INTERLOCKED BIVALENTS IN RECONSTRUCTED METAPHASE I CELLS OF BREAD WHEAT

J. S. HESLOP-HARRISON AND M. D. BENNETT
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, England

SUMMARY
Complete reconstructions of all the bivalents were made from electron micrographs of serial sections through six pollen mother cells at metaphase I of meiosis in *Triticum aestivum* (hexaploid bread wheat). At least two of these metaphases contained interlocked pairs of bivalents. In one, two ring bivalents were interlocked, while in another a rod bivalent ran through the centre of a ring bivalent. Two other groups of bivalents were too closely appressed to allow separation into individual bivalents and may have contained interlocks. Meiosis in other anthers of the same plants examined by light microscopy was considered normal. The frequency of interlocking found was much higher than reported from light-microscope spreads.

Not all interlocks in metaphase I cells need adversely affect meiosis, but knowledge of their regularity and form may facilitate understanding the processes of chromosome pairing.

INTRODUCTION
The interlocking of bivalents at metaphase I of meiosis occurs when one bivalent completely surrounds (or encircles) part of another bivalent. The phenomenon has been widely reported since early this century. Darlington (1937) reviewed the early work on this subject.

In a few species such as *Oenothera*, interlocking at metaphase I occurs regularly because the karyotype includes stable interchanges (Cleland & Blakeslee, 1931). However, in most species, interlocking was considered to be very rare at metaphase (e.g. see Yacobi, Mello-Sampayo & Feldman, 1982). In contrast to this belief, interlockings of chromosomes and bivalents have been identified in virtually all organisms where zygotene nuclei have been completely reconstructed (Holm & Rasmussen, 1984). Apparently, there is a mechanism that removes interlocks during meiotic prophase. The interlock frequency can be manipulated genetically or environmentally, as it is affected by genes (Yacobi *et al.* 1982), translocations (Holm *et al.* 1981), and temperature (Buss & Henderson, 1971).

The reconstruction of complete nuclei (or metaphases) at meiosis, using complete sets of electron micrographs of serial sections through the nucleus, has many advantages over spreading, drying or squashing techniques commonly used to examine bivalents at meiosis. Almost exact relative positions of chromatin segments within the nucleus can be found, with no distortion due to preparative artifacts (Bennett, 1984). Moreover, features such as the interlocking of synaptonemal...
Fig. 1A. For legend see p. 88.
**Interlocked bivalents in wheat**

![Diagram of interlocked bivalents in wheat](image)

Fig. 1b. For legend see p. 88.
complexes, which are often difficult or impossible to resolve using the light microscope, can be positively identified (Holm & Rasmussen, 1984). However, because of the time involved in making the reconstructions, it is impractical to examine the large numbers of cells normally used in light-microscopic studies.

MATERIALS AND METHODS

The plants used were *Triticum aestivum* L. emend. Thell. ssp. *vulgare* MacKey, cv. Chinese Spring (2n = 6x = 42). This wheat normally has 21 bivalents at metaphase I of meiosis and disomic inheritance. Seeds were germinated on moist filter paper, transferred to 12 cm pots of John Innes no. 1 compost in a heated, continuously illuminated greenhouse, and then moved into a growth cabinet (20°C, 24 h white light at about 110 W/m²) about 14 days before they reached meiosis. Fertilizers (ammonium nitrate or 'Chempak No. 2', NPK25:15:15 with seven trace elements) and a non-systemic insecticide (pyrethrum and piperonyl butoxide) were applied to the plants. The nuclei discussed in this paper came from two different plants grown in different years.

When flowers reached meiosis, sample anthers were squashed and pollen mother cells (PMCs), stained with aceto-carmine, immediately examined by light microscopy. In wheat, each floret contains three anthers with PMCs that are nearly synchronous in development at meiosis. When an anther containing PMCs at metaphase I was found, the remaining two were embedded for electron microscopy.

Conventional electron-microscopic techniques (described by Bennett, Smith, Simpson & Wells, 1979) were used to prepare photographs of 0·1 μm thick serial-sections through complete first metaphases. Between 109 and 197 sections were required to section each nucleus, and photographs of only 1% of the total number of sections were unavailable for analysis. Photographs were placed in a binder in order, and chromatin, visible in the sections, was followed through consecutive sections by eye (Finch, Smith & Bennett, 1981; Heslop-Harrison & Bennett, 1983a).

RESULTS

Meiosis appeared normal in the light-microscopic preparations of anthers synchronous to those embedded for electron microscopy and in other anthers from the same plants sampled at the same time as the metaphases used for analysis. Thus, there was no reason to believe that the cultural conditions or genotype induced any unusual meiotic behaviour.

Two pairs of interlocked chromosomes were identified in the six reconstructed nuclei. Fig. 1A shows a series of sections through two interlocked ring bivalents. Fig. 1B shows drawings of the outlines of the two bivalents. Centromeres are seen as areas of lighter grey chromatin in the sections (Heslop-Harrison & Bennett, 1983b).

---

Fig. 1. A. Serial electron micrographs of consecutive sections through two interlocked ring bivalents in a pollen mother cell from bread wheat. Sections that are not informative because they show little change from adjacent sections, or are dirty or otherwise imperfect, were omitted from the series shown. The bivalents are seen as electron-dense (dark grey) areas in the less-dense cytoplasm. Two centromeres (mid-grey areas) are on each bivalent. B. Outlines of the two interlocked bivalents shown in A; one is drawn as an unbroken, and the other as a broken, line. Crosses indicate the centromeres, and the numbers give the section numbers from the first section including one of the two bivalents. ×3200.
Interlocked bivalents in wheat

Fig. 2A and B show micrographs and drawings of a rod bivalent passing through the centre of a ring bivalent. This bivalent comes from a different plant grown in a different year from that shown in Fig. 1.

In two of the six nuclei there were groups of six chromosomes identified by six centromeres, which were presumed to be three bivalents. No junctions between them could be identified because their chromatin was so closely appressed. One of these groups was in the same cell as the pair of interlocking bivalents shown in Fig. 1. Because of the sizes, orientation and centromere positions, it was considered likely that both groups contained three bivalents and that there was one or more interlocks within each group. All other bivalents were separated into individual rods or rings without difficulty and the expected number of centromeres (42) was found in each cell.

DISCUSSION

The reconstruction of electron micrographs of serial sections through complete metaphases has shown that interlocking of both pairs of rings, and ring and rod bivalents, occurs in wheat metaphases.

In wheat meiotic metaphases of the same variety (Chinese Spring), prepared for examination in the light microscope, the frequency of cells showing interlocking bivalents has been reported as 4% (Yacobi et al. 1982). The frequency found here (at least 33%) is significantly higher than this ($P = 2\%$), although a relatively small sample was used.

Holm & Rasmussen (1984), discussing the spreading of synaptonemal complexes at pachytene of meiosis, stated that 'the spatial organization of the nuclei is disrupted and the two-dimensional projection of the nucleus does not allow a distinction between interlockings and overlapping lateral components'. The identification of interlocked, rather than overlapped, bivalents can be very difficult in the light microscope, even at metaphase I. Where only a small proportion of the cells on a slide is scored, those including an interlock may well be disregarded because the bivalents are considered to be poorly separated.

The consequences of bivalent interlocking depend largely on the orientation of centromeres at anaphase. If both centromeres from a bivalent orient so that they go to the same pole at anaphase, aneuploidy and perhaps sterility will result. If orientation is normal, so that homologous centromeres pass to opposite poles, correct chromosome segregation will occur. Thus, providing the orientation of centromeres on interlocked bivalents is correct, it will not matter if interlocks are unresolved until first anaphase. Whether the centromeres of the interlocked bivalents occurring in bread wheat tend to orient correctly is unknown. However, any general model of the process of meiosis must take into account not only the formation and 'resolution' of interlocks during prophase of meiosis (Holm & Rasmussen, 1984) but also the possibility of bivalents remaining interlocked through metaphase I.
Fig. 2. A. A similar set of micrographs to those in Fig. 1A, showing a rod bivalent (broken line in 2B) passing through the centre of a ring bivalent (unbroken line). B. Tracings of the outlines of the two bivalents shown in A. ×3000.
Interlocked bivalents in wheat

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>31</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>17</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>17</td>
<td>47</td>
</tr>
</tbody>
</table>

Fig. 2a
J.S. H.-H. thanks Peterhouse, Cambridge, for the award of a William Stone Research Fellowship. The authors thank Mr J. B. Smith for the preparation of the serially sectioned nuclei discussed in this paper.

REFERENCES

(Received 22 August 1984 – Accepted 15 November 1984)