The time and duration of female meiosis in wheat, rye and barley

By M. D. Bennett, R. A. Finch, J. B. Smith and M. K. Rao

Plant Breeding Institute, Cambridge, CB2 2LQ

(Communicated by R. Riley, F.R.S. – Received 6 December 1972)

[Plates 44 to 47]

Few recent investigations have been made of female meiosis in cereals, and almost nothing is known about the duration of female meiosis in higher plants. Consequently, the time and duration of female meiosis in *Triticum aestivum, Hordeum vulgare* and *Secale cereale* have been studied. The appearance of the embryo sac mother cell (e.m.c.) and of the meiotic nuclei during female meiosis in *Hordeum vulgare* is described and illustrated.

In the species studied, each floret contains only one ovary with a single e.m.c., and meiosis is almost synchronous in the pollen mother cells from all three anthers. Consequently, it is possible to make precise comparisons between the stages of male and female development within individual florets. Data from these comparisons, together with knowledge previously determined of the duration of male meiosis in these species, allowed the estimation of the time and duration of female meiosis fairly accurately for *T. aestivum* and *H. vulgare* and approximately for *S. cereale*. The results showed that for *H. vulgare* and *T. aestivum* grown at 20 °C, the duration of female meiosis was very similar to the duration of male meiosis. Furthermore, on average male and female meiosis occurred almost synchronously. In *S. cereale*, however, male meiosis preceeded female meiosis by about 15 h. Growing *T. aestivum* under environmental stress induced asynchrony between male and female development at meiosis. Synchrony was not re-established after a long period under normal conditions.

Nuclear DNA content and ploidy level are known to be important factors determining or affecting the duration of male meiosis. These factors appear to play an important role in controlling the duration of female meiosis also.

**INTRODUCTION**

Compared with the effort devoted to timing somatic cell cycles in higher organisms, little has been done to investigate the duration of their meiotic divisions. Furthermore, most timing has measured the duration of male meiosis. Thus, the duration of male meiosis has been estimated for about 30 species of higher plants and about 20 species of animals. As far as the authors are aware, the duration of female meiosis has been estimated only in *Xenopus laevis* (Bird & Birnstiel 1971; Coggins & Gall 1972) and partly measured in three mammal species (Lima-de-Faria & Borum 1962; Edwards 1965; Kennelly, Foote & Jones 1970). No estimates of the duration of female meiosis in higher plants have been previously published. The great importance of female meiosis has often been stressed on theoretical grounds (Darlington 1971) and some investigations of meiosis in plant embryo sac mother cells (e.m.cs) have been made (Fogwill 1958; Vosa 1972; Vosa & Barlow 1972). Nevertheless, the number of studies made of female meiosis are too few to reflect the relative importance of the second component of ‘two-track heredity’ (Darlington 1971). The main reason for this in plants is the...
technical problem associated with the location and handling of e.m.cs. Most plant species produce hundreds or even thousands of pollen mother cells (p.m.cs) for each e.m.c. Even if an e.m.c. is found, the chances are low that it will be at a required stage. Thus, in species which flower infrequently and produce few florets per inflorescence a great many individuals may be required before enough e.m.cs are located for examination of, for instance, chiasma frequency at first metaphase.

It was decided to investigate the time and duration of female meiosis in cereals for several reasons. First, timing studies of male meiosis in cereals have recently led to an increased understanding of the factors which determine the duration of meiosis in higher plants (Bennett, Chapman & Riley 1971). It has been shown that the duration of male meiosis in diploid higher plants is positively correlated with, and perhaps determined by, the species nuclear DNA amount (Bennett 1971); but that the duration of meiosis is negatively correlated with increasing ploidy level in auto- and allopolyploids and polyhaploids (Bennett & Smith 1972; Finch & Bennett 1972; G. A. Dover & M. D. Bennett, unpublished). It would be interesting to know, therefore, whether the duration of female meiosis exhibits the same relationships to nuclear DNA content and ploidy level as does male meiosis.

There are also practical reasons which make a knowledge of the timing of the initiation of the female gametophyte desirable in cereals. Chiasma frequency is known to differ considerably between the e.m.cs and p.m.cs of some higher plant species (Fogwill 1958; Vosa 1972). If such differences exist in cereals they could have implications for the controlled handling of genetic variation in breeding programmes. It was hoped, therefore, that the present study would provide a comparison of chiasma frequency in e.m.cs and p.m.cs of a cereal species. The estimation of chiasma frequency in female meiosis depends upon being able to locate e.m.cs at suitable meiotic stages. Consequently, any information about the relative synchrony of male and female meiosis in individual florets should facilitate the location of e.m.cs at diplotene, diakinesis and first metaphase.

Finally, as already noted, female meiosis has not received the attention from cytologists which its importance merits. It was hoped, therefore, that a detailed investigation of female meiosis in several cereal species would make a useful addition to the limited knowledge on this subject which would be of interest to more than plant breeders alone.

**Materials and methods**

The experiments were performed using the following species and genotypes:

**Diploids** $(2n = 2x = 14)$

_Hordeum vulgare_ L. (i) Var. Sultan – a two-row spring variety. (ii) Var. Husky (ds) – a six-row spring variety. The line used had a homozygous recessive mutation causing desynapsis after pachytene. (iii) H350-1554 – a six-row breeding line produced at the Plant Breeding Institute. (iv) A few e.m.cs from four other varieties (Bonus, Colsess V, Maris Otter and Universe) were also examined.
**Female meiosis in cereals**

*S. cereale* L. var. Petkus Spring.

*Hexaploid (2n = 6x = 42)*

*Triticum aestivum* L. var. Chinese Spring.

In each of these genotypes there is only a single e.m.c. per floret compared with about 2100 p.m.cs in *T. aestivum*, about 3000 in *H. vulgare* and about 16000 in *S. cereale* (D'Souza 1970; R. A. Finch, unpublished). There is, however, no shortage of material for examination. Flowering occurs readily within only a few weeks of sowing and there are many florets per inflorescence and usually several inflorescences per plant.

A preliminary investigation showed that it was possible to obtain both p.m.cs and e.m.cs at meiosis from the same inflorescence, and often from the same floret, in all three species.

**(a) The culture of plants**

Plants of *T. aestivum* var. Chinese Spring, *S. cereale* var. Petkus Spring and *H. vulgare* vars. Sultan and Husky were grown in pots in a warm glasshouse until about 7 days before meiosis when they were transferred to environments maintained at 20 ± 1 °C and given constant illumination. Plants of *T. aestivum* var. Chinese Spring were also grown in pots in a cool glasshouse in which the temperature during the 2 weeks before meiosis averaged 7 to 8 °C and varied between 5 and 12 °C. Some plants, however, were transferred to an environment maintained at 20 ± 1 °C with continuous illumination about 7 to 10 days before female meiosis occurred in leading tillers. Plants in the cool glasshouse were subject to natural short day length in Cambridge during the period 9 to 17 December 1971 and were not given supplementary illumination.

*H. vulgare* plants of the variety Sultan were sown in the field at Cambridge on 28 March 1972 and tillers were fixed in Carnoy's solution on either 17 or 28 June. Seed of the breeding line H350–1554 was sown in the field on 27 February 1971 at the Plant Breeding Institute and spikes were fixed on 19 May 1971.

**(b) Meiotic timing**

The sampling methods previously used for timing male meiosis in the cereals (Bennett *et al.* 1971) cannot be used for timing female meiosis because there is only a single e.m.c. per floret. While the autoradiographical methods previously used to estimate the duration of male meiosis in *T. aestivum* (Bennett *et al.* 1971) should function in e.m.cs, the technical difficulties associated with finding enough female meiocytes and performing autoradiography on them appeared too great. Consequently, another method for timing meiosis in female meiocytes was used.

Inflorescences containing female meiosis were fixed in either 1:3 acetic alcohol or Carnoy's fixative. After fixation, individual florets were removed and their anthers and ovaries dissected out, hydrolysed for 10 min in 1N HCl at 60 °C, and stained for 2 h in Feulgen. After staining the ovary was transferred to a drop of 45% acetic acid and the ovule was dissected out under a dissecting microscope and transferred to another drop of 45% acetic acid on a clean slide. A coverslip
was applied and sometimes tapped gently to spread the cells of the nucellus. If a female meiocyte or tetrad was located, and its stage determined, then the anthers from the same floret were also examined in squash preparations and the stage of development of the p.m.cs or pollen grains determined. Thus, the stage of female development was compared directly with the stage of male development in p.m.cs from anthers in the same floret.

The method used for timing female meiosis depends upon four facts. First, the cereal species which we examined have only a single e.m.c. per floret. Secondly, male meiotic development is almost synchronous both within and between the three anthers from a single floret (Bennett et al. 1971). Thirdly, female meiosis often occurs within individual florets while male meiosis is still in progress. Fourthly, the durations of the premeiotic, meiotic, and postmeiotic stages of male development in these cereals have been carefully determined (Bennett & Finch 1971; Bennett & Smith 1972).

The first three facts facilitate precise comparisons of the stages of male and female development within a floret. Comparison of the stage of male and female meiosis in an individual floret shows whether or not they are synchronous. If they are not synchronous then the developmental interval between the stage of female meiosis and the stage of male development can be expressed in hours of corresponding male development at 20 °C using the data for the duration of individual premeiotic, meiotic and postmeiotic stages in anthers previously estimated (Bennett et al. 1971; Bennett & Finch 1971; Bennett & Smith 1972). This comparison can be made graphically by plotting the stages of male and female development for individual florets against equal axes on which individual meiotic stages are given their absolute duration as measured for normal male meiosis at 20 °C. Both male and female stages are plotted at the mid-stage value unless some clear indication was seen that the e.m.c. or p.m.cs had completed more or less development or were intermediate between stages. The durations of meiotic and other developmental stages in wheat, rye and barley anthers at 20 °C are collated in table 1. If meiosis is synchronous in male and female meiocytes in each individual floret then all the points plotted should fall on a line passing through the origin and having a slope of unity (i.e. \( b = 1.0 \)). If, however, meiosis in each floret proceeds at the same rate in the e.m.c. and p.m.cs but starts earlier in one sex by a constant time period, then all the points should fall on a line having a slope of unity and cutting one axis at a value equal to the constant time differential. If meiosis normally or always proceeds at different rates in meiocytes of the two sexes then the points for individual florets should fall on a line with a slope other than unity indicating the relative durations of the two types of meiotic divisions.

Provided the duration of male meiosis is known then the duration of female meiosis can be calculated from the slope of the regression line for observed points. Whenever the points fall on a line with a slope of unity then it follows that the duration of both male and female meiosis are the same. If male meiosis is plotted
Female meiosis in cereals

on the ordinate it follows that if the slope for observed points is greater than unity then female meiosis is longer than male meiosis and, if the slope is less than unity, the opposite is true.

In practice natural variation and sampling error result in a scatter of points around the hypothetical lines just discussed, but the line required may be calculated using linear regression analysis.

Table 1. The duration in hours of premeiotic stages, meiosis and its constituent stages and the tetrad stage in barley, rye and wheat anthers at 20 °C

The data are taken from Bennett & Finch (1971), Bennett & Smith (1972).

<table>
<thead>
<tr>
<th>stage of development</th>
<th>Hordeum vulgare var. Sultan</th>
<th>Secale cereale var. Petkus Spring</th>
<th>Triticum aestivum var. Chinese Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>premeiotic interphase (stage 2)</td>
<td>18.0</td>
<td>15.0</td>
<td>10.4</td>
</tr>
<tr>
<td>premeiotic interphase (stage 3)</td>
<td>12.0</td>
<td>20.0</td>
<td>9.0</td>
</tr>
<tr>
<td>leptotene</td>
<td>15.0</td>
<td>11.4</td>
<td>8.8</td>
</tr>
<tr>
<td>zygotene</td>
<td>10.4</td>
<td>8.0</td>
<td>2.2</td>
</tr>
<tr>
<td>pachytene</td>
<td>9.0</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>diplotene</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>diakinesis</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>first prophase</td>
<td>32.6</td>
<td>41.0</td>
<td>17.0</td>
</tr>
<tr>
<td>metaphase I</td>
<td>1.6</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>anaphase I</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>telophase I</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>dyad stage</td>
<td>2.0</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>metaphase II</td>
<td>1.2</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>anaphase II</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>telophase II</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>MI–TII inclusive</td>
<td>6.8</td>
<td>10.2</td>
<td>7.0</td>
</tr>
<tr>
<td>total meiotic duration</td>
<td>39.4</td>
<td>51.2</td>
<td>24.0</td>
</tr>
<tr>
<td>tetrad stage</td>
<td>8.0</td>
<td>8.5</td>
<td>10.0</td>
</tr>
</tbody>
</table>

(c) Possible objections to the method

(1) The method used for timing female meiosis depends upon individual meiotic stages having the same relative durations in both male and female divisions. We have assumed that the relative durations of individual stages of male and female meiosis are the same. If this is not so our estimates of the duration of female meiosis may be thereby subject to error.

(2) The method of estimating the duration of female meiosis uses observations plotted at mid-stage values. While this introduces a further possible error into the method its effect is unlikely to be large. The error cannot be important for the many short stages such as diakinesis. The chances of finding meiocytes either
before or after the mid-stage value are equal, and so, even for long stages, individual errors should tend to cancel each other out, provided sufficient observations are made.

Results

(a) General description of female meiosis

In the cereals dealt with in this paper, all of which belong to the tribe Hordeae, each floret contains one ovary with a single bitegmic, tenuinucellar ovule. During meiosis in barley and wheat the ovule is longitudinally symmetrical but after meiosis it becomes anatropous. In *H. vulgare* the integuments do not enclose the nucellar dome during meiosis as they do in *S. cereale* and *T. aestivum*. Consequently, female meiosis is more easily observed in *H. vulgare* than in the other two species. At the onset of meiosis the e.m.c. is marked by its large volume, compared with other nucellar cells. In this respect it is similar to p.m.cs which, in these species, have much greater volumes than do the tapetal or epidermal anther cells. The e.m.c. is also marked by its shape, being greatly elongated and having its micropylar end much wider than its chalazal end so that it is pear-shaped. Thus, the shape of the e.m.c. differs from that of p.m.cs which are roughly spherical. Meiosis in e.m.cs of all three species examined was successive, and the appearance of the chromosomes at each stage was the same as at the corresponding stage of meiosis in p.m.cs (plates 45 to 47). The plane of the spindle at the first meiotic division is along the long axis of the nucellus and the e.m.c. In barley and wheat the planes of the second meiotic division differ in the two cells of the dyad (figure 1d, plate 44). In the micropylar cell it is at right angles to the plane of the first meiotic division, while in the chalazal cell it is in the same plane as in the first meiotic division. Consequently, the product of female meiosis is a T-tetrad.

According to Davis (1966) the e.m.c. tetrad in the Gramineae is usually linear although exceptions are known. For instance, sexual forms of *Agropyron scabrum* have a T-tetrad (Hair 1956). Percival (1921) stated that *T. aestivum* has a linear female tetrad. Our observations on intact ovules of *T. aestivum* cleared in polyvinyl lactophenol (cf. Herr 1971) differ from those of Percival (1921). More than one tetrad shape is known in *Urginea indica* (Capoor 1937) and the grass *Euchlaena mexicana* apparently has linear and T-shaped female tetrads (Cooper 1937). Perhaps both linear and T-tetrads occur in *T. aestivum* but one shape is normal and the other anomalous. It should be noted, that both normal isobilateral and anomalous T-tetrads are sometimes produced by male meiosis in *H. vulgare* (Finch & Bennett 1972). T-shaped female tetrads were, however, found in two

Description of Plate 44

Figure 1. Wheat ovule squashes showing: (a) early prophase meiotic nucleus seen through the nucellar epidermis in partly squashed ovule; (b) pachytene nucleus; (c) diakinesis nucleus; (d) metaphase II nuclei showing different division planes; (e) two haploid tetrad nuclei surrounded by somatic cells. (N.B. (b) to (e) are at the same magnification.)
Figure 1. For legend see facing page.
Figure 2. (a, b) Lateral views of excised, unsquashed barley ovules at the start of meiosis (a), and near the end of meiosis (b). The micropylar nucellar dome protrudes well beyond the developing integuments throughout meiosis. (c) e.m.c. nucleus at leptotene. (d) e.m.c. nucleus at zygotene.

(N.B. (a) and (b) are at the same magnification, and (c) and (d) are at the same magnification.)
Female meiosis in cereals

species, one grown both in the field and in the glasshouse and so it seems likely that this shape is normal.

Most recent investigations of megasporogenesis and subsequent female development in cereals have concentrated on postmeiotic organization and development such as embryo sac structure (Vazart 1955), fertilization and embryogenesis (Pope 1937, 1943; Morrison 1955, Weatherwax 1955). Because of this, and because there seem to be some discrepancies between our observations and those of, for instance, Percival in *T. aestivum*, it seemed worth while to describe female meiosis in some detail for at least one cereal species and *H. vulgare* was chosen.

(b) Female meiosis in *Hordeum vulgare*

Figure 2a, plate 45 shows the ovule as it typically appeared at the onset of female meiosis consisting of a round nucellus with two integuments growing up from the base but not enclosing the nucellar dome. During meiosis, the size of the nucellar dome and integuments increased, but the height of the nucellar apex above the inner integument remained almost constant throughout female meiosis (figures 2a, b, plate 45). The e.m.c. was pear-shaped with its long axis coincident with that of the ovule. It was located centrally within the nucellus with its dilated micropylar end clearly visible immediately below the epidermis of the nucellar dome. During meiosis the e.m.c.'s chalazal end extended down to about the level of the top of the outer integument, and was usually obscured from view in the intact stained ovule. Like p.m.cs the e.m.c. was surrounded by a thickened callose wall (figure 4b, plate 47) which was laid down before meiosis and persisted throughout the meiotic division.

The appearance of the chromosomes at all stages of female meiosis was the same as in p.m.cs at corresponding stages (plates 45 to 47) and there was no obvious difference in chromosome size between e.m.cs and p.m.cs.

During first prophase the e.m.c. nucleus was usually located near the middle of the dilated micropylar end and had a single large peripheral nucleolus. At diakinesis the bivalents were arranged on the equatorial plate and at first metaphase their centromeres became coorientated (figures 3d, e, plate 46). The plane of the spindle at the first meiotic division was along the long axis of the e.m.c. so that at first anaphase the chromosomes moved to poles at either end of the e.m.c. (figure 3f, plate 46).

After first telophase a cell wall was laid down separating the products of the first meiotic division (figure 4c, plate 47). The two cells produced had unequal diameters in the long axis of the nucellus, since the cross-wall formed much nearer to the micropylar end than to the chalazal end. The planes of the second meiotic division have already been described. The division, which occurred synchronously in both cells of the dyad, gave rise to a T-tetrad. Microdensitometry of Feulgen stained ovules showed that the nuclear DNA content was reduced from the 4C amount in first prophase to the 2C amount at first anaphase, and to the 1C amount at second anaphase.
(c) **Comparison of chiasma frequency in Hordeum vulgare p.m.cs and e.m.cs**

Wherever possible the chiasma frequency was estimated in e.m.cs at diakinesis or first metaphase, and in p.m.cs at corresponding stages either from the same floret or, more often, from the same inflorescence. Enough results for meaningful comparisons were obtained for H 350-1554 grown in the field and for Sultan grown at 20 °C in continuous light. These results (table 2) show that in both genotypes at both meiotic stages the chiasma frequency per cell was very similar in both male and female meiocytes. For instance, at first metaphase in H 350-1554 the mean chiasma frequency per cell was 14.00 for seven e.m.cs and 13.81 for two hundred and eighty p.m.cs.

<table>
<thead>
<tr>
<th>genotype</th>
<th>meiotic stage</th>
<th>e.m.c.</th>
<th>p.m.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H 350-1554</td>
<td>diakinesis</td>
<td>13.67 (3)</td>
<td>13.65 (69)</td>
</tr>
<tr>
<td></td>
<td>metaphase I</td>
<td>14.00 (7)</td>
<td>13.81 (280)</td>
</tr>
<tr>
<td>Sultan</td>
<td>diakinesis</td>
<td>13.67 (3)</td>
<td>14.00 (8)</td>
</tr>
<tr>
<td></td>
<td>metaphase I</td>
<td>13.33 (3)</td>
<td>14.05 (20)</td>
</tr>
</tbody>
</table>

(d) **Time and duration of female meiosis**

Because of the favourable morphology of the ovule in *H. vulgare* many more (215) e.m.cs at meiotic stages were observed than in *S. cereale* (30) and *T. aestivum* (31).

Conclusions based upon the results for *H. vulgare* should be reliable since they are based on a large sample of e.m.cs. The relations between the stages of meiotic development in the e.m.c. and p.m.cs from individual florets are plotted in figures 5a to d for each of the three genotypes. It is immediately obvious that in all three genotypes meiosis occurred in an e.m.c. within several hours of its occurrence in p.m.cs in the same floret, since in 175 of the 215 florets with female meiotic stages the anthers also contained some stage of meiosis. Often, both the e.m.c. and p.m.cs from a single floret contained the same stage of meiosis. For instance, in Sultan grown at 20 °C with continuous illumination, 42% of the florets with female meiosis contained an e.m.c. and p.m.cs at the same meiotic stage. The greatest asynchrony (table 3) between male and female development in a
Figure 3. For legend see facing page.

(Facing p. 308)
Figure 4. For legend see facing page.
Female meiosis in cereals

single floret was recorded for H 350–1554 grown in the field in which female meiosis preceded male meiosis by an interval corresponding to 25 h of male development in Sultan grown at 20 °C (Bennett & Finch 1971).

The stages of male and female meiosis in individual florets are plotted for each of the three genotypes as described in the section on methods (figures 5a to d). On

<table>
<thead>
<tr>
<th>genotype</th>
<th>percentage of florets with synchronous male and female meiosis</th>
<th>percentage of florets with both female and male meiosis</th>
<th>largest asynchrony in hours of corresponding male development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hordeum vulgare var. Sultan</td>
<td>66</td>
<td>73</td>
<td>18</td>
</tr>
<tr>
<td>(a) grown at 20 °C</td>
<td>41</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>(b) grown in the field</td>
<td>49</td>
<td>84</td>
<td>18</td>
</tr>
<tr>
<td>var. Husky</td>
<td>59</td>
<td>95</td>
<td>25</td>
</tr>
<tr>
<td>H 350–1554</td>
<td>59</td>
<td>95</td>
<td>25</td>
</tr>
<tr>
<td>(b) grown under stress</td>
<td>27</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>(c) grown under stress then 7 to 10 days at 20 °C</td>
<td>18</td>
<td>78</td>
<td>49</td>
</tr>
<tr>
<td>Secale cereale</td>
<td>30</td>
<td>87</td>
<td>37</td>
</tr>
</tbody>
</table>

each graph the calculated regression line for the points plotted is shown together with the regression line expected if male and female meiosis were completely synchronised in each floret. As explained previously, if both male and female meiosis have the same duration then the slope of the regression line for observed points will be unity, irrespective of whether male and female meiosis occur synchronously within individual florets. The slopes of the regression lines for Sultan \( b = 1.09 \pm 0.07 \) and for Husky \( b = 1.05 \pm 0.07 \) grown at 20 °C are not significantly different from unity. The slopes of the regression for Sultan grown in the  

**Description of Plate 47**

**Figure 4.** Barley ovule squashes showing; (a) e.m.c. at dyad stage before cross-wall formation; the cytoplasm outline is unusually round; (b) excised apex of nucellar dome at dyad stage showing callose thickening on the e.m.c. side walls seen in t.s. in the centre; (c) nuclei, cross-wall and all but the most chalazal part of the cytoplasm of e.m.c. at dyad stage; (d) prophase II nuclei; (e) anaphase II nuclei; (f) the four haploid tetrad nuclei. (N.B. (c) to (f) are at the same magnification.)
Figure 5. The stages of male and female development in individual florets of *H. vulgare* together with the relationship between the times and durations of male and female meiosis. Individual stages of development are given their relative durations as previously determined for male development at 20 °C (Bennett & Finch 1971) and a time scale in hours relative to the duration of male meiosis is shown. **Key:** ——, the
regression line for male meiosis on female meiosis expected \((b = 1.0; r = 1.0)\) if male and female meiotic development were completely synchronous. ———, the regression line for male meiosis on female meiosis obtained for the observations plotted. (a) Var. Sultan, grown in the field \((b = 1.14 \pm 0.07; r = 0.92)\). (b) Var. Sultan, grown at 20 °C \((b = 1.09 \pm 0.07; r = 0.88)\). (c) Var. Husky, grown at 20 °C \((b = 1.05 \pm 0.07; r = 0.91)\). (d) H 350-1554 grown in the field \((b = 0.73 \pm 0.05; r = 0.87)\).
field \( (b = 1.14 \pm 0.07) \) is just significantly different from unity at the 5\% level, and the slope for \textit{H}350–1554 grown in the field \( (b = 0.73 \pm 0.05) \) is significantly different from unity at the 0.1\% level. These slopes indicate that, compared with the duration of male meiosis in Sultan at 20 °C (39.4 h), female meiosis lasted about 43 h in Sultan and about 41.5 h in Husky grown at 20 °C. The remaining slopes indicate that the duration of female meiosis in Sultan grown in the field was 14\% longer than male meiosis, while in \textit{H}350–1554 grown in the field the duration of female meiosis was about 15\% shorter than male meiosis. Thus, if the mean temperature in the field was 20 °C the duration of female meiosis would have been about 45 h in Sultan and, assuming that the duration of male meiosis in Sultan and \textit{H}350–1554 is the same, the duration of female meiosis in \textit{H}350–1554 would have been about 33.5 h. The results show that the durations of male and female meiosis were not significantly different in the two genotypes grown under controlled conditions at constant temperature. In the two genotypes grown in the field, however, the durations of male and female meiosis were significantly different. It seems, therefore, that male and female meiosis were approximately synchronous in plants grown at 20 °C, but some asynchrony occurred in plants grown in the field and this may have been an environmentally determined effect.

\textit{(ii) T. aestivum}

The relationships between the stages of meiotic development in e.m.c. and p.m.cs from individual florets of Chinese Spring plants grown in three very different environmental treatments are shown in figure 6a to c. The results for plants grown at 20 °C with continuous illumination (figure 6a) showed that under these conditions male and female meiosis occurred at about the same time in a single floret. Thus, 16\% of the florets with female meiosis also contained p.m.cs at identical meiotic stages and 94\% of the florets with female meiosis also contained p.m.cs at some stage of meiosis. The largest asynchrony found in a single floret was between an e.m.c. at late leptotene and anthers containing p.m.cs at the start of leptotene and synchronously dividing tapetal cells. This asynchrony corresponds to male development lasting about 11 h at 20 °C. The slope of the regression line calculated for the plotted observations \( (b = 1.09) \) does not differ significantly from unity. Consequently, the duration of female meiosis in hexaploid wheat is very similar to that of male meiosis already estimated (Bennett \textit{et al.} 1971). The relative positions of the regression lines expected and obtained show that in individual florets female meiosis occurred on average about 2 h before male meiosis.

Figure 6b illustrates the results for individual florets in plants grown under continuous stress conditions, that is low temperature and short days. In these conditions only 4\% of florets contained synchronous meiotic development and the largest asynchrony observed in a single floret corresponded to male development lasting about 43 h at 20 °C. In these conditions the average asynchrony between male and female development within individual florets was greater than
the largest asynchrony observed at 20 °C. Figure 6b shows that under stress conditions female meiosis tended to occur before male meiosis in the same floret. The cause for this is unknown. Perhaps the supply of essential molecules is limited under stress conditions and their availability to the e.m.c. and the p.m.cs is unequal.

Figure 6c shows the stages of male and female meiosis in individual florets from plants grown first under stress conditions and then in an environment at 20 °C with continuous light for 7 to 10 days before the onset of female meiosis. In these plants male and female meiotic development remained highly asynchronous. Indeed, the maximum asynchrony between male and female development within a single floret corresponded to about 49 h of male development at 20 °C. This was slightly larger than the maximum asynchrony observed in plants grown continually under stress conditions. However, the incidence of synchrony between male and female meiosis was increased compared with that found in plants grown continually under stress conditions. Identical male and female meiotic stages were found in 16% of the florets examined. This percentage is identical with that found in florets from plants grown at 20 °C with continuous light.

The results show that environmental stress imposed more than 7 days before meiosis but not during the 7 days before meiosis induced asynchrony between the development of male and female germ line cells. The results indicate that once asynchrony between male and female development is induced, it is substantially maintained thereafter.

(iii) S. cereale

It was much harder to locate e.m.cs at meiosis in S. cereale than in T. aestivum or H. vulgare and, consequently, relatively few observations were made in this species. The observations for individual florets (figure 6d) show that, as in wheat and barley, both male and female meiotic stages were often found in a single floret. The greatest asynchrony seen between male and female development in a single floret corresponded to male development lasting about 37 h at 20 °C which is less than the duration of male meiosis at 20 °C (table 1). In 27% of the florets both the e.m.c. and p.m.cs contained the same meiotic stage. This figure is lower than the mean for H. vulgare (38%) but higher than that for T. aestivum (16%) at 20 °C. In individual florets male meiosis preceded female meiosis by about 15 h on average (figure 6d). Only in this species did male meiosis generally occur before female meiosis in individual florets.

The slope of the regression line for the points plotted in figure 6d is 0.41 (P < 0.001) which is significantly different from unity. This slope indicates that male meiosis takes more than twice as long as female meiosis in S. cereale. While this may be true, the deviation from a slope of unity probably resulted from the extreme difficulty of locating and identifying e.m.cs at early stages of meiosis in this species.
FIGURE 6. The stages of male and female development in individual florets of *T. aestivum* and *S. cereale* showing the relationship between the times and durations of male and female meiosis. Individual stages of development are given their relative durations as previously determined for male development at 20 °C (Bennett & Smith 1972) and a time scale in hours relative to the duration of male development is shown. Key: ———, the
regression line for male meiosis on female meiosis expected ($b = 1.0; r = 1.0$) if male and female meiotic development were completely synchronous. The regression line for male meiosis on female meiosis obtained for the observations plotted. (a) *T. aestivum* grown at 20 °C ($b = 1.09 \pm 0.15; r = 0.79$). (b) *T. aestivum* grown under stress conditions ($r = 0.49$). (c) *T. aestivum* grown under stress conditions then at 20 °C for 7–10 days before meiosis ($r = 0.22$). (d) *S. cereale* grown at 20 °C ($b = 0.41 \pm 0.11; r = 0.58$).
DISCUSSION

Several factors are known which affect or determine the duration of male meiosis in plants. These include: (a) nuclear DNA content (Bennett 1971); (b) ploidy level (Bennett & Smith 1972); (c) genotypic characters (Bennett 1973); and (d) environmental factors (Bennett, Smith & Kemble 1972). Comparisons of male and female meiotic duration in the same individual are interesting because nuclear DNA content, ploidy level, and the genotype are constant for meiocytes of both sexes. Consequently, any difference in meiotic behaviour between e.m.cs and p.m.cs must be caused by differences either in their development, or in the external plant environment to which they are subjected.

Differences in meiotic behaviour between male and female meiocytes from the same individual have been noted. For instance, Fogwill (1958) reported that in *Fritillaria meleagris*, chromosomes in the e.m.cs were larger than the chromosomes in p.m.cs. Differences in the mean chiasma frequency per cell in p.m.cs and e.m.cs have been described in *F. meleagris*, several *Lilium* species, several *Tulbaghia* species and *Listera ovata* (Fogwill 1958; Vosa 1972; Vosa & Barlow 1972).

Differences in chiasma frequency may be correlated with differences in meiotic duration (Darlington 1940; Fogwill 1958), especially in the time spent in chromosome pairing, that is in zygotene. Meiotic duration was not measured, however, in any of the species where e.m.cs and p.m.cs are known to have different mean chiasma frequencies. If meiotic time and mean chiasma frequency per cell are positively correlated then no difference in meiotic duration between e.m.cs and p.m.cs would be expected in species with very similar chiasma frequencies in meiocytes of both sexes. There are similar mean chiasma frequencies in p.m.cs and e.m.cs of *H. vulgare* (table 2), and the timing data for *H. vulgare* show that the durations of male and female meiosis were very similar. This result is not proof of a relationship between meiotic time and chiasma frequency, but it does not contradict Darlington's theory and may be interpreted as lending support to it.

It has been shown that for species with a common ploidy level, male meiotic duration shows a positive linear relationship to nuclear DNA content (Bennett 1971; Bennett 1973), and that *H. vulgare* obeys the relationship demonstrated for diploid species (Bennett & Finch 1971). In *H. vulgare* and *T. aestivum* both e.m.cs and p.m.cs contain the same DNA amount at corresponding stages of meiosis (M. D. Bennett, unpublished). If nuclear DNA content and meiotic duration are related for female meiosis in the same way as for male meiosis, then a similar meiotic time for both processes in the same species is expected when meiocytes of both sexes have the same DNA content. That both male and female meiosis have very similar durations in *H. vulgare* and *T. aestivum* supports this view.

In cereal species, ploidy level is negatively correlated with the duration of meiotic development so that the duration of meiosis decreases with increasing ploidy level in related allo- and autopolyploid forms even though the increase in ploidy level is accompanied by an increase in nuclear DNA content (Bennett & Smith
Female meiosis in cereals

1972; Bennett 1973; Finch & Bennett 1972). Thus, although the 4C nuclear DNA amount for *T. aestivum* (72 pg) is much greater than for its related diploid, *T. monococcum* (28 pg), meiosis at 20 °C takes only 24 h in the former compared with 42 h in the latter. The meiotic times of the diploids, *T. monococcum* and *H. vulgare*, are very similar (42.0 and 39.4 h, respectively) as are their 4C nuclear DNA amounts (28.0 and 27.0 pg, respectively). At 20 °C the duration of male meiosis in hexaploid *T. aestivum* (24 h) is much shorter than in diploid *H. vulgare* (39.4 h).

In these species the duration of female meiosis was similar to, or the same as, the duration of male meiosis at 20 °C. It appears, therefore, that polyploidy has a similar affect on the duration of female meiosis and on male meiosis since the durations of both male and female meiosis were much shorter in the polyploid species with a high nuclear DNA content than in the diploid species with a much lower nuclear DNA content.

As already noted differences in meiotic behaviour between male and female meiocytes of the same individual must be influenced by developmental or environmental factors. Lawrence (1958) suggested that differences in meiotic behaviour between e.m.cs and p.m.cs may be determined by the availability of molecules essential for their development. Rees & Naylor (1960) found developmental gradients in anthers of *Secale cereale*. They noted that the most advanced meiocytes were nearest to the filament insertion point and suggested that precocity may be determined by a substance diffusing inwards along the vascular strand. Male and female meiocytes may differ with respect to their physical properties so that the shape and size of the meiocytes may vary. It was shown that the shape of the female meiocyte is very different from that of the male meiocyte in the three cereal species examined. Fogwill (1958) reported large differences in the size of meiotic cells, nuclei and chromosomes between e.m.cs and p.m.cs of *Lilium* and *Fritillaria* species. She suggested that nuclear size and chiasma frequency may be causally correlated in these species. Similar correlations between physical properties of somatic nuclei and their behaviour are well known (Van't Hof & Sparrow 1963; Alfert & Das 1969; Bennett 1972; Bennett, Smith & Smith 1972).

The ovule and the anthers within a single floret of wheat, rye or barley, are enclosed within the same lemma, palea, glumes and leaf sheaths and share the same microclimate. If meiosis occurs synchronously in both male and female meiocytes then the external plant environment will not affect male and female meiosis unequally. If, however, male and female meiosis occur in an individual floret at different times then they may be subjected to different conditions determined by the external plant environment. Corresponding stages of meiosis, may for instance, be subjected to different temperatures. The results for *T. aestivum* and *H. vulgare* grown at 20 °C show that male and female meiosis have very similar durations and on average occur almost synchronously. Consequently they are normally subject to the same external environment.

The present results demonstrate that environmental factors can break down the normal synchrony between male and female meiosis. In *T. aestivum* plants grown
under stress conditions, male and female meiosis became asynchronous. Furthermore, synchrony was not re-established after a long period under normal conditions. Even though the environmental stress imposed was much greater than that normally encountered by *T. aestivum* in the field the degree of asynchrony induced was equivalent to only 48 h or less of normal male development at 20 °C. Meiosis is very temperature sensitive and has a *Q*₁₀ of 2.3 in *T. aestivum* var. Chinese Spring for the temperature range 15 to 25 °C (Bennett, Smith & Kemble 1972). Consequently, the asynchrony between male and female meiosis in individual florets would correspond to a much longer period of male development at 10 °C than at 20 °C.

Asynchrony between male and female meiosis, if it were maintained until dehiscence might affect fertility. Wheat and barley pollen remains viable for only a few hours after dehiscence (Pope 1944; D'Souza 1970). In many wheat and barley genotypes stigmas are normally pollinated by pollen from the same floret. In such genotypes if the time period between dehiscence and the onset of stigmatic receptiveness should exceed the pollen viability time then some infertility would probably result. Asynchrony between male and female gametophytic development would be important only if male development became precocious. The onset of stigmatic receptiveness two days before dehiscence would not result in infertility since the stigma in wheat and barley remains receptive for pollination for much longer than two days (Riddle & Suneson 1944).

Little is known concerning the relative timing and rate of male and female meiotic and gametophytic development in higher plants. Studies of these processes are necessary if we are to understand the control of reproductive development. The improved performance of many crop plants must partly depend on the controlled manipulation of their reproductive behaviour to overcome problems such as incompatibility and partial sterility. Investigations into the reproductive behaviour of crop plants seem, therefore, particularly worth while since they may lead to increased understanding of breeding systems.

**References**


Female meiosis in cereals 319


Fogwill, M. 1958 Differences in crossing-over and chromosome size in the sex cells of *Lilium* and *Fritillaria*. *Chromosoma* 9, 483–504.


FIGURE 1. For legend see facing page.
FIGURE 2. (a, b) Lateral views of excised, unsquashed barley ovules at the start of meiosis (a), and near the end of meiosis (b). The micropylar nucellar dome protrudes well beyond the developing integuments throughout meiosis. (c) e.m.c. nucleus at leptotene. (d) e.m.c. nucleus at zygotene. (N.B. (a) and (b) are at the same magnification, and (c) and (d) are at the same magnification.)
FIGURE 3. For legend see facing page.
FIGURE 4. For legend see facing page.