

# A Cytological Study of F<sub>1</sub> Hybrids Between Swiss Chard and Beta Webbiana<sup>1</sup>

HELEN SAVITSKY<sup>2</sup> AND JOHN O. GASKILL<sup>3</sup>

## Introduction

The three species comprising the section *Patellares* Tr. of the genus *Beta* L. (26)<sup>4</sup>, *B. procumbens* Chr. Sm. (13, 21, 22), *B. webbiana* Moq. (1, 5, 22), and *B. patellaris* Moq. (22) are considered as potential sources of certain valuable characters not known to occur in the sugar beet (*B. vulgaris* L.). Of special importance among those characters are high resistance to leaf spot (*Cercospora beticola* Sacc.) and to sugar beet nematode (*Heterodera schachtii* Schmidt) (7, 12, 27).

Attempts to transfer genes from the section *Patellares* directly to the sugar beet have failed consistently, the F<sub>1</sub> hybrids usually dying while very small seedlings (27). Recent reports by two workers have shown that viable hybrids can be obtained rather readily from matings of *B. procumbens* and *B. webbiana* with Swiss chard (*B. vulgaris* *cicla* L.) (19) and with certain wild species of the section *Vulgares* T.<sup>2</sup> (9, 23). Since Swiss chard and the latter wild species are compatible with sugar beets, it is hoped that such material may be made to serve as a bridge for transfer of desirable genes from the section *Patellares* to the sugar beet. Interest in the possibility of transferring nematode resistance to the sugar beet by such means has been stimulated further by preliminary evidence indicating that the resistance of *B. procumbens* and *B. webbiana* was passed on to F<sub>1</sub> hybrids resulting from matings of those species with highly susceptible Swiss chard (9).

In general the F<sub>1</sub> hybrids, Swiss chard X *B. procumbens* and Swiss chard X *B. webbiana*, have been sterile, and the investigations reported in this paper were undertaken in order to study the causes and to search for a possible solution. F<sub>2</sub> plants, Swiss chard ♀ X *B. webbiana*, like those previously described (9), were grown at Fort Collins, Colorado, as the source of material, and the cytological studies were made by the senior author at Salt Lake City, Utah.

## Materials and Methods

Young floral axes of F<sub>1</sub> hybrid plants were fixed in chrom-acetoformol, dehydrated, and sectioned. Slides were stained by iron-hematoxylin. The samples were taken from eight F<sub>1</sub> hybrid plants, but the necessary meiotic stages were found in only five plants. Camera lucida drawings and photomicrographs are presented.

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<sup>2</sup> Cytogeneticist, Beet Sugar Development Foundation and Collaborator, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Salt Lake City, Utah.

<sup>3</sup> Plant Pathologist, Field Crops Research Branch, Agricultural Research Service, U. S. Dept. of Agriculture, Fort Collins, Colorado.

<sup>4</sup> Numbers in parentheses refer to literature cited.

### Experimental Results

The number of chromosomes was previously studied in the species of section *Patellares* and the results obtained were reported by Helen Savitsky at the meeting of the Beet Sugar Development Foundation in January 1953 (25).

The number of chromosomes in *B. procumbens* and in *B. webbiana* had not been previously established<sup>5</sup>. *B. patellaris*, according to Bleier (4), is a diploid species ( $2n = 18$ ). Samples of these three species from different origins were used by H. Savitsky for chromosome study. *B. procumbens* and *B. webbiana* appeared to be diploid species, with 18 chromosomes in mitotic cells and 9 bivalents in meiosis. But the plants of *B. patellaris* in all samples were tetraploid. They had 36 chromosomes in mitosis and 18 bivalents in meiosis.

The  $F_1$  hybrids between *B. vulgaris* ( $n = 9$ ) and *B. webbiana* ( $n = 9$ ) all showed 18 chromosomes in the early meiotic stages. The early prophase stages in the meiosis of  $F_1$  hybrids developed as usual in diploid beets. At the leptotene stage, the chromatin threads were freely disposed in the nucleus. Some of them were intertwined and thickened at certain points. At the zygotene, the spiralization of the chromatin threads was more frequent and sometimes doubleness of the threads or segments of threads was observed (Figure 1). Contraction in synapsis showed the usual picture: Chromatin threads were gathered in a ball at one side of the nucleolus so that only the ends of the threads could be seen sometimes outside of the ball (Figure 2).

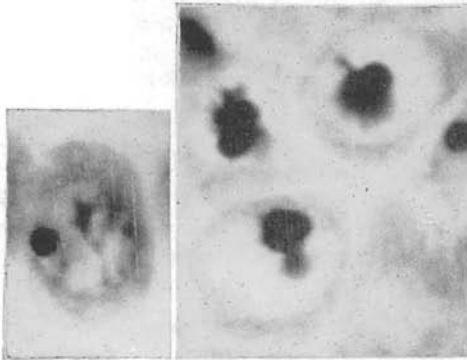


Figure 1.

Figure 2.

Figure 1.—Zygotene in P.M.C. X 2318. Figure 2.—Synapsis in P.M. Cells X 2098.

The pairing of chromosomes was studied at diakinesis where the primary spiralization of chromosomes in early prophase could not obscure the picture. The ability of chromosomes to pair was different in different pollen mother cells (P.M.C.). Some nuclei showed typical asyndesis. They

<sup>5</sup> The number of chromosomes for *B. procumbens* ( $2n = 18$ ) was reported in Sveclovodstvo 1:6. 19, 1940. But Russian collections of wild *Beta* species did not include *B. procumbens*, therefore, this species could not be studied in Russia. The number of chromosomes in *Beta* species, known at that time in Russia, were reported by H. Savitsky in the same book (Chapter "Karyology of Genus *Beta*" p. 516). The species *B. procumbens* was not mentioned there.

contained 18 unpaired univalents which were scattered over the nuclei (Figure 3). In other P.M.C. from one to five paired chromosomes were observed. The distribution of bivalents is shown in Table 1.

Table 1.—Distribution of Bivalents in Pollen Mother Cells of  $F_1$  Hybrids

Number of bivalents	0	1	2	3	4	5	Total
Number of P.M.C.	2	3	11	15	13	6	50
Percent of P.M.C. with corresponding number of bivalents	4	6	22	30	26	12	



Figure 3.



Figure 4.

Figure 3.—Asyndetic nucleus with 18<sup>1</sup> X 3255. Figure 4.—Nucleus with 3<sup>11</sup> and 12<sup>1</sup> X 3255.

Three and four bivalents were observed in the majority of P.M.C. The cases with one or five bivalents were found rather seldom. The same concerns P.M.C. in which the pairing of chromosomes was absent. Nuclei with 18 univalents and with three bivalents are shown in Figures 3 and 4. All chromosomes at diakinesis were split lengthwise, so that the four chromatids in paired chromosomes and the two chromatids in univalents could be distinguished. The univalents usually had the shape of rods; seldom were they contracted like droplets. The bivalents were distinguished by their larger size, by the number of chromatids, and by the chiasmata connecting them. The shape of bivalents was typical for *Beta* in diakinesis. The majority of them had one chiasma located close to one end of the chromosomes, which could be called a "sub-terminal" chiasma (Figure 5—c, d, e, f, g, j, l). The majority of chiasmata was symmetrical. Quite frequently the bivalents showed one or two interstitial chiasmata (Figure 5-a). The interstitial chiasmata were very often observed in cross-shaped bivalents (Figure 5—i, n). Sometimes bivalents had one interstitial chiasma and one terminal chiasma (Figure 5-k). The terminal chiasmata were seldom observed (Figure 5-h). The chiasmata frequency was estimated at 3.04 per P.M.C. In some P.M.C., fragmentation of chromosomes could be observed (Figure 6). Several chromosomes in some nuclei showed the accentuated constrictions, and the arms of such chromosomes lay wide apart. Two chromatids, more or less

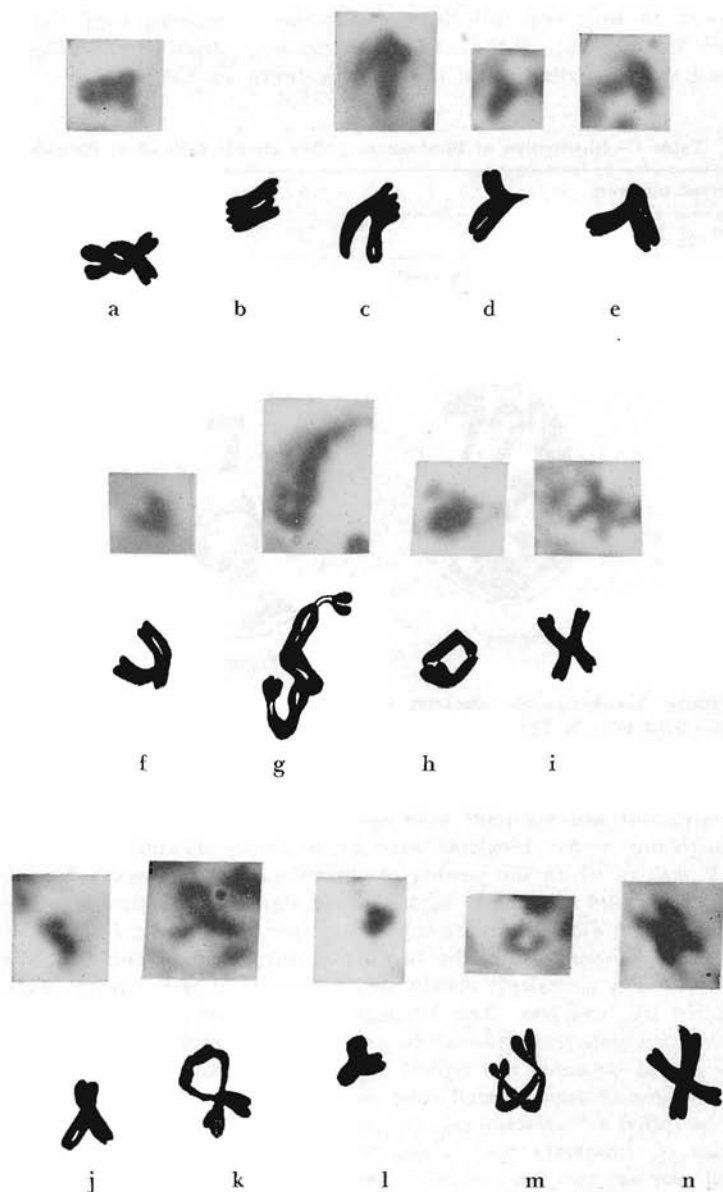


Figure 5.—The types of bivalents; bivalents with:  
 1 "sub-terminal" chiasma—c, d, e, f, g, j, l  
 1 interstitial chiasma—i, n  
 2 interstitial chiasmata—a, m  
 2 terminal chiasmata—h  
 1 terminal and 1 interstitial chiasmata—k X 3890

separated, were clearly visible in the majority of arms. The wide constrictions were evidently due to unspirialized understained (thin) heterochromatin threads. If the breakage occurs in the elongated constriction region the chromosome will be divided into two fragments. The diakinetinuclei containing fragments were observed in the hybrids studied. Figure 7 shows one of such nuclei containing 18 univalents and one fragment.



Figure 6.



Figure 7.

Figure 6.—Fragmentation of chromosomes X 3528. Figure 7.—Nucleus with  $18^1 + 1$  fragment X 3255.

At the first metaphase the chromosomes were crowded at the equatorial plate, but the contraction of the chromosomes and their orientation on the spindle was not so complete as in *B. vulgaris* or in other *Beta* species. In *B. vulgaris* the first metaphasic plate consists of small, nearly round bivalents. In  $F_1$  hybrids many chromosomes represented shortened rods, and the ends of chromosomes were often directed to the outside of the plate. The late stages of the first meiotic division in  $F_1$  hybrids showed many specific peculiarities deviating from the normal type of meiosis in diploids. In diploid *Beta* species the chromosomes move toward the poles in the first anaphase (IA), simultaneously, forming two narrow strips of chromosomes between the equator of the spindle and the poles. For the  $F_1$  hybrids it was typical that the chromosomes in the first anaphase were scattered over the whole spindle. This was caused by the random orientation, non-simultaneous moving apart of the chromosomes, the majority of which were univalents, and also by the lagging of some chromosomes. Figure 8 shows a typical view of the first anaphase where some chromosomes have reached the poles while others are lying in different parts of the spindle between two poles. The distribution of chromosomes at different poles was very irregular. The total number of chromosomes lying on the spindle often exceeded 18, the number of chromosomes in the first anaphase usually observed in normal meiosis of a diploid.

In  $F_1$  hybrids the number of chromosomes in the first bipolar anaphase varied from 18 to 28. This means that some univalents divided in the first meiotic division and others proceeded to the poles undivided.

In several P.M.C some large chromosomes could be observed at the first anaphase. Sometimes these large chromosomes consisted of four chromatids resembling in size and shape the diakinetinuclei. In two cases there was no doubt that such large chromosomes represented bivalents.



Figure 8.



Figure 9.

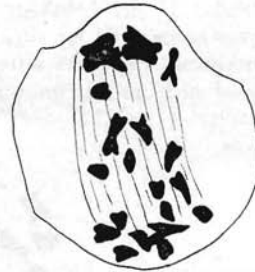


Figure 10.

Figure 8.—1 bipolar anaphase with 23 chromosomes X 3500. Figure 9.—1 anaphase nondisjunction of a bivalent X 3500. Figure 10.—1 anaphase with two bivalents at the pole X 3500.

Figure 9 shows nondisjunction of a bivalent. The bivalent is situated on the spindle near one pole and consists of two chromosomes connected by "subterminal" chiasma. Both partners of bivalents showed split ends indicating that they consisted of two chromatids. In Figure 10, two bivalents were distinguished from all other chromosomes by their larger size. At certain positions the split ends of one partner in each chromosome could be seen, which, besides their size, confirmed the fact that they were bivalents. The bivalents observed at the anaphase resembled in size (they were a little smaller) and appearance the bivalents seen in diakinesis, that is, contraction at the first metaphase (IM) was not strong enough. Nondisjunction was frequently observed by Goodspeed in X-rayed *Nicotiana tabacum* (10).

The interkinetic nuclei also sometimes contained bivalents which have been previously observed at the IA. Figure 11 shows an interkinetic nucleus with a large bivalent. One end of the bivalent had two splits, showing that it consisted of four chromatids. The curvature of the spindle was observed in a few cells.

Occasionally, during interkinesis, P.M.C. with large, non-reduced nuclei were found among binuclear P.M.C. The number of chromosomes in such nuclei equaled 18 (Figure 12). Such nuclei may give rise to a diploid pollen grain containing one genome of *B. vulgaris* and one of *B. webbiana*. The case is analogous to *Raphano-brassica* hybrids (14) and some others.

The first amphidiploid plant reported in the genus *Beta* was obtained by Zossimovitch (28, 29) after hybridization of *B. lomatosogona* ( $n = 9$ ) to *B. corolliflora* ( $n = 18$ ). When non-reduced gametes of  $F_1$  hybrids ( $n = 27$ ) united during fertilization an amphidiploid plant arose which contained 54 chromosomes in somatic cells and resembled very much in its appearance the hexaploid species *B. trigyna*.



Figure 11.

Figure 11.—Two interkinetic nuclei with 11 and 13 chromosomes; one nucleus contains a bivalent X 3500. Figure 12.—Non-reduced nucleus with 18 chromosomes X 3255.



Figure 12.

An important deviation from the normal meiotic division in diploids was the formation of multipolar spindles at the IA of  $F_1$  hybrids. In many P.M.C. the spindle developed three, four, or more poles. In most cases the multipolar spindle developed three or four poles which were connected by the continuous fibres, forming one common spindle. In the cells with a tri-polar spindle, the chromosomes proceeded at IA to three different poles and three nuclei were formed. Sometimes these nuclei were of approximately the same size (Figure 13), but the number of chromosomes which reached their respective poles varied in some degree. In other cases the number of chromosomes at different poles and the size of nuclei varied considerably (Figure 14). In the four-polar spindles, sometimes four symmetrical poles were formed (Figure 15), the chromosomes were distributed



Figure 13.

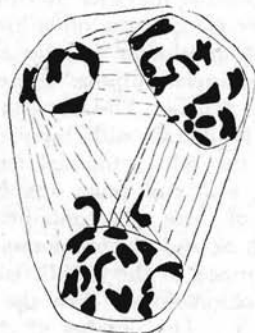


Figure 14.

Figures 13 and 14.—Late multipolar anaphase with three poles X 3500.





Figure 15.

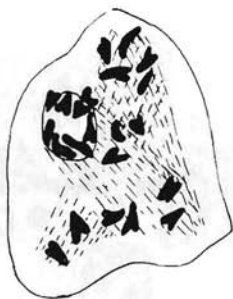


Figure 16.

Figures 15 and 16.—Late multipolar anaphase with four symmetrical poles X 3500.

between poles very irregularly, and many of them remained on the spindle outside of the forming nuclei. Figure 16 shows the distribution of chromosomes between the poles of a four-polar spindle in another P.M.C. One group of chromosomes reached the pole, and the nucleus was already formed, while other chromosomes were on the way to the other poles.

In many P.M.C. the shape of the spindle was asymmetrical. In some cases three poles were found at one end of the spindle and the fourth pole at the opposite end (Figure 17). Sometimes the spindle formed a triangle (Figure 18), and the fourth or fifth pole was at the side on another level and the chromosomes which are marked only by their contour lines were still moving forward toward the pole. The two nuclei lying side by side could be considered either as nuclei formed at the third and fourth poles, or one of them could be a micro nucleus derived from the group of chromosomes which remained on the spindle. In some cells there were five, six, or seven poles and the chromosomes were distributed among them (Figure 19). The distribution of chromosomes in P.M.C. with a multipolar spindle was much more irregular than in the cells with a bipolar spindle and the size of formed nuclei varied considerably.

The activity of chromosomal fibers from different poles was not similar. In many P.M.C. the majority of chromosomes moved to one or two poles and only a few reached the other two poles (Figures 14, 15, 17). But the activity of the multipolar spindle in general was much higher than the activity of the bipolar spindle. More chromosomes were moving apart and reached poles than in P.M.C. with a bipolar spindle. The number of chromosomes in P.M.C. with bipolar anaphase varied from 18 to 28; the great part of the cells contained from 18 to 24 chromosomes (Table 2). In the P.M.C. with multipolar anaphase, more chromosomes were observed. The majority of these cells contained from 24 to 29 chromosomes. In two cases cells with 32 and 36 chromosomes were found. At the same time more nuclei were formed in the P.M.C. with a multipolar spindle, but they contained fewer chromosomes than the interkinetic nuclei after bipolar anaphase (Table 3). The number of chromosomes in the nuclei after bipolar anaphase varied from eight to fourteen. The nuclei after multipolar anaphase contained from one to eighteen chromosomes. Many of them had three, four, five, or six chromosomes; in the majority of these nuclei the



number of chromosomes was lower than nine, although the number of nuclei with nine chromosomes was about the same when compared with bipolar anaphase. More lagging chromosomes were observed at multipolar anaphase than in bipolar. More univalents were oriented on the multipolar spindle than on the bipolar—therefore, more chances existed for some univalents to be oriented later at the late metaphase or even at anaphase. The late-oriented chromosomes may not have time to complete their "polarization" toward two different poles or some of them will not be able to reach the poles until the nuclei are formed and they remain as laggards

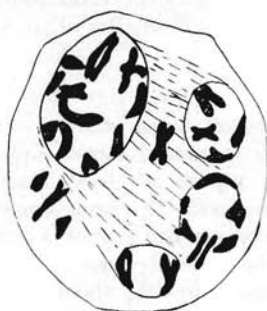


Figure 17.



Figure 18.



Figure 19.

Figure 17.—Late multipolar anaphase with four asymmetrical poles X 3500. Figures 18 and 19.—Late multipolar anaphase with more than four poles X 3500.

Table 2.—Number of Chromosomes in Pollen Mother Cells with Bi- or Multipolar Spindle in the First Anaphase.

	P.M.C. With Number of Chromosomes as follows:																		
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
P.M.C. with bipolar anaphase	3	1	3	1	3	1	1	1				1							
P.M.C. with multi-polar anaphase							4	1		3		5			1				1

Table 3.—Number of Chromosomes in Nuclei Formed After Bipolar and Multipolar Anaphase.

	Nuclei with Number of Chromosomes as follows:																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Nuclei after bipolar anaphase								1	5	5	7	5	2	1				
Nuclei after multipolar anaphase	1	8	6	3	3	8	6	8	6	4			1	2				2

on the spindle. At any rate, three, four, or five chromosomes often were seen on the spindle at the time the nuclei were formed at the poles after multipolar anaphase instead of one or two after bipolar anaphase. The bivalents which were observed in the bipolar anaphase and sometimes in the interkinetic nuclei after bipolar anaphase never were seen on the multipolar spindle or in the nuclei derived after the multipolar anaphase. This also confirms the fact that the moving of chromosomes to the poles and the co-orientation of the bivalents were more easily completed by the multipolar spindle. The chromosome fibres extending from the poles in different directions could more easily co-orient the kinetochores of a bivalent towards two different poles, because the angle of turning of centromeric chromomeres and of the kinetochore itself may be much smaller than on the bipolar spindle.

In some multipolar spindles certain poles were situated very close to each other, so that the nuclei formed at the poles often lay side by side (Figures 18, 19). In some cells the fusion of two groups of chromosomes lying at closely situated poles could be observed. Figure 21 shows fusion of two groups of chromosomes to form one nucleus with 18 chromosomes. Figure 22 represents another similar case. An enormous nucleus of a very irregular shape and not yet completely formed lies at one pole. It also contains 18 chromosomes. The number of chromosomes in both these nuclei considerably exceeded the number usually observed in one nucleus after anaphase. Such nuclei with high chromosome numbers may originate either from the fusion of two groups of chromosomes at two closely located poles, or from partial bipolarization of the multipolar spindle, as was observed in a *purpurea* haploid of *Nicotiana* (6).



Figure 20.



Figure 21.



Figure 22.

Figure 20.—P.M.C. with 36 chromosomes containing a hexad formed after meiosis X 3500. Figure 21.—Fusion of two groups of chromosomes at closely located poles in multipolar anaphase X 3500. Figure 22.—Incompletely formed nucleus with 18 chromosomes derived from fusion of two groups of chromosomes; the poles with chromosomes marked with contour lines are at different level X 3500.

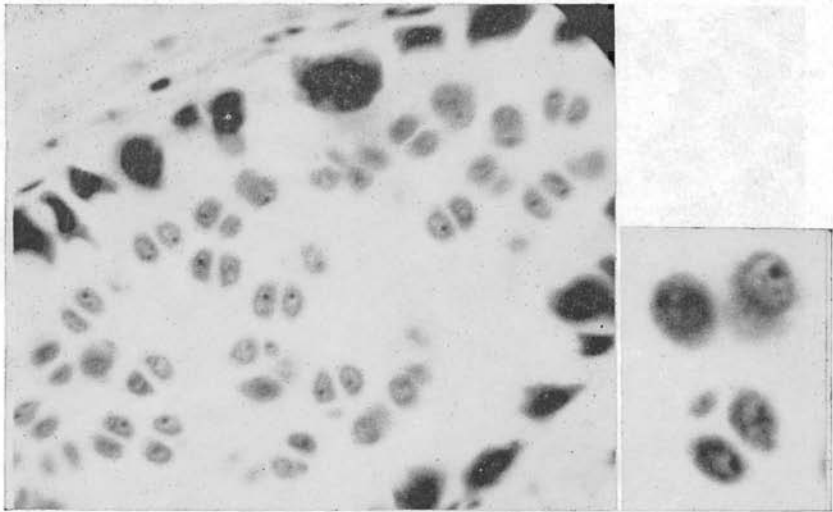


Figure 23.

Figure 24.

Figure 23.—Locule of an anther containing almost exclusively dyads formed after 1 bipolar anaphase X 607. Figure 24.—Dyad and a dyad with a little additional cell or a triad X 1600.

The second meiotic division did not take place in  $F_1$  hybrids. After the interkinetic nuclei were formed, the walls of pollen mother cells collapsed and the nuclei surrounded by cytoplasm were freely disposed in the locules of anthers where they gradually developed into pollen grains. The pollen mother cells which had two nuclei after bipolar anaphase produced dyads (Figure 23). If, besides the two nuclei, the micronucleus was formed, the dyads also contained a small third cell (Figure 24). The dyads were not rare; in some locules of anthers the majority of pollen mother cells produced dyads almost exclusively (Figure 23). In other locules, besides dyads, triads, tetrads, pentads, etc., were formed which developed after multipolar anaphas (Figure 25). Both types of the anaphase (bipolar and multipolar) occurred with about the same frequency, and often the tetrad-stage in pollen mother cells lying side by side showed different types of anaphas (Figure 26).

There were great variations in the size of nuclei and cells in tetrads, pentads, etc., as well as in the size of pollen grains derived from them (Figure 27). Very small pollen grains developed no nucleoli and were abortive just after their formation. In open flowers some anthers dehisced and some did not. In certain flowers the anthers stood on outstretched filaments for a longer than usual period of time without dehiscing. The pollen grains in the anthers of open flowers were yellow, with both intine and extine being developed. Some of them were round, but most had irregular shapes, sometimes they were crescent in shape, resembling pollen grains of the synaptic forms in *Allium amplexans* (16). The size of pollen grains varied from  $6\mu$  to  $26\mu$ , the majority of them equalling  $15\mu$ . In diploid sugar

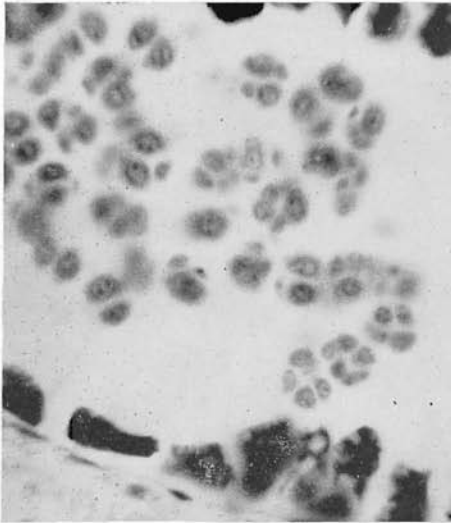


Figure 25.

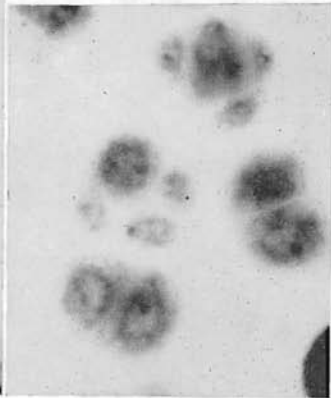


Figure 26.

Figure 25.—Locule of an anther containing tetrads, pentads, hexads, etc. formed after 1 multipolar anaphase X 607. Figure 26.—Two dyads, tetrad and a pentad lying side by side X 1600.

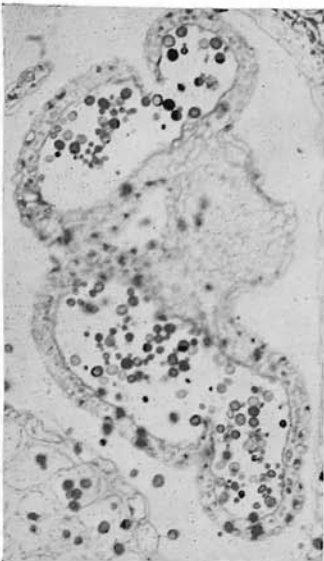


Figure 27.



Figure 28.

Figure 27.—Sectioned matured anther containing pollen grains of different sizes X 109. Figure 28.—Sectioned pollen grains from an opened flower; empty pollen grain and pollen grains with one or two degenerating nuclei X 2000.

beets the size of pollen grains usually varies from  $19\mu$  to  $21\mu$ . Examination of sectioned pollen grains from matured buds and open flowers showed that 74.7 percent of them still remained at the one-nucleus stage instead of the three-nuclei stage which is normal at this period of development (Table 4 and Figure 28). In many mononuclear microspores the nuclei were dead (dark-stained or destroyed) and 21.8 percent of pollen grains were empty without plasma and nuclei. Only three percent of the microspores developed a tube nucleus and a generative nucleus. But, in spite of the picture of general disorganization, 0.5 percent contained three nuclei, two sperm nuclei and a tube nucleus. Some of these pollen grains may be functional.

Table 4.—Pollen Grains in the Anthers of Open Flowers.

	Empty	With One Nucleus	With Generative and Tube Nuclei	With 2-Sperm Nuclei and Tube Nucleus	Total
Number of pollen grains	218	747	30	5	1000
Percent	21.8	74.7	3.0	0.5	100

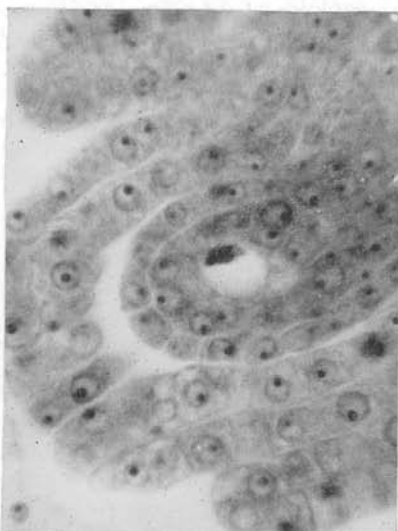


Figure 29.—Synapsis in megaspore mother cell X 833.

#### Discussion

In remote interspecies hybridization, such as a hybridization of *B. vulgaris* with *B. webbiana*, the absence of chromosome association in  $F_1$  hybrids could be regarded as normal. However, cytological study showed that in most P.M.C. of  $F_1$  hybrids, three or four pairs of chromosomes were observed.

This appearance must be considered as a positive one. The final purpose of such hybridization consists of transferring desirable genes, such as those providing for resistance to nematodes, from species of the section *Patellares* (including *B. webbiana*) to the species *B. vulgaris*.

If the chromosomes of *B. vulgaris* and of species of the section *Patellares* could not associate, almost no chance would exist of obtaining nematode-resistant sugar beets by use of interspecies hybridization. Although diploid hybrids between these species were sterile, and preferential association between chromosomes of the same species must be expected in tetraploid hybrids, yet the ability of chromosomes of *B. vulgaris* to associate with chromosomes of *B. webbiana* is a very hopeful sign that chromatin transfer between these diverse species can be expected, provided fertile hybrids are obtained.

The shape of bivalents in  $F_1$  hybrids closely resembled the shape of bivalents usually observed in *Beta* species. Therefore, it could be assumed that the chiasmata frequency in the bivalents of hybrids did not greatly differ from the chiasmata frequencies in the bivalents of *Beta* species in diakinesis.

It may be suspected that part of the bivalents in  $F_1$  hybrids may result from association of chromosomes within a haploid set. Some haploids (or monoploids), such as haploids of *Nicotiana tabacum* (6), of *Datura stramonium* (2, 3), etc., did not show chromosome pairing, but in other haploid plants *Antirrhinum* (11), *Zea mays* (20), *Secale cereale* (17), *sorghum* (15, 8), and *B. vulgaris* (18), the pairing of chromosomes were observed. A. Levan (18) described the pairing of chromosomes in a haploid sugar beet plant, but the pairing in haploid rye, sugar beets, and some other species is mainly a spiralization pairing through early prophase stages and gradually disappears during diplotene-diakinesis.

The great distinction, according to Levan, between pairing in diploids and haploids is that the haploid gives rise to only a few chiasmata and these chiasmata may have been caused by purely chance factors and were not formed between homologous segments. The chiasmata frequency in sugar beet haploid plants varied for different fixations from about 0.5 to 0.1 per cell. In the  $F_1$  hybrids studied the chiasmata remained until the first metaphase and their frequency was much higher; it was evaluated at 3.04 per P.M.C. The chiasmata frequency might be reduced but little during the first metaphase. It is impossible to prove that not one bivalent arose in  $F_1$  hybrids from pairing of chromosomes within a haploid set, but the chiasmata frequencies and the late meiotic stages, where they were observed, indicated that it is much more probable that the pairing took place between homologous chromosomes of *B. vulgaris* and *B. webbiana*. In many interspecies hybrids the pairing between chromosomes of two parental species was usually observed.

Among different abnormalities in meiosis of  $F_1$  hybrids the defects of the spindle could be mentioned. Nondisjunction of bivalents in bipolar anaphase could be explained by insufficient activity of the poleward forces of the spindle. The arrangement of chromosomes at the equatorial plate at the first metaphase and the moving of chromosomes toward the poles



depend upon the poleward forces (the a-factor) of the spindle and upon the orientation of kinetochores of chromosomes. Nondisjunction of bivalents could occur when the kinetochores of such bivalents were not co-oriented on the equatorial plate because one chromosome of the pair was not attached to the chromosome fibre, or because of the insufficient activity of the pulling forces (poleward attraction) of one of the poles.

Development of multipolar spindles with three or four or sometimes a higher number of poles is also a deviation from the normal type of division. But, in spite of this, the multipolar spindle functioned more efficiently than the bipolar. More chromosomes were moved apart and transported to the poles in multipolar anaphase. It is probable that more chromosomal fibres crossing the nucleus in different directions were developed in P.M.C. with a multipolar spindle resulting in more effective movement of the chromosomes to the poles. Higher activity of the multipolar spindle can be explained on the basis of the hypothesis of orientation by pulling (24). The majority of chromosomes in the P.M.C. of  $F_1$  hybrids are univalents. The kinetochores of the univalents are oriented at random in various directions. On the multipolar spindle the poleward attraction (the a-factor) working on the kinetochores of the univalents can more easily arrange them in various positions on the spindle. The chromosomal fibres extending from poles in different directions can more easily meet the centromeric chromomeres of the univalents (which often show an angular separation), arrange the univalents on the spindle, and lead to their "polarization." As a result more univalents may divide in the multipolar anaphase than in the bipolar.

The negative consequence of the activity of a multipolar spindle was the formation of a higher number of nuclei, each containing a lower number of chromosomes. This led to the formation of a large quantity of non-viable pollen grains. The total number of chromosomes in P.M.C. of  $F_1$  hybrids was lower after meiosis than in the normal diploid beet. Thirty-six chromosomes were observed in only one cell. In the majority of P.M.C. the highest number of chromosomes varied from 27 to 32. Evidently not all univalents were divided during the first division. The formation of dyads in these hybrids was due to the failure of the second meiotic division.

A cytological study of  $F_1$  hybrids between Swiss chard and *B. webbiana* revealed the causes of their sterility. Because of the high sterility of the gametes in these  $F_1$  hybrids, there were but few chances to obtain the progeny from intercrosses between  $F_1$  plants. But, even so, the diploid hybrids between *B. vulgaris* and species of section *Patellares* are not completely hopeless. The pollen grains developed from the nuclei with a low chromosome number such as 2, 3, 4, 5, etc., will be nonfunctional. It is quite doubtful that all haploid pollen grains (with nine chromosomes) and the diploid pollen grains (with 18 chromosomes) derived from the union of two groups of chromosomes in anaphase will be completely viable. Their viability will depend upon cooperation of different chromosomes obtained from both species, *B. webbiana* and *B. vulgaris*, and their physiological activity, therefore, may be limited.

The non-reduced nuclei (with 18 chromosomes) will produce viable pollen grains. If two non-reduced gametes of  $F_1$  hybrids could meet, am-



phidiploid fertile hybrid plants could develop. Only a few non-reduced gametes were observed in  $F_2$  hybrids between Swiss chard and *B. webbiana*. To increase the possibility of the formation of non-reduced gametes, it might be reasonable to involve in crosses species of the section *Patellares*, and the diverse races of *B. vulgaris*. On the other hand, the chances of development of amphidiploid plants will become higher if more  $F_1$  hybrid plants are produced. It may also be desirable to grow a clone of a self-sterile, tetraploid sugar beet, together with an isolated group of  $F_1$  hybrids. In this way the non-reduced gametes of hybrids may be more likely to meet the diploid female or male gametes of the sugar beet and produce viable tetraploid offspring, with three genomes of *B. vulgaris* and one genome of *B. webbiana* or *B. procumbens*. Another way, which seems more promising, consists of the production of tetraploid hybrids either by obtaining amphidiploid plants from colchicine treatment of diploid  $F_1$  hybrids, or by crosses of tetraploid sugar beets with tetraploid plants from the species of the section *Patellares*.

The  $F_1$  tetraploid hybrids will represent amphidiploids and will be fertile or semi-fertile. Their intercrosses and backcrosses to tetraploid sugar beets will produce many new combinations of chromosomes of parental species as well as the interchanges between segments of chromosomes. At the same time, a number of chromosomes higher than in the diploid hybrids will provide for a broader genetic base and higher viability of the offspring.

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