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Chiasma variation and control in pollen mother cells and embryo-sac mother cells of rye

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SUMMARY

Mean chiasma frequencies and the amounts of between-bivalent chiasma variation were computed for pollen mother cells (p.m.c.) and embryo-sac mother cells (e.m.c.) of five distinct inbred rye lines. Despite the considerable differences for both these metrics shown by the different lines, very little variation was seen between p.m.c. and e.m.c. belonging to the same lines. It is concluded that chiasma formation in p.m.c. and e.m.c. of rye is governed and regulated by a single controlling system of genes and that variation in this genetic system is expressed identically in the two sexes.

1. INTRODUCTION

Most studies of chiasma variation in natural and experimental populations of plants and animals are based exclusively on observations of meiosis in male meiocytes. The reasons for this bias are largely technical and associated with the problems involved in locating and identifying female meiocytes at the correct cytological stage for chiasma observations. Despite these difficulties several studies have established that chiasma frequency and distribution may be different in the two sexes (see John & Lewis, 1965 for review) and can sometimes be very different (e.g. Watson & Callan, 1963). The knowledge that these differences can arise creates a need for information on meiosis in *both* sexes before meaningful conclusions can be drawn about such things as the regulation of recombination and its relation to the expression of genetic variation in populations of plants and animals.

In addition to the practical question of whether meiosis, in specific cases, differs in the two sexes, a more fundamental question relates to whether meiosis in the two sexes is jointly or separately controlled. This question is complicated since it cannot be answered directly from a simple comparison of meiosis in the two sexes as the type of control does not necessarily bear a direct relationship to the presence or absence of a sex difference. For example, similar chiasma conditions in the two sexes need not imply a joint control but might result from two independent control systems acting convergently to produce the same effect. Similarly, sex differences at meiosis need not imply separate controls but could reflect instead the differential response of a joint control to the differing conditions of male and female meiocytes. However, where it is possible to study both male and female meiosis in a

range of genetically different types (e.g. meiotic mutants or inbred lines) it should be possible to assess the influence of the genotype in relation to chiasma control in the two sexes.

A large body of data has been published on meiosis and especially on chiasma frequency and distribution in rye pollen mother cells (p.m.c.) (e.g. Rees, 1955; Rees & Thompson, 1956; Jones & Rees, 1964; Jones, 1967). By contrast the properties of female meiosis in rye embryo-sac mother cells (e.m.c.) are practically unknown and there is even less indication of whether meiosis in the two sexes is jointly or separately controlled. For this reason a parallel study was undertaken of chiasma frequency and distribution in both p.m.c. and e.m.c. of several inbred lines of rye.

2. MATERIALS AND METHODS

Five inbred lines of rye were used in this study, including 4 lines (J_{33}, J_{93}, J_{113}, J_{115}) derived originally from an interspecific cross (Jones, 1974) and one line (P_2) derived by self-pollination from the variety Stålrag (Rees, 1955). Whole inflorescences containing male and female meiocytes were fixed in Carnoy's (6:3:1) fixative and stored in the refrigerator until needed. For the study of chiasmata in the p.m.c., anthers containing Metaphase I stages were squashed in aceto-carmine and temporary slide preparations made.

Metaphase I in the e.m.c. was found to correlate most closely with the tetrad stage in the p.m.c. of the same floret and this was used as a basis for the location of first metaphase stages in e.m.c. for chiasma observations. This relative timing is therefore consistent with the findings of Bennet *et al.* (1973) who observed that while meiosis was synchronous in the e.m.c. and p.m.c. of about 27% of all florets, male meiosis preceded female meiosis by about 15 h on average. Ovaries were dissected from florets in which p.m.c. were at the tetrad stage, hydrolysed for 30 min in 5 N-HCl at room temperature and stained for 30 min in Feulgen reagent. After staining, the ovary was transferred to a drop of 45% acetic acid and the single ovule dissected out before squashing in 45% acetic acid. About 10% of the ovules treated in this way yielded e.m.c. at the first metaphase stage of meiosis (Plate I). Bivalent chiasma frequencies were scored in 25 p.m.c. and 10 e.m.c. from each of the five lines.

3. RESULTS

The chiasma data obtained from p.m.c. and e.m.c. from each of the five lines studied is summarized in Table 1.

(i) *Mean chiasma frequency.* It can be seen from Table 1 that the mean chiasma frequency per cell in the different lines ranges from 8·20 to 12·88 chiasmata per cell in the p.m.c. and from 9·00 to 12·60 chiasmata per cell in the e.m.c. An analysis of variance (Table 2) shows that this variation in mean chiasma frequency between lines is highly significant. However, the chiasma frequency variation between sexes is not significant, thus showing that despite the very considerable between-line variation, p.m.c. and e.m.c. within individual lines have very similar chiasma frequencies.

First metaphase of meiosis in a rye embryo-sac mother cell showing the seven bivalents surrounded by somatic nuclei of the nucellus.

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Table 1. *A summary of the chiasma data obtained from p.m.c. and e.m.c. of five rye lines*

Lines	Chiasmata per bivalent 0	1	2	3	Number of cells	Mean chiasma frequency per bivalent	per cell	Unpartitioned bivalent mean-square
p.m.c.								
P_2	2	33	131	9	25	1·840	12.880	0·241
J_{93}	8	55	110	2	25	1·617	12·489	0·355
J_{113}	23	100	51	1	25	1·170	8·190	0·459
J_{115}	21	81	60	13	25	1·377	9·039	0·625
J_{33}	18	71	58	28	25	1·549	10·843	0·777
e.m.c.								
P_2	0	17	50	3	10	1·800	12·600	0·199
J_{93}	0	24	46	0	10	1·657	11·599	0·229
J_{113}	8	34	28	0	10	1·286	9·002	0·439
J_{115}	9	33	23	5	10	1·357	9·499	0·634
J_{33}	6	32	27	5	10	1·443	10·101	0·569

Table 2. *Analysis of variance of mean chiasma frequencies in p.m.c. and e.m.c. of five rye lines*

Item	*N*	Mean square	Variance ratio	*P*
Lines	4	97·609	44·655	< 0·001
Sexes	1	0·002	0·001	n.s.
Lines × Sexes	4	2·534	1·159	n.s.
Within sexes–within lines	165	2·186		

(ii) *Chiasma distribution.* Two quite different aspects of chiasma distribution may be considered, namely chiasma *frequency* variation among cells and bivalents and chiasma distribution along bivalents which is *positional* in nature. Positional chiasma distribution within bivalents is difficult to measure in rye where reliable chiasma scores must be obtained on condensed first metaphase bivalents, and this is especially true of poorly squashed preparations such as the e.m.c. preparations studied here. For this reason chiasma positional distribution was not recorded in this study and attention was concentrated instead on chiasma distribution between cells and bivalents. Total chiasma frequency variation within anthers is normally partitioned into between-cell and within-cell components (Mather, 1936) but since it has been shown that these components measure the same kind of variation and are highly correlated in rye (Jones, 1974) it is preferable to take the total, unpartitioned mean-square of all the bivalent chiasma frequencies in the sample (bivalent mean-square) as a measure of within anther chiasma distribution.

Table 1 shows that the lines differ considerably with respect to bivalent mean-squares. Line P_2 shows the least variation in bivalent chiasma frequencies, which is reflected in a relatively low bivalent mean-square (0·241 in p.m.c.) while line J_{33} shows at the other extreme much more variable bivalent chiasma frequencies

and a correspondingly higher bivalent mean-square (0·777 in p.m.c.). An analysis of variance of the bivalent mean-squares (after square-root transformation) confirms that the five lines studied do indeed differ significantly with respect to the amount of between-bivalent chiasma variation (Table 3). However, as in the case of

Table 3. *Analysis of variance of bivalent mean-squares (after square-root transformation) in p.m.c. and e.m.c. of five rye lines*

Item	*N*	Mean square	Variance ratio	*P*
Lines	4	0·047	22·254	< 0·001
Sexes	1	0·007	3·429	n.s.
Lines × sexes	4	0·002	—	—

mean chiasma frequencies, the between-sexes item is not significant, showing that the amount of between-bivalent chiasma variation is very similar in p.m.c. and e.m.c. of individual lines.

(iii) *Correlation analyses.* In the preceding analyses, both mean chiasma frequencies and bivalent mean-squares show highly significant differences between lines but non-significant amounts of variation between sexes. It follows that these two parameters should be correlated to a high degree over the five lines studied. In Figs. 1 and 2 the male values for these parameters are plotted against the female values and it is clear that pronounced positive relationships exist between the male and female values, which is confirmed in both cases by significant correlation coefficients ($r = 0{\cdot}941$; $P < 0{\cdot}02$ and $0{\cdot}909$; $P < 0{\cdot}05$ respectively).

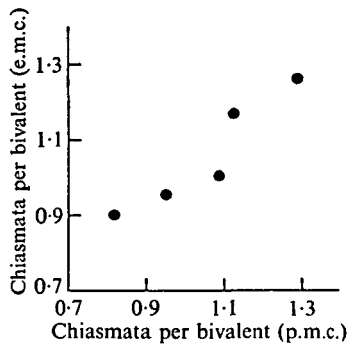

Fig. 1

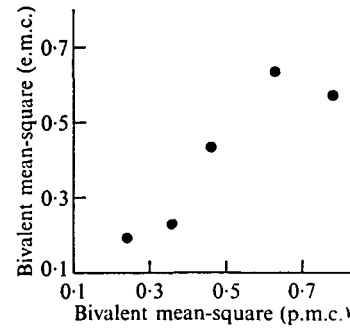

Fig. 2

Figs. 1 and 2. Male (pollen mother cell) values of mean bivalent chiasma frequency (Fig. 1) and bivalent mean-square (Fig. 2) plotted against female (embryo-sac mother cell) values for the five inbred rye lines studied.

4. DISCUSSION

In the relatively few cases where observations have been made on both male and female meiosis, it is unusual to find identical chiasma conditions in the two sexes. Where meiosis in both sexes is chiasmate, it is commoner to find higher mean

chiasma frequencies on the female side (e.g. Pastor & Callan, 1950; Fogwill, 1958; Slizinsky, 1960; Watson & Callan, 1963; Ved Brat, 1969; Vosa, 1972) although the reverse has been found in at least one case (Watson & Callan, 1963). The situation in rye, with identical mean chiasma frequencies and chiasma distributions in the two sexes, is therefore rather unusual but it is by no means unique as similar findings have been reported for lilies (Darlington & La Cour, 1940; Brock, 1954) and barley (Bennet *et al.* 1973).

In the context of chiasma control, the most important finding from this study is the *consistent* absence of a sex difference throughout the five inbred rye lines, despite considerable between-line differences in chiasma frequency and distribution. In other words, genetically determined variation in meiotic behaviour is expressed identically in p.m.c. and e.m.c. The unavoidable conclusion is that meiosis, or at least chiasma formation, in the p.m.c. and e.m.c. of rye is under a single, joint, control. Comparable evidence from other organisms is scarce, with the notable exception of *Drosophila melanogaster*, where the existence of numerous meiotic mutants has enabled a detailed study of this question (Sandler *et al.* 1968). The evidence here indicates that the first meiotic division is separately controlled in the two sexes. Meiotic mutants C(3)G, mei-S51, mei-S282 and mei-S332b all affect the first meiotic division and have pronounced effects on recombination, but are effective only in the female. Another group of mutants (mei-081, mei-58 SD and RD) also affect meiosis I only but are effective only in males. There is evidence that the control systems converge again during the second meiotic division since mei-S332a, which acts during the second division, produces a similar effect in both sexes.

It was stressed in the introduction to this study that the presence or absence of a sex difference in meiosis need not be indicative of the type of control governing meiosis in the two sexes. Nevertheless, the contrasting situations in rye and Drosophila, although admittedly a very small sample, do show a complete correlation of these factors. Drosophila, which displays an extreme form of sex difference with complete achiasmy in the male, also has separate controls governing meiosis I in the two sexes, while rye which has identical chiasma conditions in p.m.c. and e.m.c. has a single joint control governing both sexes. Further studies of this type in a range of different species are clearly needed to determine the extent of this correlation and whether therefore all cases of sex differences in meiosis are indicative of separate meiotic controls in the two sexes, and vice-versa.

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