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Meiotic mutants of rye *Secale cereale* L.

I. Synaptic mutant *sy*-1

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Summary. A mutant form of weedy rye characterized by male and female sterility and having a hereditary block in the chromosome synapsis has been found and described. Genetic analysis has shown the synapsis block to be determined by the recessive allele of a gene designated as *sy-1*. Electron microscopy of surface-spread microsporocyte nuclei revealed the complete absence of the synaptonemal complex over the whole meiotic prophase I, although the axial cores were perfectly formed by each chromosome. Only univalents were observed at metaphase I, their average number ranging from 13.1 to 14.0 per cell. A precocious distribution of univalents at the poles is observed at metaphase I. All of the later stages of meiosis were irregular and resulted in the formation of abnormal microspores. Thus, the mutant proves to be asynaptic because of the blocked initiation of synapses at prophase I.

Key words: Meiotic mutant – *Secale cereale* – Light and electron microscopy – Synaptonemal complex – Genetic analysis

Introduction

A number of dominant genes are known to control normal meiosis in higher plants. The function of these genes became clear after their recessive mutations, which determine various meiotic abnormalities, were studied (Golubovskaya 1979; 1989; Gottschalk and Kaul 1980; Kaul and Murthy 1985).

The synapsis of chromosomes is controlled by numerous genes found in various plant species (Gottschalk and Kaul 1980; Koduru and Rao 1981; Kaul and Murthy 1985). Both terms, asynapsis and desynapsis, are used for the description of abnormalities in chromosome pairing during meiotic prophase I. Mutations which cause a complete or partial block in chromosome pairing at the zygotene-pachytene stages are referred to as asynaptic (*as*), whereas those causing a precocious separation of homologous chromosomes from bivalents and thus inducing the appearance of univalents are termed as desynaptic (*ds*). The distinction between these two types of mutants is undoubtedly important. However it is difficult to study the early stages of meiotic prophase in some species. Bearing this in mind, Riley and Law (1965) suggested that both types of mutants should be designated as synaptic mutants with the symbol *sy*. Some authors have used this system of designation (Iwanaga 1984; Kaul and Murthy 1985).

In this paper we analyze the expression and inheritance of asynapsis in one of the synaptic mutants of diploid rye *Secale cereale*. This is the first case in which a true asynpatic mutant of this species has been described.

Materials and methods

A spontaneous sterile synaptic mutant *sy-1* was found in the F_2 progeny from the hybrid F_1 plant obtained in a cross between the plant from a weedy rye population and a self-fertile inbred line F-1 a. In the progeny which segregated male sterile plants several fertile plants were self-fertilized. In some of the lines obtained, sterile plants with abnormal meiosis segregated again. Meiosis was studied in sterile and fertile plants from the two succeeding generations of inbreeding, I_2 and I_3. To study meiosis by means of the light microscope, squash preparations were made from anthers fixed with Newcomer fluid. The preparations were stained with acetocarmine.

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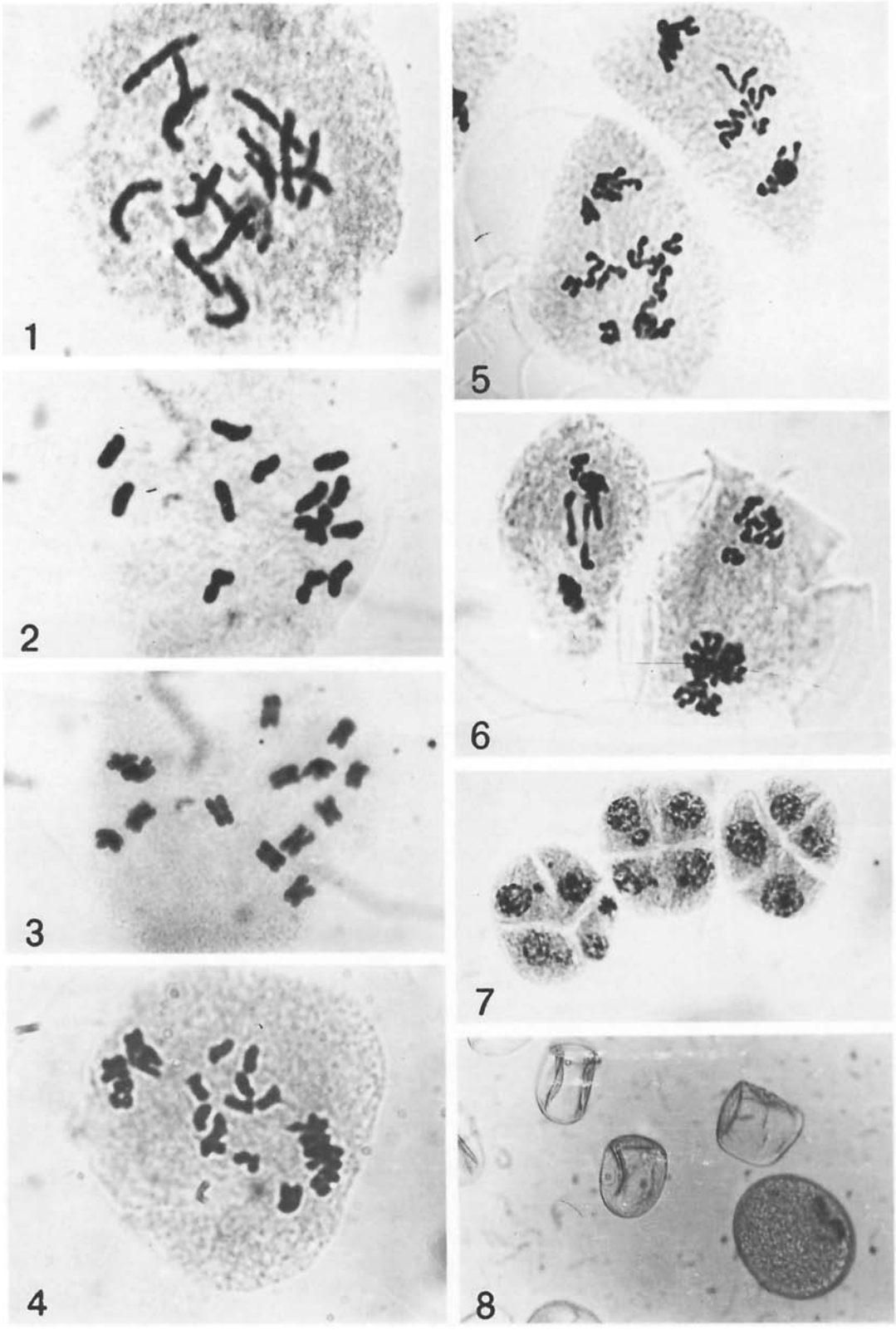

Figs. 1–8. Light micrographs of the microsporocytes of rye mutant *sy-1*. **Fig. 1** Diakinesis with 14 univalents. **Fig. 2** Metaphase I. Polar distribution of 10 univalents (2+8) and 4 univalents in the center. **Fig. 3** Anaphase I. Chromatids in each univalent are easily observed. **Fig. 4** Anaphase I. Equational division of 5 univalents. **Fig. 5** Anaphase II. Several chromosomes are seen to be lagging in each of the two cells. **Fig. 6** Anaphase II and telophase II. **Fig. 7** Tetrads of microspores with nuclei of different sizes and micronuclei. **Fig. 8** Sterile pollen grains in anthers of *sy-1* plant

To obtain the surface spreads of meiotic nuclei for electron microscopy, anthers from the plant were placed in a cold isolation medium (Gillies 1981) (Eagle's medium to which 0.1% bovine serum albumin and 2 m*M* EDTA were added; pH was adjusted to 7.7 with 0.5 *M* NaOH) and sectioned with a razor blade. Their content was squeezed out with forceps. The cell suspension obtained was homogenized with a pipette and dropped onto a hypophase (0.2 *M* sucrose solution on a paraffin-polished glass). After 1.5–2 min, the spread nuclei were picked up by touching the hypophase surface with a glass slide precoated with a plastic film (a 0.5–0.7% solution of Falcon Optilux petri dish dissolved in chloroform). A uniformly distributed suspension was fixed in formalin vapor in Petri dishes and placed in the refrigerator for several hours. The slides were subsequently air-dried for at least 3 days; washed in distilled water and stained at 60 °C for 2–4 h with a 70% solution of $AgNO_3$ in distilled water adjusted to pH 4.5 with HNO_3 (Fedotova et al. 1989a, b). Preparations containing well-spread, stained nuclei were scanned under the light microscope. Film areas containing the spread nuclei were cut out and put onto single-hole grids. The spreads were then examined under the JEM 100B electron microscope.

Inheritance of meiotic abnormality was studied in two ways: (1) in the segregating inbred progenies and (2) in progenies of the hybrids obtained by crossing fertile plants from the segregating inbred lines with a self-fertile inbred line, Ky-2/63, from the genetic stock collection available at the Laboratory of Plant Genetics, Biological Institute of the University of St. Petersburg.

Results

Light microscopy

Fertile plants from the segregating progenies were characterized by normal, regular meiosis. The number of chiasmata per bivalent at diakinesis ranged from 2.03 to 2.27, and the number of occasional univalents per cell at metaphase I varied in different plants from 0 to 0.19. Later stages of meiosis also showed only occasional abnormalities. These fertile plants had a relatively high seed set by both the open and self-pollination (Sosnikhina et al. 1989).

Sterile plants were found to have only sterile pollen. Since these plants did not set any seeds either in self- or open pollination and, thus, were also female sterile, we suggest that meiosis in megasporogenesis was also abnormal. Meiosis in the anthers of sterile plants appeared to be abnormal even at early prophase I. We observed a number of the pachytene nuclei with numerous chromosomal asynpatic regions at this stage, although it was impossible to perform a detailed light microscopic analysis. At diplotene and diakinesis, complete asynapsis was observed in the sterile plants studied (Fig. 1).

At metaphase I, the chromosomes were mostly univalent. Only univalents could be seen in 94–100% of the cells from 9 mutant plants, obtained from the inbred progenies; the remaining cells contained a few open bivalents. The mean number of bivalents per cell varied from 0 to 0.06. The univalents were not organized at the equatorial plate but rather became distributed into two groups in the regions of the poles (Fig. 2). Only occasional univalents remained at the center of the cell at this stage. The frequency of cells with different distributions of univalents at the poles was evaluated in mutant plants (Fig. 9). The most frequent distributions observed were 8:6 (28.1%) and 9:5 (20.8%). Cells with several univalents at the center of the cell (20%) were observed relatively frequently. Thus, the non-uniform distribution of chromosomes at the poles occurs at metaphase I.

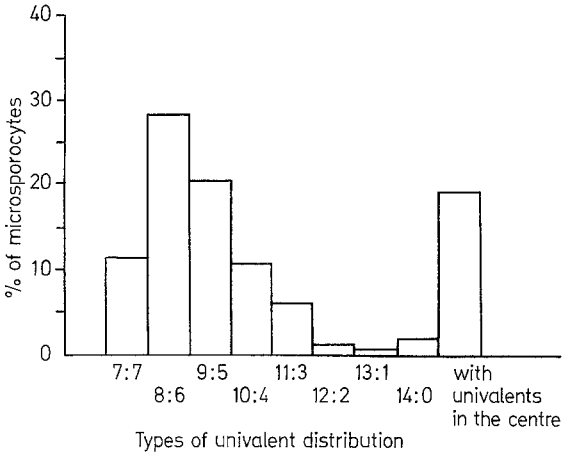

Fig. 9. Frequencies of microsporocytes at metaphase I with different types of univalent distribution in *sy*-1 mutant

At anaphase I cromatids were easily observed (Fig. 3). The number of chromosomes at the two poles was unequal in almost, all of the cells; only occasional cells had a regular 7:7 distribution (0.92%). In different mutant plants the percentage of cells at anaphase I in which some of the chromosomes remained at the center of the cell and may eventually divide equationally (Fig. 4) varied from 2% to 21%. The total frequency of cells at anaphase I with equational division of some chromosomes (at the center and/or at the poles) varied from 33% to 62% in different mutant plants. Multipolar anaphases I were also relatively frequent (from 3.9% to 17.0% in different mutant plants).

Dyads with unequal nuclei size of as well as triads, tetrads and monads (4–10%) and three-nuclear cells (48.5%) were observed at telophase I. Of the cells 33–75% were found to have micronuclei.

As a rule, almost all of the cells at metaphase II were abnormal; only occasional pairs of sister cells contained seven chromosomes each. In some plants 20–25% of the dyads formed one common metaphase plate at metaphase II. This may result in the formation of unreduced spores. However, we failed to find unusually large pollen grains.

Anaphases II were completely abnormal: most of the cells differed in their number of chromosomes; some cells differed in the axis of division; up to 50% of the cells had

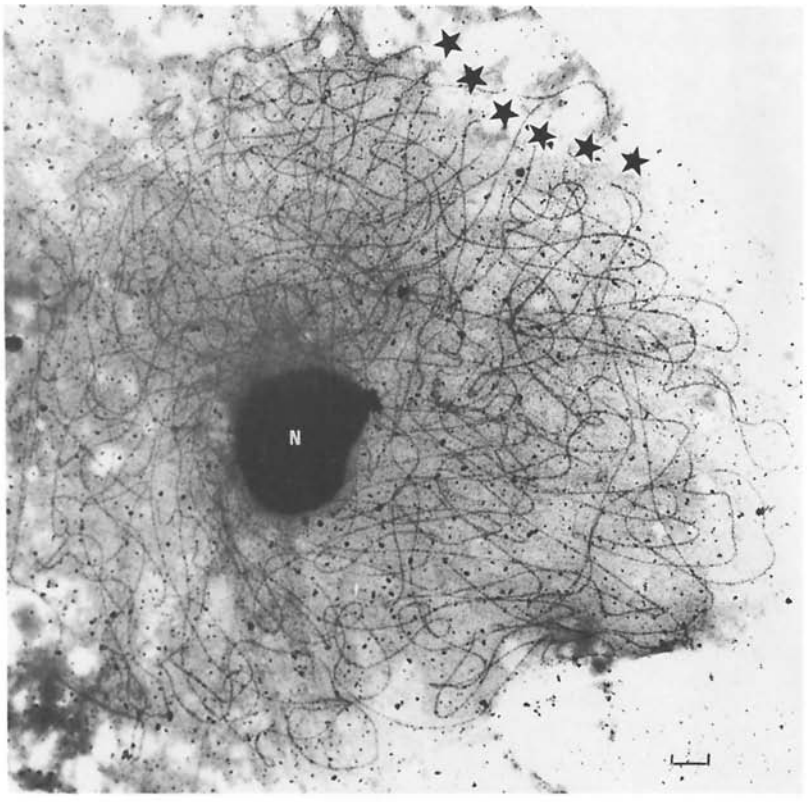

Fig. 10. Electron micrographs of the surface-spread microsporocyte of mutant *sy-1*. This stage is analogous to zygotene: telomeres of the axial elements are located in the restricted part of the nucleus (denoted by darkened stars), and axial elements show "the bouquet" configuration. No synapsis initiation was found. *N* Nucleolus. *Bar:* 1 μm

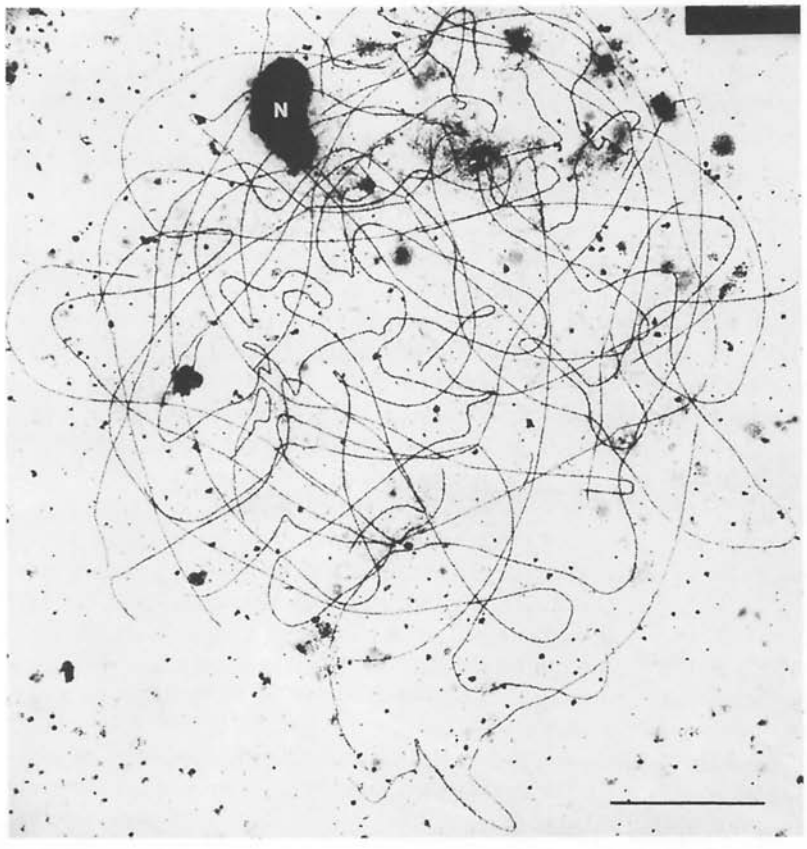

Fig. 11. This stage is analogous to pachytene. Axial elements of univalents are visible, but no synapsis is recognizable. *Bar*: 10 μm

lagged chromosomes (Fig. 5). In some plants the separating cell walls were not revealed in up to 16% of the dyads at anaphase II and as a result the dyads had a common spindle for both cells.

At telophase II, nuclei of unequal size and micronuclei were observed at a high frequency. At the end of meiosis abnormal tetrads had been formed. They contained nuclei of different sizes and micronuclei (Fig. 7). Polyads (5–80% in different plants), dyads (13.1%), and monads (4.9%) were also observed at this stage. All these result in the formation of sterile, empty pollen grains (Fig. 8).

Electron microscopy

Sixty-four meiocytes from 9 fertile plants and 183 meiocytes from 17 sterile plants were studied.

By the end of leptotene, axial elements of the chromosomes were generally formed in the nuclei of pollen mother cells. The leptotene stage in the *sy-1* plants did not differ from that typical of normal plants. In the mutant plants stage was observed that we considered to be analogous to zygotene on the basis of the formation of a typical "bouquet" of axial elements (Fig. 10). However, as seen in Fig. 10, no signs of synapsis between the axial elements were observed at this stage, and, therefore, no synaptonemal complexes (SCs) were formed.

The next stage, analogous to pachytene, was identified on the basis of the behavior of the telomeres of the axial elements: they are distributed over the nucleus in the same way as telomeres of the SCs are distributed at the pachytene stage in normal plants. Only the axial elements of univalents were still revealed (Fig. 11). At the stages analogous to zygotene and pachytene, hooks, entanglements, and breaks in the axial elements were observed in the nuclei of the mutant cells. Sometimes axial elements formed short hairpin-like configurations. We failed to find any SCs in such "hairpins".

At the stages analogous to diplotene, the axial elements started to degrade, and many fragments of axial elements appeared (Fig. 12). None of the coiled unpaired axial elements found at the diplotene stage in normal plants were seen in the mutant plants.

In conclusion, no signs of SCs were observed during the whole meiotic prophase I in the mutant *sy-1* plants. This led us to a conclusion that the mutant is a true asynaptic one.

Inheritance

In the inbred progenies segregating sterile plants several plants with normal fertility were selfed. In some of these progenies sterile plants were found to segregate again. A study of meiosis in sterile plants revealed them to be asynpatic (Table 1). The ratio of plants with normal meiosis to those with asynapsis fitted a monogenic 3:1 ratio ($P>0.50$). Segregations in all of the progenies under study were found to be homogeneous ($\chi^2=3.66$; $P>0.30$). Thus, we conclude that this meiotic abnormality is determined by homozygosity for the recessive allele of the gene designated as *sy-1*. This conclusion was also supported by our study of the crosses between plants with normal meiosis from the segregating I family (N 655) and those of the self-fertile line *Ky-2/63*. All the F_1

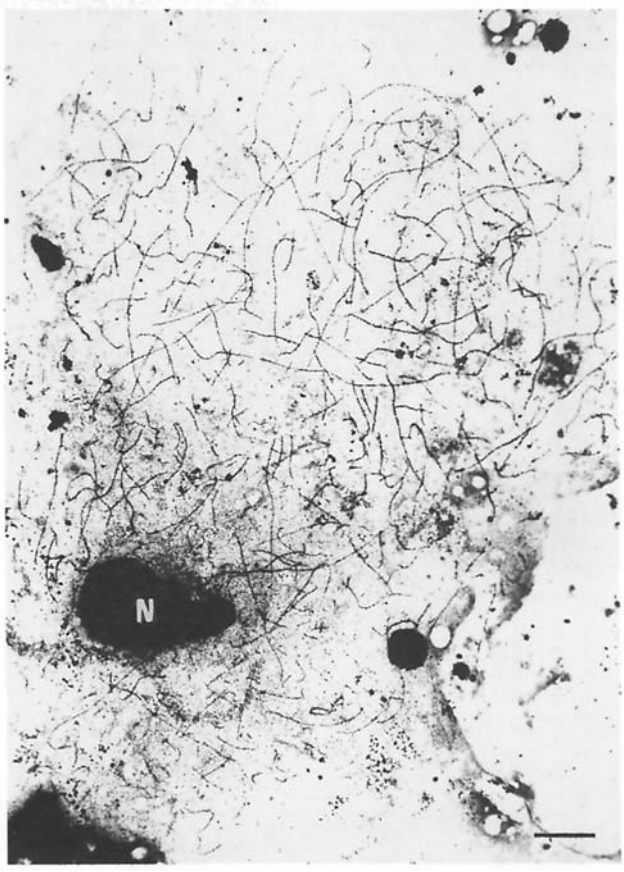

Fig. 12. A fragment of the nucleus at the stage analogous to diplotene. Remnants of unpaired axial elements are visible. *Bar:* 2 μm

Table 1. Segregation of phenotypes with normal and asynaptic meiosis in the inbred progenies

Progeny number	Generation of inbreeding	Number of plants	
		Normal	Asynaptic
406	I_2	5	1
453	I_2	7	5
483	I_3	6	4
653	I_3	6	2
655	I_3	15	3
Total		39	15

Table 2. Segregation of fertility and type of meiosis in the self-pollinated F_2 progenies of the F_1 hybrids between heterozygous plants *Sy1/sy1* and self-fertile line Ky-2/63 (*Sy1/Sy1*)

Progeny number	Individual number of heterozygous F_1 plant	Number of F_2 plants		Probability of a 3:1 ratio	Number of F_2 plants cytologically observed	
		Fertile	Sterile		Normal	Asynaptic
255	655/1	18	8	0.45	2	3
256	655/1	23	2	0.02	13	3
257	655/1	21	7	1.00	8	1
258	655/1	23	9	0.50	5	2
259	655/1	21	5	0.45	4	1
260	655/1	25	8	0.90	5	2
261	655/1	23	9	0.50	6	2
Total		154	48	0.50	43	14
269	655/11	19	9	0.30	7	1
271	655/11	18	5	0.70	3	0
272	655/11	26	7	0.50	3	1
Total		63	21	1.00	13	2
279	655/13	16	4	0.50	–[a]	–
280	655/13	19	7	0.80	–	–
Total		35	11	0.80	–	–
283	655/14	15	6	0.50	–	–
290	655/22	26	6	0.30	–	–
Grand total		293	92	0.50	56	16

[a] Not observed

plants were found to be fertile; these were subsequently selfed, and in some of the F_1 inbred progenies the sterile plants segregated (Table 2). Segregation in each inbred progeny as well as that taken in total fitted a monogenic 3:1 ratio, the sterile plants being homozygous recessive. The segregation into fertile and sterile plants was homogeneous in all of the 14 progenies analyzed ($\chi^2 = 7.30$; $P > 0.80$). Meiosis was studied in 73 of the 385 F_1 plants, and sterile plants were confirmed to be asynpatic. The ratio of plants with normal meiosis to that of the asynaptic ones also fitted a monogenic (3:1) ratio, and the segregation ratios were homogeneous in all of the progenies studied ($\chi^2 = 5.48$; $P > 0.50$).

Discussion

The characteristics of several asynaptic mutants described in the literature suggest that these mutants have a block in initiating the synapsis and SC formation and, as a result, there is a suppression of crossing over (Kaul and Murthy 1985). On the basis of a study of pachytene by means of light microscopy, Gottschalk and Kaul (1980) described mutations that cause complete or partial synapsis. The latter is normally characterized by a low degree of chromosome synapsis at pachytene.

An "asynaptic" mutant in rye *S. cereale* studied under the light microscope by Prakken (1943) has to be qualified, according to recent terminology, as a desynaptic one: all the chromosomes were paired at pachytene, but thereafter, at diakinesis, three to five pairs were precociously dissociated into univalents.

The meiotic abnormality in rye described in the present paper is true asynapsis. This is proven by the absence of chromosome synapsis at pachytene and also by a very high frequency of univalents at diplotene, diakinesis, and metaphase I (13.1 to 14 per cell). The mutant plants practically do not form bivalents. Our data show that asynapsis is determined by a homozygous state of the recessive allele of the *Sy-1* gene. However, since our previous data (Sosnikhina et al. 1989) showed that occasional mutant plants appeared in segregating progenies from bivalents at a somewhat higher frequency (such as 2.3 per cell), some other genes may modify the expression of the *sy-1* allele.

For a detailed study of meiotic prophase I in normal and mutant plants of rye we used the surface microspreading technique, which is commonly applied for investigating the synaptonemal complex (Gillies 1981, 1985; Hasenkampf 1984; Holm 1986). Our observations concerning formation of the SC in normal plants are in good agreement with data reported earlier in rye (Gillies 1985).

We found that in all of the rye plants studied (both normal plants and mutants) meiotic prophase I begins with a similar pattern, namely, with the formation of axial elements in unpaired chromosomes at leptotene. However, as meiotic prophase I proceeds, normal plants and the *sy-1* mutants begin to be significantly different: the axial elements of homologous chromosomes do not participate in the SC formation in *sy-1*. Moreover, no synapsis initiation sites are observed throughout the entire meiotic prophase I, which is especially obvious in the surface spreads (Figs. 10 and 11).

Similar results were obtained in the *Triticum durum* asynaptic mutant when prophase I microsporocytes were studied in ultrathin sections (La Cour and Wells 1970). In addition to unpaired axial cores, La Cour and Wells found abnormal polycomplexes in the late prophase I microsporocytes of the mutant. These polycomplexes lacked the central element, and the conclusion was drawn that this element was not assembled in the mutants (La Cour and Wells 1970). A similar conclusion can be drawn with respect to the possible cause of asynapsis in the rye mutant studied here, though no structures resembling polycomplexes were found in the spread cells. It is also possible that the asynaptic mutants lacked some specific molecular factor responsible for the initiation of synapsis. Another possibility is that the fine structure of axial elements in the asynaptics may be changed significantly, preventing the formation of "microcomplexes"

analogous to "the association sites" observed in the triploid *Allium*. According to some authors, these association sites are important for the initiation of synapsis and for SC formation in diploids (Loidl and Jones 1986). These "microcomplexes" probably appear to be a complex of axial elements with some factor involved in the synapsis.

At the stage corresponding to diplotene, some specific events probably occur in the *sy-1* mutant that distinguish it from the normal plants and also from some of the other synaptic mutants (*sy-3* and *sy-9*) examined by us (Fedotova et al. 1989a). It is only in this mutant that we have failed to find any coiling of the unpaired lateral elements at diplotene (compare Fig. 12 with Figs. 2–4 in Fedotova et al. 1989b).

The results of our cytological investigation of microsporogenesis in the mutant plants studied here show that their sterility is caused by the abnormalities that occur in meiosis, such as the absence of chromosome synapsis at prophase I and the inability to form SCs. Some of the abnormalities seen at other stages probably result from this asynapsis.

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