemed to be associated, the shapes were different from the normal bivalent, showing pan, ring, or rod types (Fig. 3g, h), with no chiasmata. In DMA[l MrMm], most of the MI cells showed two univalents as expected (Table 3, Fig. 3e, f). This line also had a few bivalents at low frequency (Table 3, Fig. 3i, j).

We investigated the localizations of the two univalents at MI because some of the cells showed the univalents positioned in symmetrical places across the equatorial plate (Fig. 3d, f), whereas others were randomly placed near the equatorial plate (Fig. 3c, e). About half of the DMA[l Mm] cells showed symmetrical positioning of univalents, and considerable numbers of the DMA[l MrMm] cells showed univalents in symmetrical positions (Table 3).

In both lines, the two univalents in random positions moved to one of the poles (Fig. 4d, h) or stayed on the equatorial plate then the sister chromatids separated and then moved to each pole (Fig. 4e, f, i, j). On the other hand, the univalents in symmetrical positions moved to each pole as ordinary chromosomes (Fig. 4c, g). One chromosome shown in Fig. 4c shows four bright red signals of telomeric heterochromatin of L. racemosus, indicating absence of recombination.

**Discussion**

**Genetic background affects meiotic chromosome pairing**

Since both L. racemosus and L. mollis are tetraploid, carrying the NsNsXmXm genome, they have four chromosomes belonging to homoeologous group 2. If we arbitrarily designate these chromosomes as 2Ns’ and 2Xm’ for L. racemosus and as 2Ns” and 2Xm” for L. mollis, the possible chromosome combinations of DMAs are 2Ns’–2Ns”, 2Xm’–2Xm”, 2Ns”–2Xm”, and 2Xm’–2Ns”. The former two combinations are homologous, the latter two are homoeologous. We produced three of the possible four DMAs in this study, meaning that at least one relationship must be homologous and one must be homoeologous. Because DMA[l MrMm] showed the highest coefficient of homology (h), the two alien chromosomes in this line must be in a homologous relationship. Similarly, the alien chromosomes in DMA[A Mm] were not completely homologous, though Kishii et al. (2003) reported that the F1 hybrid between L. racemosus

---

**Table 3. Frequency (% ± SD) of interactions between two alien chromosomes in double monosomic addition (DMA) lines.**

<table>
<thead>
<tr>
<th>DMA Line</th>
<th>Diakinesis</th>
<th>Metaphase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univalent</td>
<td>Bivalent</td>
</tr>
<tr>
<td></td>
<td>Univalent</td>
<td>Bivalent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA[l MrMm]</td>
<td>38.6 ± 5.2</td>
<td>61.4 ± 5.2</td>
</tr>
<tr>
<td>DMA[f MrMm]</td>
<td>65.7 ± 1.2</td>
<td>34.4 ± 1.2</td>
</tr>
<tr>
<td>DA[Mm Mm]</td>
<td>0.8 ± 0.8</td>
<td>99.2 ± 0.8</td>
</tr>
</tbody>
</table>

---

**Figure 4. Behavior of alien chromosomes in meiotic anaphase I. GISH shows L. racemosus chromosomes in red and L. mollis in green. a, b: DA[Mm Mm]. c–f: DMA[f MrMm]. g–j: DMA[f MrMm]. Wheat chromosomes are blue. Scale bar, 10 µm.**
and *L. mollis* produced clear bivalents from pachytene to MI, irrespective of terminal heterochromatin composition. This difference indicates that the genetic background of common wheat affected the pairing between the homologous chromosomes that originated from different species. It is known that common wheat possesses *Ph1* gene that strictly inhibits the pairing of homoeologous chromosomes (Riley and Chapman 1958; Chen et al. 1994; Martinez-Perez et al. 2001). This condition may distinguish chromosomes \([l\] and \([M\]) and inhibit their normal pairing. We suggest that pairing is not strictly controlled in the genetic background of *Leymus* species. This may be the case in DMA\([A^R M^M]\), even though the two chromosomes are in homologous relationship.

The two alien chromosomes of DMA\([A^R M^M]\) must be homoeologous, because the line had the lowest \(h\). Although this value of \(h\) indicates independent segregation, more spores carried a single alien chromosome than carried either two or no chromosomes (Table 2). This result suggests that these chromosomes interacted slightly with each other. However, the non-homologous combination DMA\([F^R M^M]\) also showed this tendency, and the prophase cells of this line showed association of the two chromosomes. Chromosomes \([F^R]\) and \([M^M]\) are non-homologous, but both come from *Leymus* species. We used probes for both *L. racemosus* and *L. mollis* to distinguish the
two *Leymus* chromosomes, but either set can discriminate the *Leymus* chromosomes from the wheat ones (Fig. 1c, d). This indicates that both *Leymus* species carry common dispersed repetitive sequences that are not present in wheat. In DMA[FrMm] where wheat chromosomes form clear bivalents, the two alien chromosomes may associate because of the presence of the common dispersed repetitive sequences.

**Relation between chromosome association and segregation**

The association of homologous chromosomes is important for homologous recombination and accurate segregation of chromosomes (Ding et al. 2004). However, we did not observe normal bivalents during MI even in DMA[FrMm], in which the two alien chromosomes were homologous. This line showed association of the alien chromosomes until diakinesis, but the association was released before MI. Two alien chromosomes showing no association become univalents with random positioning, and two alien chromosomes maintaining pairing till diakinesis become univalents with symmetrical positioning. During MI, the univalents took symmetrical positions across the equatorial plate, because the associated chromosomes in prophase do not form chiasmata. We did not observe recombined sister chromatids in the alien chromosomes. The two chromosomes moved to opposite poles during MI (Fig. 3d), while the wheat bivalents were held on the equatorial plate by chiasmata (Carpenter 1994; LeMaire-Adkins et al. 1997; Bozza and Pawlowski 2008; Cai et al. 2010). The non-homologous chromosomes in DMA[FrMm] showed similar behavior (Fig. 3f), although the frequencies of association in diakinesis and of symmetrical positioning in MI were lower than those in DMA[FrMm] (Table 3).

Symmetrical positioning is the process of normal movement to the pole, and in the second division these alien chromosomes will be segregated normally to the daughter cells, as are the chromosomes of wheat. So from the data of symmetrical positioning in Table 3, we can deduce the distribution of alien chromosomes in tetrad spores: If we expect 52.8% (the rate of symmetrical positioning) of univalents in DMA[FrMm] to segregate normally to each of the daughter cells and take part in the

---

Figure 6. Diakinesis chromosomes in DA[Mm Mm] (a–c), DMA[FrMm] (d, e), and DMA[FrMm] (j, k). GISH shows *L. racemosus* chromosomes in red and *L. mollis* in green. f–i: Representative bivalents in DMA[FrMm]. l–o: Representative bivalents in DMA[FrMm]. Wheat chromosomes are blue. Scale bar, 10 µm.
second division together with wheat chromosomes, these PMCs must show segregation in tetrad cells at a 0:1:1:0 ratio of no chromosome: *L. racemosus* chromosome:*L. mollis* chromosome: both chromosomes. However, in the other PMCs, the two alien chromosomes segregate independently, giving a segregation ratio of 1:1:1:1 in the tetrad. Based on this expectation, $h$ is calculated to be 0.53. This value is close to that calculated by observation of tetrads ($h = 0.62$, Table 2). A similar calculation in DMA$[F^\text{M}M^\text{F}]$ gives $h = 0.27$. This value does not fit the observation of tetrads ($h = 0.05$, Table 2), and indicates that the symmetrical positioning in the MI cells of the non-homologous DMA$[F^\text{M}M^\text{F}]$ was false and not the process of normal movement to the poles.

**Acknowledgment**

This work was partly supported by a Grant-in-Aid from the Japanese Society for the Promotion of Science (No. 226380003).

**References**


Larson SR, Kishii M, Tsujimoto H, Qi LL, Chen PD, Lazo GR, Jensen KB, Wang RRC (2011) *Leymus* EST linkage maps identify 4Ns–SnsL reciprocal translocation, wheat–*Leymus* chromosome introgressions, and functionally important gene loci. (in press)

