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Synaptic mutant affecting only megasporogenesis in potatoes

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THE TERMS asynapsis and desynapsis have been used to describe pairing failure during meiotic prophase. Mutant genes that influence the initial pairing of chromosomes are referred to as asynaptic, whereas those that alter the maintenance of pairing between synapsed chromosomes are designated desynaptic. To differentiate between these two conditions, detailed analyses of early prophase stages are required in conjunction with analyses of later stages of the first meiotic division. Although this distinction is clearly important, it is often difficult in practice, since early prophase stages of meiosis are often indistinct and unresolvable in many species.

Either asynapsis or desynapsis are often applied to mutants that produce elevated frequencies of univalents at metaphase I without paying specific attention to the importance of the distinction between them. The situation is further complicated, since the terms asynaptic and desynaptic have not been used consistently. Soost[23] recognized the difficulty of making a cytological distinction between the two, and suggested that asynapsis be used in a general sense to indicate a lack of pairing during any stage of the first meiotic division. Thus, the name of a mutant does not necessarily indicate the nature of the pairing defect. Riley and Law[21] proposed an alternative term, synaptic, to describe the activities of major genes that influence the extent of meiotic pairing. In accord with their proposal, synaptic is used in this report to describe a meiotic mutant that results in univalents at diakinesis and subsequent abnormal chromosome distribution during later stages of megasporogenesis in some diploid ($2n = 2x = 24$) potatoes.

Some potato species produce $2n$ gametes that function in tetraploid ($2n = 4x = 48$) × diploid ($2n = 2x = 24$) crosses, resulting in mainly tetraploid offspring[5]. The cytological mechanisms of $2n$ pollen formation have been described[14]. The important genetic consequence is that $2n$ gametes can be highly heterozygous when they are produced by a mechanism genetically equivalent to first division restitution (FDR) and highly homozygous when they are a result of second division restitution (SDR). The importance of FDR $2n$ gametes in breeding and evolution has been discussed[9,11,15,19].

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Although superiority of FDR $2n$ eggs in potato breeding has been indicated[12], no cytological results in regard to the origin of $2n$ eggs have been reported. Genetic approaches, however, have been used in attempts to detect the mode of $2n$ egg formation. Ross and Langton[22] reported the possible origin of FDR $2n$ eggs based on segregation for virus resistance in progenies from diploid ($Rxrx$) × tetraploid ($rxrxrxrx$) crosses. Their reasoning, however, was circular since they did not know the location of the gene Rx (virus × resistance) in relation to the centromere. In one clone of *Solanum tuberosum* group Andigena ($2n = 4x = 48$), Taylor[25] reported that the tetraploid parthenogenetic plants may be produced by a mechanism equivalent to SDR.

A synaptic mutant affecting only megasporogenesis was found in the course of our studies of megasporogenesis in some diploid potatoes that produce $2n$ eggs. This paper describes megasporogenesis of the synaptic mutant and discusses its possible relation with FDR $2n$ egg formation.

Materials and Methods

The synaptic mutants were found among progeny from the cross W5295.7 (I) × W5337.3 (J), where both I and J are diploids ($2n = 2x = 24$) derived from crosses between group Phureja and haploid group Tuberosum. Ovaries from two mutant clones, IJ-31 and IJ-32, were used for cytological observation of megasporogenesis.

Young ovaries were fixed in CRAF V, embedded in paraplast, and sectioned at 10–15 μ. The sectioned materials were stained with a rapid safranin-crystal violet-light green staining sequence[3].

Results

Inheritance of synaptic mutant

The mutant appears to be inherited as a simple Mendelian recessive. Preliminary findings of discrete segregation among hybrids from I × J crosses suggested that the synaptic abnormality was controlled genetically. Since both parents have normal synapsis, the production of hybrid progeny that are synaptic mutants suggests that a recessive gene controls the abnormality. To test this hypothesis additional progeny from crossing I × J were obtained. Cytological analysis of megasporogenesis in 16 hybrids indicated that 12 were normal and 4 were mutant. The data fit a 3:1 ratio, and are thus compatible with the hypothesis that the synaptic abnormality is probably conditioned by a single recessive allele, designated as *sy*.

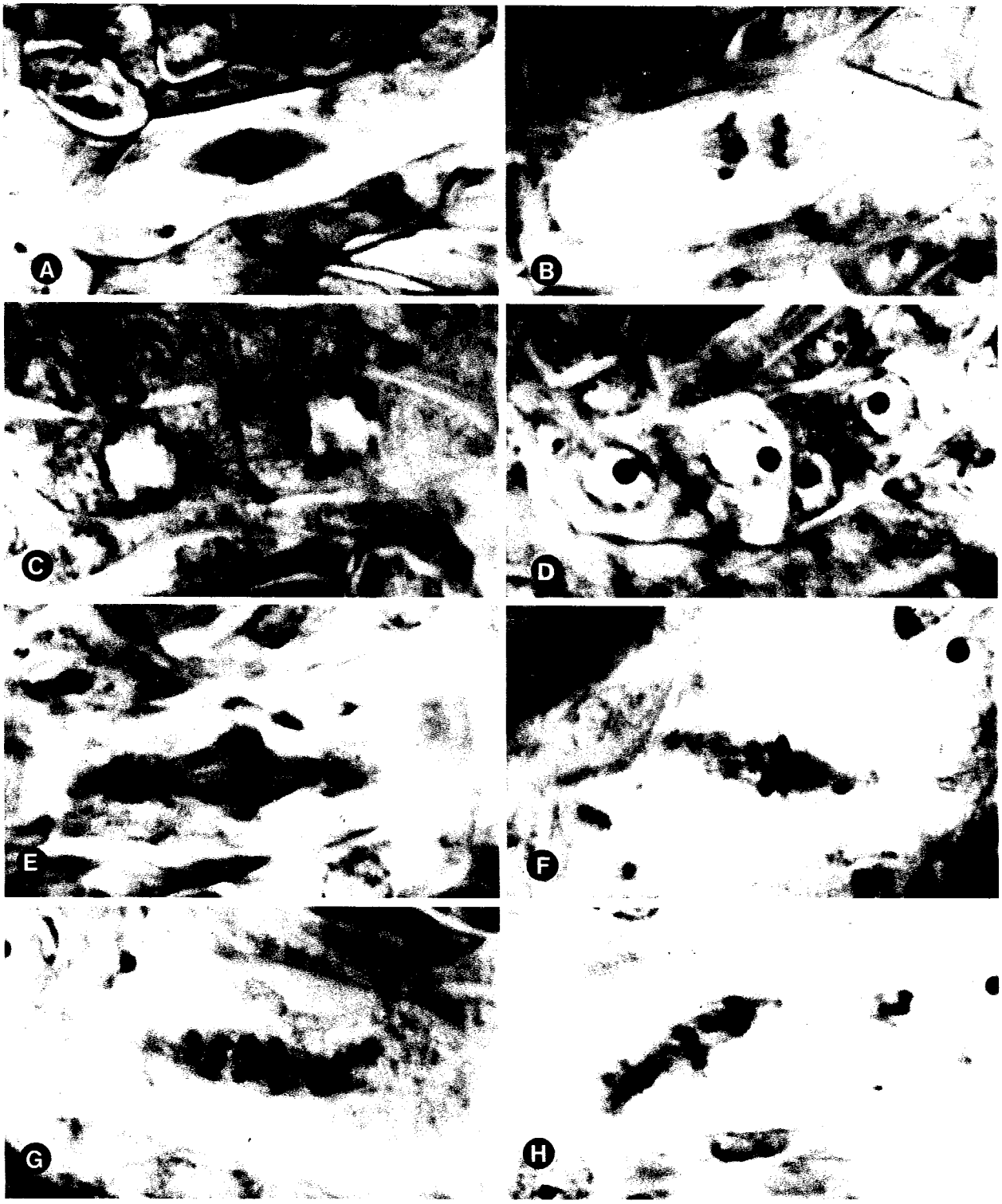

FIGURE 1—Megasporogenesis in normal and synaptic mutant plants. *A–D*—megasporogenesis in a normal plant: *A*—metaphase I; *B*—anaphase I; *C*—telophase I; *D*—telophase II. *E–H*—megasporogenesis in a synaptic mutant: *E* and *F*—metaphase I; *G* and *H*—anaphase I.

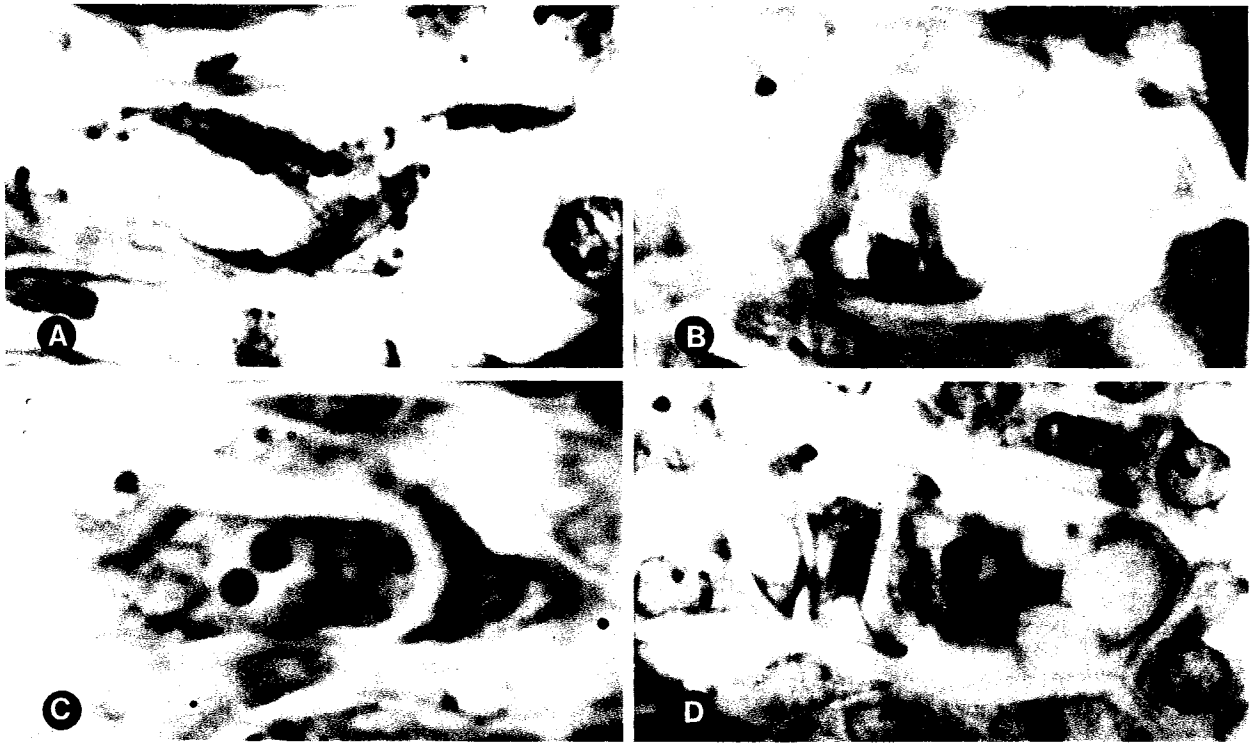

FIGURE 2—Megasporogenesis in a synaptic mutant. *A*—anaphase I; *B*—restitution nucleus; *C*—dyad at tetrad stage; *D*—triad at tetrad stage.

Cytology

Normal megasporogenesis: Although megasporogenesis in nonmutant I × J hybrids follows a normal pattern for potatoes, a brief description of the normal sequence is included so that the distinctive cytological manifestation of the synaptic mutant can be clearly appreciated. At metaphase I the axis of the spindle is parallel with the long axis of the megaspore mother cell (Figure 1*A*). Chromosome movement to each pole at anaphase I usually is synchronized (Figure 1*B*), but irregularities occur at a low frequency. A cell plate is formed at telophase I dividing the megaspore mother cell into two daughter cells of approximately equal size (Figure 1*C*). After telophase II (Figure 1*D*) a linear tetrad of megaspores is formed; the chalazal megaspore of the linear tetrad becomes the functional megaspore.

Megasporogenesis of the sy homozygotes: Whether the univalents of postdiplotene stages are due to asynapsis or desynapsis is not known, since early prophase stages are not amenable to cytological analysis in paraffin sections. Therefore, the term synaptic rather than asynaptic or desynaptic more appropriately describes the present mutant.

Meiotic abnormalities of the synaptic mutant were first detected at diakinesis. More than 12 chromosome bodies were present at this stage. From the size and structural differences of the chromosome bodies, some appeared to be univalents. At metaphase I univalents, precocious chromatid separation and unoriented bivalents were sometimes observed (Figure 1*E* and *F*). Accurate scoring of the frequencies of univalents at either diakinesis or metaphase I was not possible. Abnormalities at anaphase I (Figures 1*G* and 2*A*), included elongated, curved and multiple spindles, lagging univalents, dividing univalents, and bivalents going to one pole. Extreme abnormalities like those shown in Figures 1*H* and 2*A* were rather exceptional; most abnormalities were less drastic (Figure 1*G*). Restitution nucleus fromation was found in one meiocyte (Figure 2*B*). If a normal second division follows the restitution nucleus formation, first division restitution (FDR) 2*n* eggs could be formed. As a result of abnormal chromosome distribution at anaphase I, micronuclei were often formed, so that the number and size of nuclei at the end of meiosis varies (Figure 2*C* and *D*). Moreover, cytokinesis was so irregular that most of megaspore mother cells do not generate normal linear tetrads (Figure 2*C* and *D*). These observations suggest that female fertility of *sy* plants should be very low when crossed with diploids.

Whether or not the second meiotic division occurred was not determined. Only two figures of the second division were found in the mutants. Since even in normal plants the second division was rarely found, the rare observation of second division in *sy* plants was either due to an effect of *sy* or was simply due to the difficulty of finding the second division.

The expressivity of *sy* was evaluated from the frequency of meiocytes with abnormal chromosome movement such as precocious anaphase chromosome movement, unoriented bivalents at metaphase I and at anaphase I. Since the transition from metaphase I to anaphase I was not always clear in *sy* plants, the scoring for the presence of abnormalities was made without separating these two stages. Results with three mutant clones

along with normal plants are given in Table I. The frequency of abnormalities at metaphase I and anaphase I varied from 93 to 99 percent in synaptic mutants, and was only 16 percent in normal plants. The expressivity of *sy* was almost complete, since very few megasporocytes with normal meiosis were found in the synaptic mutants.

The expression of *sy* was specific to megasporogenesis, since microsporogenesis in IJ-31 and IJ-32 was normal as far as synapsis was concerned. According to Mok[13], however, these clones produced 2*n* pollen; their genotypes being *psps pclpcl* and *psps Pcl*–, respectively, in regard to the parallel spindle and premature cytokinesis loci. It can be argued that *sy* was not expressed in microsporogenesis simply due to the existence of another mutant gene, i.e., *ps* or *pcl*. These mutants, however, are expressed after anaphase I. Therefore the effects of *sy*, which were expressed as early as diakensis in megasporogenesis, would be detected even in the presence of *ps* or *pcl*. Thus, it appears that *sy* was affecting only megasporogenesis.

Discussion

Most of the meiotic abnormalities found in *sy* have been reported in other synaptic mutants. In a recent review, Baker et al.[1] reported that in addition to the univalents by which mutants were characterized, most synaptic mutants also exhibited elongated or multiple spindles, failure of congression, lagging univalents, precocious chromatid separation, and the formation of micronuclei and/or restitution nuclei. It appears that prolongation of metaphase I and/or anaphase I occurred in *sy* plants. Within any single ovary of normal plants only a few meiocytes were at metaphase I or anaphase I, whereas in *sy* plants more than 20 meiocytes per ovary at these stages were found. The high frequency of meiocytes at metaphase I and anaphase I suggests that development could be arrested at these stages. Wagenaar[26] related poor pairing to prolongation of meiosis in F_1 hybrids between *Triticum crassum* and *T. turgidum*. He also suggested that restitution nucleus formation was probably due to prolonged metaphase I and lagging chromosomes during anaphase I.

This is the first report to describe cytological abnormalities in megasporogenesis due to a synaptic mutant. Although there have been many reports of synaptic mutants (see reviews by Katayama[7] and Baker, et al.[1]), only a few[2,20,23] made brief comments on the effect of synaptic mutants on megasporogenesis. When a synaptic mutant has reduced female fertility, it generally has been assumed that the synaptic mutant is expressed in megasporogenesis also, but no cytological evidence was provided. There are several reasons why cytological studies have been restricted to microsporogenesis. One is the technical difficulties of observing megasporogenesis that usually needs to be done by time-consuming paraffin sections. Even after the tedious work, it is not easy to determine the effects of a mutant allele. For example, it is impossible in most species to either score the frequency of univalents at metaphase I, or to determine if the univalency is due to asynapsis or desynapsis. Therefore, it has been acknowledged that megasporogenesis is not appropriate for studying the effects of synaptic mutants on meiosis. However, synaptic mutants affecting megasporogenesis will become important in relation to their genetic consequences (i.e., 2*n* gamete formation and transmission of extra chromosomes), since it is probable that their expression will not always be identical in micro- and megasporogenesis.

The *sy* allele is unique among synaptic mutants in that it is the first example where the effect is expressed in megasporogenesis but not in microsporogenesis. A reciprocal situation was reported in maize[17], where a desynaptic mutant affected microsporogenesis but not megasporogenesis. Reasons for the differential effect with respect to sex are unknown.

It is possible that megasporogenesis in *sy* homozygotes might result in FDR 2*n* egg formation. Clones IJ-31, IJ-32, and AI-18 (normal synapsis), which are heterozygous for the yellow flesh gene, *Y*, were crossed as female with nulliplex tetraploids to detect the mode of 2*n* egg formation in diploid female parents by half-tetrad analysis[10]. Since the gene *Y* is about 13 map units from the centromere[16], the expected frequencies of white flesh progeny (*yyyy*) are 6.5 percent and 37 percent for FDR and SDR, respectively. The combined results obtained by our group[13,18] and the present authors is as follows. Among 50 individuals from IJ-31 × tetraploids, 4 percent had white flesh, while 8 percent of 108 progeny from IJ-32 × tetraploids had white flesh. These initial results indicate that IJ-31 and IJ-32 could produce 2*n* eggs by FDR. On the other hand, AI-18, which has normal synapsis, appears to produce 2*n* eggs by SDR, since 40 percent of the progeny have white flesh. In addition to the genetic results, the observation of first division restitution (Figure 2*B*), the almost complete expressivity of *sy*, and the finding of no other abnormality related to 2*n* egg formation in *sy* plants suggests that the synaptic abnormality might result in FDR 2n egg formation. However, more cytological evidence is needed to establish the mode of 2n egg formation.

Several reports indicate first division restitution can follow synaptic abnormalities. In *Allium amplectens*[8] complete lack of metaphase pairing, formation of restitution nuclei at the end of anaphase I, and equational division of the chromosomes at the second division results in balanced pollen dyads. Hickok[6], working on a synpatic mutant in the fern *Ceratopteris*, found that failure of bivalent formation at metaphase I frequently led to the formation of restitution nuclei, normal second division, and the production of a dyad of diploid spores. These reports support our extrapolation that FDR 2*n* eggs are formed in the ovaries of *sy* homozygotes.

Based on the results of half-tetrad analysis from 2*x* × 4*x* crosses, it is possible to speculate whether the ac-

Table I. Frequencies of abnormal meiocytes at metaphase I or anaphase I in three synaptic mutant clones and normal plants

Clone	No. meiocytes observed	No. meiocytes with abnormalities	Percentage of abnormal meiocytes
IJ-31	97	96	99
IJ-32	100	93	93
JI-66	22	21	95
Total	219	210	96
Normal plants	50	8	16

tion of *sy* is asynaptic or desynaptic. If FDR 2n eggs are formed following complete asynapsis, no nulliplex progeny are expected. On the other hand, if FDR 2*n* eggs are formed following desynapsis after normal crossing over, 6.5 percent nulliplex progeny are expected. The recovery of 4 percent and 8 percent nulliplex progeny in the two families suggests *sy* is desynaptic, but larger families are needed to verify this.

The synaptic mutant is significant from several standpoints; 1) this is the first report to describe cytologically an inherited synaptic abnormality in megasporogenesis, 2) it is an example of a synaptic mutant that has an effect on megasporogenesis but not microsporogenesis, 3) *sy* might be useful as a source of aneuploids, and perhaps most important to us 4) abnormal synapsis may be followed by first division restitution resulting in FDR 2*n* egg formation.

We emphasize the desirability of searching for meiotic mutants that affect meiosis so that 100 percent of the heterozygosity of the parent is transmitted to the progeny. The following cytological situations are expected to produce such 2*n* gametes: 1) completely asynaptic univalents divide equationally at anaphase I to produce symmetrical dyads that mature into functional spores without undergoing a second meiotic division as described in the asynaptic mutant affecting microsporogenesis in *Brassica campestris*[24]; 2) a similarly desirable situation results from complete asynapsis at prophase I, the formation of restitution nuclei at anaphase I and equational division of the chromosomes at anaphase II resulting in balanced 2*n* gametes as reported in an asynaptic mutant affecting microsporogenesis in *Allium*[8]; and 3) in megasporogenesis, apospory or diplospory with no bivalent formation, as reported in some apomictic species[4] would be equally advantageous. By using these modes of 2*n* gamete formation, along with *ps* (FDR) and *pc* (SDR)[14], it should become feasible to manipulate the proportion of heterozygosity to be transmitted and the amount of variation in the progeny.

Summary

A synaptic mutant was found among progeny from the cross W5295.7 (I) × W5337.3 (J), where both I and J are diploid potatoes ($2n = 2x = 24$) derived from crosses between group Phureja and haploid group Tuberosum. The mutant is inherited as a simple Mendelian recessive, designated *sy*. Meiotic abnormalities of the mutant during megasporogenesis include univalents at diakinesis; univalents, unoriented bivalents, and precocious chromatid separation at metaphase I; elongated, curved, and multiple spindles; abnormal chromosome distribution at anaphase I; restitution nucleus formation at telophase I; and abnormal cytokinesis at the tetrad stage. The expression of *sy* is specific to megasporogenesis, since normal synapsis occurs in microsporogenesis. It is possible that some of the abnormalities in megasporogenesis could result in first division restitution (FDR) 2*n* egg formation. Two mutant clones that are heterozygous for the yellow flesh gene (*Yy*) were crossed as females with nulliplex (*yyyy*) tetraploids to detect the mode of 2*n* egg formation by half-tetrad analysis. Initial results indicate that the clones produced 2*n* eggs by FDR. The *sy* mutant is the first report of an inherited synaptic abnormality in megasporogenesis; it is valuable as a source of both aneuploids and 2*n* eggs for genetic and breeding investigations.

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