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Cytogenetic studies on the artificially raised trigenomic hexaploid hybrid forms in the genus *Brassica* *

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Introduction

A number of cultivated forms in the genus *Brassica*, to which many vegetables, forages, and as well as certain oil crops belong, could be classified according to their genomic constitutions into the following two main groups (Morinaga, 1928, 1929a,b,c, 1931, 1933, 1934; Manton, 1932 ; Haga, 1938; Sikka, 1940; Fukushima, 1945; Mizushima, 1952 ; etc.) :

1. Monogenomic group

a genome species (n= 10) ;	*B. campestris* L., *B. rapa* L., *B. pekinensis* Rupr., *B. japonica* Sieb., *B. nipposinica* Bailey, etc.
b genome species (n=8) ;	*B. nigra* Koch
c genome species (n= 9);	*B. oleracea* L., *B. alboglabra* Bailey

2. Digenomic group

ab genome species (n=18);	*B. juncea* Hemsel., *B. cernua* Coss.
bc genome species (n=17);	*B. carinata* Braun
ac genome species (n=19);	*B. napus* L., *B. integrifolia* Auth., etc.

It remains still uncertain, however, whether or no the genus has any one natural species having trigenomic *a b c* composition.

Many cultivated forms, e.g., species belonging to *Triticum*, *Nico-*

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tiana, or *Gossypium*, are known to be of allopolyploidy. So that many workers considered that the artificial synthesis of the allopolyploid or amphiploid forms would be used profitably for the plant breeding measure. The discovery of a new technique of chromosome doubling by the use of colchicine, acenaphthene, and some other chemicals had decisively promoted the effective production of the certain artificial polyploid plants. Remarkable successes have been achieved in such artificial production of allopolyploid forms in the genus ***Brassica***, particularly in the experimental synthesis of the digenomic species, and the experimental verifications of the result of genome analyses already obtained have been effected. Examples of such new plant breeding through the synthesis of the new species in *Brassica* are as follows: Artificial *napus* by U (1935), Frandsen (1947), Rudorf (1950), Hosoda (1950), Hoffman and Peters (1958), and by Olsson (1960); artificial *juncea* by Frandsen (1943), Mizushima (1952), Olsson (1960), and by Ramanujam and Srinivasachar (1943); artificial *carinata* by Frandsen (1947), and Mizushima (1952). The synthesis of the trigenomic form, ***a*** *b* **c**, has also been achieved for its cytogenetical importance by Howard (1942) and Mizushima (1952).

However, the artificial allopolyploid individuals so far produced were of little practical use, mainly because the artificial allopolyploids usually took more or less undesirable phenotypic appearances under the newly introduced genotypic condition, which would establish the genic balance not occurring in their parental forms, and also because the desirable phenotypic characters in the parental forms would be rather easily blended in many cases with one or more undesirable ones in the formation of allopolyploidy. The latter reason will be quite justifiable in the cases when a certain wild species has participated in the synthesis. It is, therefore, necessary, as many workers have pointed out, that any one synthesized allopolyploid form would be treated on its own level for the purpose of new breeding. It must be noted, moreover, that the allopolyploid forms in ***Brassica*** have a considerable handicap in their pedigree cultures, because there prevail the partially homologous genomes in *Brassica*, accompanying the formation of multivalent chromosomes at meiosis and the consequent various irregularities inducing to the poor fertility. The trigenomic form, ***a*** *b* **c**, synthesized by the present author was practically intolerant of pedigree culture on account of its extreme meiotic irregular behavior. The plant showed very low fertility and many aneuploid individuals have occurred in the progeny.

In the present paper the author intends to give the results of cytogenetic studies carried out during 1950 to 1957 on his trigenomic form of ***Brassica***.

Materials and methods

The names of those 6 species of the genus *Brassica* which were used as the materials, their genome constitutions, the mode of chromosome pairing at meiotic metaphase-I of their PMCs, and the grade of their fertilities are shown in Table 1. The 4x-B. *pekinensis* was obtained through several successive selfing generations, and the 4x-B. *carinata* from the first generation raised by the artificial chromosome doubling. The 4x-R. *pekinensis* formed several quadrivalents, with rare exception with some trivalents and univalents, at metaphase-I of PMCs, and in 71 per cent of the daughter MII plates observed had the exact nurnber of chromosomes. The pollen-fertility of the 4x-*B*. *pekinensis* was as high as that of the diploid individual, but the seed-fertility was definitely lower in the former than in the latter. In the 4x-B. *carinata* used, all the PMCs observed contained exclusively quadrivalents at metaphase-I, accompanying not infrequent occurrence

Table 1. Materials used in the experiment.

Species	Horticultural varieties	Ploidy and genome constitution (n)	Somatic chromosome (n)	Chromosome pairing at metaphase-I	Pollen fertility (%)
B. pekinensis Rupr.	Kashin-hakusai	2x *a*	*10*	10_{II}	*98.5*
		4x *aa*	20	(E-O), $_{V}+(8\text{–}20)_{II}$	*97.0*
B. nigra Koch	Unknown	2x *b*	8	8_{II}	99.0
B. oleracea L.	Miike-kanran	2x *c*	9	9_{II}	99.5
B. carinata Braun	Unknown	2x *bc*	17	17_{II}	97.3
		4x *bbcc*	34	$(10\text{–}4)_{IV}+(14\text{–}26)_{II}$	78.1
B. juncea Hemsel.	Miikeaka-takana	2x *ab*	18	18_{II}	99.3
B. *napus* L.	Unknown	2x *ac*	19	19_{II}	98.7

of tri- and univalents, so that the number of chromosomes per daughter MII plate was 34 (normal) in 48 per cent of the plates examined, and (l-4) below or (l-5) above 34 in the remaining ones. The 4x-B. ***carinata*** plant was less flourishing and less fertile as compared to the diploid form, and could produced a large number of aneuploid individuals in its progeny, making its pedigree culture utterly infeasible. Since such a considerable meiotic irregularity accompanying to the tetraploid forms was likely to induce certain chromosomal aberration or the aneuploid composition with any trigenomic hexaploid F_1 hybrid forms produced by a cross between any two different 4x-species, the trigenomic hexaploid forms to be submitted to the detailed cytogenetical investigation would be exclusively confined to the trigenomic amphidiploid forms obtained by the chromosome doubling of the F_1 hybrid, produced by a cross between any two different 2x-species.

The crossing was effected in the form of bud-pollination. The meiosis was studied in pollen mother-cells smeared in the aceto-carmine or acetic-orcein. The buds to be used for the purpose were fixed in Carnoy's fluid, kept in absolute alcohol overnight for hardening, and stored in 70 per cent alcohol. The pollen-fertility was estimated with the mature pollen-grains smeared in a mixture of aceto-carmine and glycelin, and the pollen-grains taken as fertile were those stained well and appearing normal in shape. The seed-fertility was denoted by the number of viable seeds produced per silique set or by the percentage occurrence of viable seeds with the ovules developed under the open-pollination.

Table 2. Results of the crossing experiments and number of the F_1 hybrids obtained.

Cross combination	No. of flowers used	No. of seeds obtained		No. of seeds sown		No. of seeds germinated	No. of F_1 hybrids		No. of false hybrids		Exp. No. of F_1
		L[1)]	S	L	S		L	S	L	S	
pekinensis (2x) x *carinata* (2x)	8	2	—	2	—	2	—	—	2	—	
carinata (2x) x *pekinensis* (2x)	27	31		31		26	14		12		$cpF_1 1$, $cpF_1 2$[2)]
pekinensis (4x) x *carinata* (4x)	36	31	14	2	12	13	—	11	2	--	$cpF_1 3$
carinata (4x) x *pekinensis* (4x)	36	1	2	1	1	2	—	1	1	—	$cpF_1 4$
juncea (2x) x *oleracea* (2x)	32	10		10		8			8		
napus (2x) x *nigra* (2x)	36	6		6		5			5		

N.B. 1) L, Large seeds. S, Small seeds.
2) cpF_1 2 was obtained from cpF_1 1 by the chromosome doubling.

Results

1. The result of experimental crossing

A series of crosses between the monogenomic and the digenomic species resulted in the production of the hybrids mentioned in Table 2. The trigenomic F_1 hybrids could be obtained only as the result of *carinata-pekinensis* combination. In tribe ***Bmssiceae,*** the true F_1 seeds raised by interspecific or intergeneric crosses are, as a rule, so remarkably reduced in their sizes as to be easily distinguishable from any false matrocrinous ones (U and Nagamatsu, 1933; Hosoda, 1946; Mizushima, ***1952).*** The present true F_1 seeds (cpF_13 and cpF_14) differed clearly in size from false matrocrinous ones. The ratio in weight between these two kinds of seeds was 2 : 1.

2. Trigenomic triploid hybrid

a. Morphology

In the stage of young seedlings the F_1 hybrids obtained were clearly distinct from their parental species in the form of their young foliage leaves. The main morphological features of the two parental species and the F, hybrid, ***a b c,*** were as follows:

(1) The *B. pekinensis* was of rosette-shape at its vegetative growth stage ; its leaves, whose upper ones grasping the stem, were spatulate in shape, with the broad midrib, haired on surface, and little efflorescent with some waxy substance; its flowers with broad petals commonly crowded at end of raceme and overtopping unopened buds (Figs, A-1, B-l). (2) The *B.* ***carinata*** showed steady increase in height of its stem during its vegetative growth stage; its leaves, with no hairs on the surface and with small lobes often on the petiole, and the upper leaves partly grasping the stem, were lyrate and remarkably efflorescent with a waxy substance; its flowers with narrow petals opened on short pedicels in lengthening racemes, and the base of each pedicel was set by a small ligulate leaf (Figs. A--3, B-4). (3) The F_1 hybrid showed only a little growing in its stem height during the vegetative growth stage ; its leaves, with hairs on the surface, were more lyrate in shape and covered with smaller extent of waxy substances than those of the parental *B.* ***carinata;*** its flowers and siliques were intermediate in shape of the parental species; and the small ligulate leaves at the base of the pedicels showed no less variation in the mode of their occurrence with the different individuals (Figs. A-2, B-2, C-3). In short, the F_1 hybrid was somewhat intermediate morphologically between its parental species, showing still much resemblance to the *B. carinata* than to the *B. pekinensis.*

b. Cytology

The process of meiosis in PMCs was remarkably irregular in the triploid F_1 hybrid. The chromosome pairings at metaphase-I are given in Table 3. The number of bivalents per PMC varied between

Table 3. Chromosome pairings at metaphase-I in PMCs of triploid F_1 hybrid derived from *B. carinata* (2x) x *B. pekinensis* (2x).

Configuration	Frequency	Percentage
$2_{II}+23_I$	2	3.8
$3_{II}+21_I$	1	1.9
$4_{II}+19_I$	6	11.5
$5_{II}+17_I$	11	21.2
$6_{II}+15_I$	13	25.0
$7_{II}+131$	10	19.2
$8_{II}+11_I$	8	15.4
$9_{II}+9_I$	1	1.9
Total	52	100

Table 3. Distribution of chromosomes at metaphase-II in PMCs of triploid F_1 hybrid derived from B. *carinata* (2x) x B. *pekinensis* (2x).

Number of chromosomes	9	10	11	12	13	14	15	16	17	18	Total	Average number of chromosomes per plate
Frequency	1	2	1	9	10	7	1	3	--	1	35	13.1
Percentage	2.9	5.7	2.9	25.7	28.6	20.0	2.9	8.6	---	2.9	100	

2 (Fig. 1) and 9 (Fig. 6), 6 being the mode (Fig. 4). At anaphase-I and its subsequent stages, the most univalents moved towards the nearer pole and the rest ones showed splitting. Some univalents, as it happened often, were left lagging in the cytoplasm (Fig. 11). Moreover, some other meiotic aberrations such as the formation of chromosome bridges and as the small extra spindles were observed occasionally at anaphase-I. The number of chromosomes at metaphase-II varied between 9 and 18, 13.1 being on an average (Table 4), and some AI chromosome bridges were found persisting throughout the

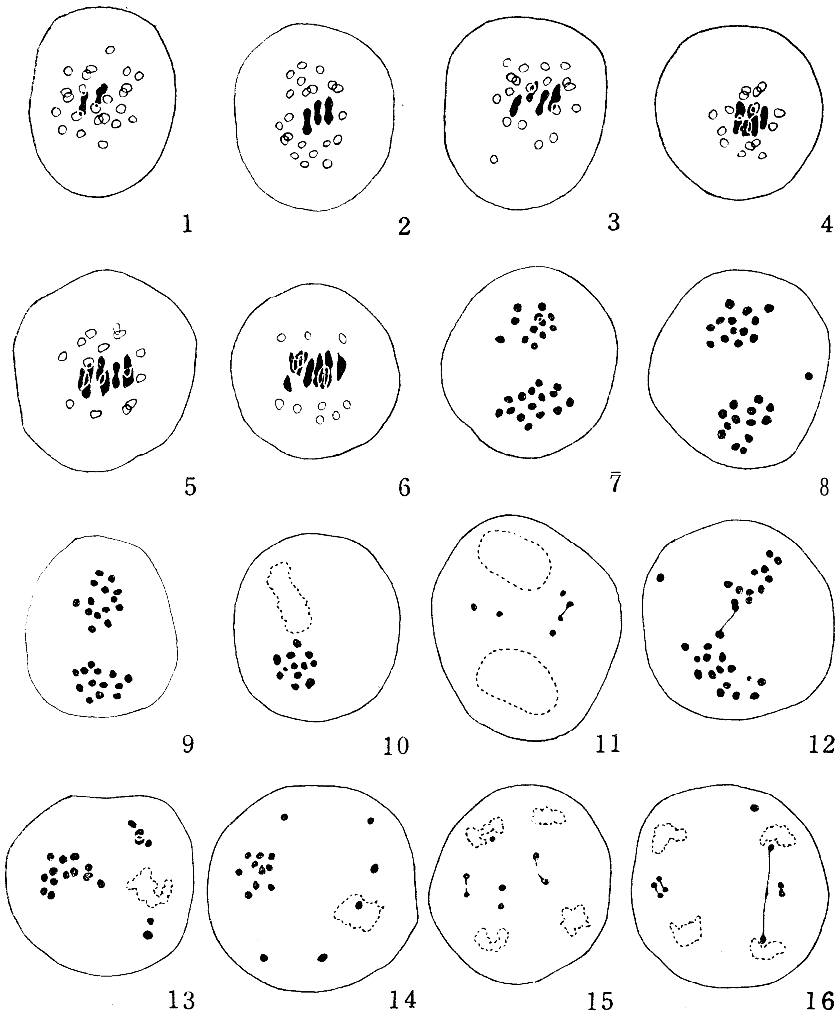


Fig. 1-16. Meiotic divisions of PMCs in the triploid F_1 hybrids. x 1300. Fig. 1. metaphase-I, $2_{II}+23_{I}$. Fig. 2. metaphase-I, $3_{II}+21_{I}$. Fig. 3. metaphase-I, $4_{II}+19_{I}$. Fig. 4. metaphase-I, $6_{II}+15_{I}$. Fig. 5. metaphase-I, $7_{II}+13_{I}$. Fig. 6. metaphase-I, $9_{II}+9_{I}$. Fig. 7. metaphase-II, 12-15 segregation. Fig. 8. metaphase-II, 13-1-13 segregation. Fig. 9. 13-14 segregation. Fig. 10. a fragment-like small chromosome is discernible in the daughter nuclear plate having 12 chromosomes. Fig. 11. anaphase-I, 5 laggards. Fig. 12. metaphase-II, persistent bridge from anaphase-I. Figs. 13 and 14. metaphase-II, irregular segregation. Fig. 15. anaphase-II, 3 laggards. Fig. 16. anaphase-II, bridge and laggards.

second division (Fig. 12). Some chromosomes at metaphase-II occurred as the diads, and the other as the monads, rarely accompanying few or none of undersized small chromosome fragments (Figs. 10, 12). The meiotic division behaved also very irregularly at anaphase-II, forming some lagging chromosomes and chromosome bridges (Figs. 15, 16). At the tetrad stage there appeared various kinds of sporads, i.e., monads, diads, triads, abnormal tetrads, pentads, and other polyads, in addition to the normal tetrads (Table 5, Fig. 17).

Table 5. Occurrence of abnormal types of sporads in F_1 plants and their amphidiploid plants.

Plant exp. NO.	Slide number	Frequency of various abnormal types of sporad 1	2	3	4	5	6	7	Normal tetrad	Total	% of abnormality	Genome constitution (2n)
cpF_1 1-3		1	2	7	120	—	--		465	496	6.3	*abc*
-5	a	1	2	1	2	74	6	—	586	672	12.8	〃
	b	1	1	1	137	1	—		373	415	10.1	〃
	c	1	5	5	1	76	2	--	605	695	12.9	〃
-26		—	2	4	2	22	2	--	526	558	5.7	〃
cpF_1 2-10		—	—	—	—	31	1	—	612	644	5.0	*aabbcc*
-11		—	—	--	—	107	13	1	565	686	17.6	〃
-16	a	—	—	—	—	36	1	—	751	788	4.7	〃
	b	—	—	—	—	81	4	—	585	670	12.7	〃
	c	—	--	--	—	60	4	—	600	664	9.6	〃

c. Fertility

The above-described meiotic irregularity in the trigenemic triploids brought down the fertility of those plants almost to nil, the stamens were malformed and the anthers remained unopen. Only 0.4 per cent of pollen-grains was found to be fertile (Fig. E-2). Those viable pollen-grains were somewhat equal in size to those of the hexaploid,

Table 6. Comparison of sizes of pollen-grains between *abc*-trigenomic tri-and hexaploid plants.

Ploidy	Fertile pollen-grains (micro-meter unit) min.-mean-max.	Number of pollen grains measured	Sterile pollen-grains (micro-meter unit) min.-mean-max.	Number of pollen-grains measured	Pollen fertility (%)
Triploid	0.54—1.02—1.76	27	0.25—0.36—0.47	40	0.4
Hexaploid	0.76-1.03-1.30	39	0.45—0.61-0.88	39	38.8

indicating presumably that all the pollen-grains remaining fertile in the triploid were of 2n chromosome composition (Table 6, Figs. E-l, 2). The well-developed seeds were so scarce in amount in the triploids that all the seeds produced under open-pollination attained to 27.8 per plant, and those obtained by the crossing, triploid F_1 (♀) × B. *pekinensis* (♂), to only one per 365 flowers treated.

d. Chromosome constitution in F_2 progeny of the trigenomic triploid hybrid

Cytological observation of PMCs in some of the F_2 plants obtained by backcrossing and as well as under open-pollination disclosed the following facts :
(1) Only one F_2 plant could be obtained by the backcrossing. It showed $10_{II}+17_I$ in its chromosome configuration at metaphase-I, revealing that its genome constitution was *aabc* and also that it had been derived from the F_1's unreduced egg cell. (2) The 4 plants of the F_2 progeny produced under open-pollination were found to have 54, 53, 45, and 32 chromosomes in somatic, respectively. One plant with 54 and the other with 53 chromosomes showed 27_{II} and $26_{II}+1_I$ at metaphase-I, respectively, and they showed rather morphological resemblance to the trigenomic hexaploid form, suggesting in all probability that these two plants had developed from the triploid (2n) or (2n−1) egg fertilized by a hexaploid (n) or (n-l) pollen-grain. One of the remaining two had 45 chromosomes in somatic, showing $20_{II}+5_I$ configuration at metaphase-I. Its morphological features made it conceivable that it may be a plant produced from a triploid (2n−1) egg fertilized by a (n) pollen-grain of B. *napus*. The other plant with 32 chromosomes, though its exact karyological nature could not be observed, was deplorably undergrown and showed marked sterility. It was morphologically more resemblant to the B. ***carinata*** than to any other species in *Brassica*, not giving any evidences with its parentage at all. Through the cytological examinations carried out with 4 F_2 plants treated above and through the morphological observations of their sister plants, it was made clear that the majority of the fertile female gametes formed in the trigenomic triploid hybrid have been composed of 27 chromosomes or a little more or less.

3. Trigenomic hexaploid

The major diameter of a stomatal guard-cell and the number of chloroplasts in a pair of cells in the original F_1 triploid, in its F_1 amphidiploid, and in their parental species are given for comparison in Table 7. The hexaploid F_1 plant was vegetatively well-grown and more vigorous than the original triploid F,.

Table 7. Comparison of major diameters of stomata1 guard-cells and number of chloroplasts in a pair of cells.

Species and hybrids	Genome (2n)	Average diameter of guard-cells (micrometer unit)	Number of cells measured	Average number of chloroplasts in a pair of cells	Number of cells observed
B. pekinensis	*aa*	14.5 ± 1.1	60	10.6 ± 1.5	92
B. carinata	*bbcc*	19.3 ± 1.0	59	19.9 ± 2.1	101
cpF_1 1	*abc*	16.0 ± 0.9	40	15.8 ± 2.0	102
cpF_1 2	*aabbcc*	22.0 ± 1.7	118	23.8 ± 2.8	91

a. Cytology

In the hexaploid form the meiotic divisions in PMCs were expected to proceed rather regularly, but at metaphase-I there appeared some quadri-, tri-, and univalents together with a number of bivalents, indicating that the selective pairing of homologous chromosomes was not correctly; the regular configuration with 2'7 bivalents was encountered in 36.4 per cent of the cells examined (Table 8, Fig. 18).

Table 8. Chromosome pairings at metaphase-I in F_1 amphidiploid hybrid.

Configuration	Frequency	Percentage
27_{II}	8	36.4
$26_{II}+2_{I}$	3	13.6
$1_{III}+25_{II}+1_{I}$	2	9.1
$1_{IV}+25_{II}$	5	22.7
$2_{IV}+23_{II}$	1	4.5
$3_{IV}+21_{II}$	2	9.1
$3_{IV}+20_{II}+2_{I}$	1	4.5
Total	22	100

Table 9. Occurrence of univalents at metaphase-I in F_1 amphidiploid hybrid.

No. of univalents	0	1	2	3	4	5	Total	Average number of univalents per cell
Frequency	37	6	9	1	2	1	56	0.71
Percentage	66.1			33.9			100	

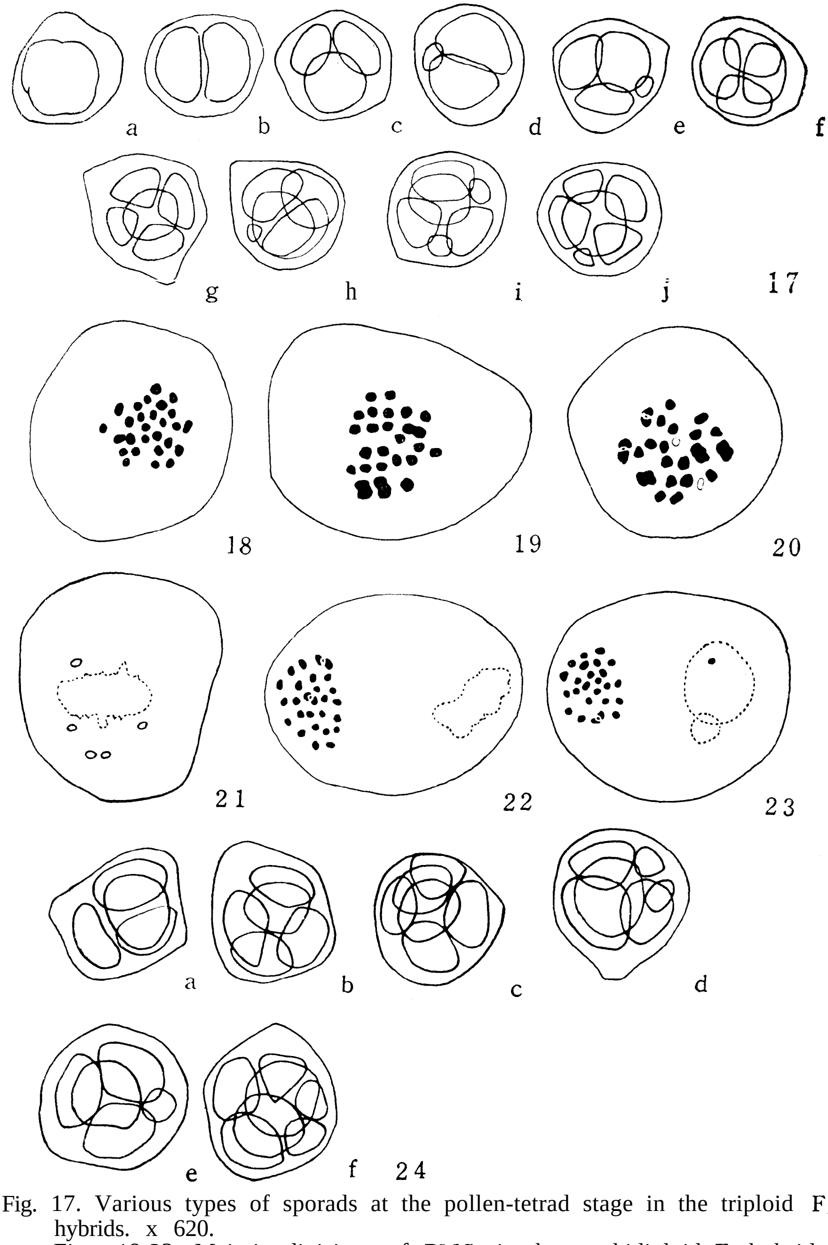


Fig. 17. Various types of sporads at the pollen-tetrad stage in the triploid F_1 hybrids. x 620.

Figs. 18-23. Meiotic divisions of PMCs in the amphidiploid F_1 hybrids. x 1300. Fig. 18. metaphase-I, 27_{II}. Fig. 19. metaphase-I, 3_{IV} t-21,. Fig. 20. metaphase-I, $3_{IV} + 20_{II} + 2_{I}$. Fig. 21. metaphase-I, 5 univalent chromosomes. Fig. 22. metaphase-II, daughter nuclear plate having 27 chromosomes. Fig. 23. metaphase-II, tripolar segregation. Fig. 24. various types of sporads in the pollen-tetrad stage in the amphidiploid F_1 hybrids. x 620.

Multivalents appeared in 50.0 per cent of the cells examined, and univalents in 33.9 per cent of the cells (Tables 8, 9; Figs. 19–21). The regular number of chromosomes, 27, at a metaphase-II plate, was observed only in 48.8 per cent of the plates, and one or two above or below 27 in the rest plates (Table 10, Figs. 22, 23). The hexaploid plant, unlike the triploid one, produced normal tetrads with certain abnormal sporads, i.e., pentads, hexads and heptads, but any one monad, diad, or triad was not encountered at all (Table 5, Fig. 24).

Table 10. Distribution of chromosomes at metaphase-II in F_1 amphidiploid hybrid.

No. of chromosomes	25	26	27	28	29	Total	Average number of chromosomes per plate
Frequency	3	8	21	10	1	43	27.0
Percentage	7.0	18.6	48.8	23.3	2.3	100	

b. Fertility

Contrary to the author's expectation, the trigenomic hexaploid form induced was not fairly fertile and its pollen-fertility was much reduced and unstable, with its stamens deteriorated in a peculiar way. This decline of stamens, a phenomenon common to all individuals belonging to three different strains (cpF_1 2, 3 and 4), varied in its extent from individual to individual, and from flower to flower in the same plant: all the 6 stamens were malformed in extreme case; and the filaments were normal with some stamens, but the anthers shriveled in some others. Such retrogression was far more extensive in the earlier half of the flowering season than in the later half, and its degree varied with plants in different environmental conditions. The average pollen- and the average seed-fertility of the hexaploid F_1 plants were 38.8 per cent and 36.3 per cent, respectively (Table 18).

4. Progeny of trigenomic hexaploid form

a. Progeny obtained by successive self-pollination

The F_2 progeny obtained by self-pollination showed no small individual difference in the color of leaves, in the size of leaf-lobes, and in the shade of the anthocyan color developed on stalks. These morphological variations were found not only in the aneuploid, but also in the eu-hexaploid individuals. Generally speaking, the retrogression of stamens was observed in the F_2 euploid progeny, but in a very slight or in hardly traceable degrees in the F_2 aneuploid progeny.

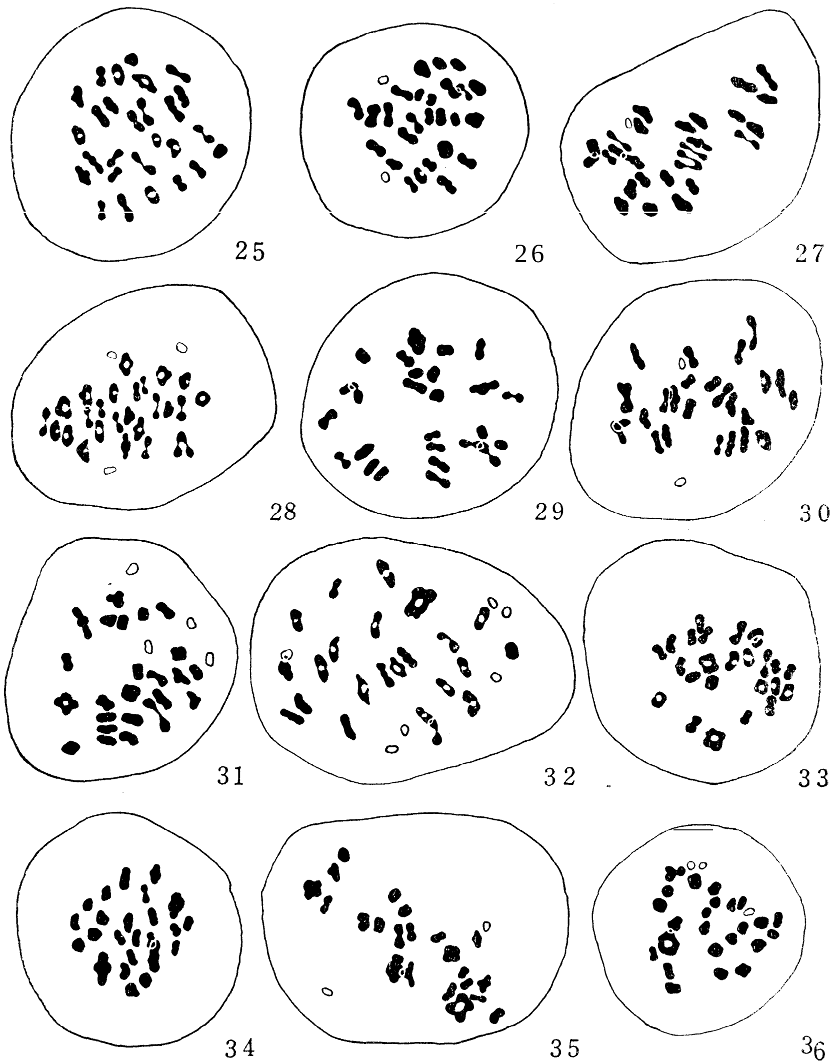
This tendency of the retrogression of stamens in the F_2 was observed exclusively in the successive F_3–F_5 generations.

The meiotic irregularity, as mentioned above, in the hexaploid F_1 brought about the formation of aneuploid individuals in the F_2 progeny. The number of chromosomes was exactly 54 (6x) with 10 out of 13 F_2 plants examined and 55 or 53 (6x$\pm$1) with the rest 3 plants. The results of the observation of these 6x plants at metaphase-I of PMCs, are given in Table 11, showing that the appearance of the normal 27_{II} configuration (Fig. 25) was less frequent, i.e., 24.4 per cent on the average, in the F_2 than in the F_1, and that the appearance of multivalent (tri- and quadrivalent) chromosomes was more frequent, 6 or

Table 11. Frequency occurrence of various chromosome associations at metaphase-I of PMCs in F_2 eu-hexaploid plants.

Configuration	F_2 Plants examined									Total	Percentage
	4-1	4-2	4-4	4-8	4-9	4-11	4-13	4-15	4-16		
27_{II}	3	5	7	3	10	17	4	5	10	64	24.4
$26_{II}+2_{I}$	3	3	2	1	7	6	2	2	5	31	11.8
$25_{II}+4_{I}$	—	--	1	-	-	1	1	1	1	5	1.9
$1_{III}+25_{II}+1_{I}$	4	2	2	—	7	—	—	1	—	16	6.1
$1_{III}+24_{II}+3_{I}$	3	1	—	—	2	—	—	—	—	6	2.4
$1_{IV}+25_{II}$	2	7	5	2	6	10	5	2	3	42	16.0
$1_{IV}+24_{II}+2_{I}$	5	--	1	--	4	9	1	1	5	26	9.9
$1_{IV}+23_{II}+4_{I}$	—	—	—	—	—	—	1	–	–	1	0.4
$1_{IV}+22_{II}+6_{I}$	—	—	—	—	1	—	—	—	—	1	0.4
$2_{IV}+23_{II}$	4	4	3	2	4	6	6	3	4	36	13.7
$2_{IV}+22_{II}+2_{I}$	2	1	1	—	1	1	—	—	2	8	3.1
$3_{IV}+21_{II}$	1	-	1	1	-	1	2	1	1	8	3.1
$3_{IV}+20_{II}+2_{I}$	—	—	—	—	--	1	-	-	-	1	0.4
$4_{IV}+19_{II}$	—	—	—	—	—	—	—	—	1	1	0.4
$4_{IV}+18_{II}+2_{I}$	—	—	—	—	—	1	—	--	—	1	0.4
$1_{IV}+1_{III}+23_{II}+1_{I}$	1	1	1	—	3	—	1	1	—	8	3.1
$1_{IV}+1_{III}+22_{II}+3_{I}$	—	—	—	1	—	—	—	—	—	1	0.4
$2_{IV}+1_{III}+21_{II}+1_{I}$	—	1	—	—	3	—	—	—	—	4	1.5
$3_{IV}+1_{III}+19_{II}+1_{I}$	—	—	—	—	1	—	—	—	—	1	0.4
$4_{IV}+2_{III}+15_{II}+2_{I}$	—	—	—	—	1	—	—	—	—	1	0.4
Total	28	25	24	10	50	53	23	17	32	262	100
Number of trivalent chromosomes per cell	0.29	0.20	0.13	0.10	0.36	0.0	0.04	0.12	0.0	0.14	(Average)

less per cell, in the F_2 than in the F_1 (Figs. 26, 27). On the other hand, the frequency appearances of trivalents in the F_2 varied from 0 to 0.36 per cell with the 9 individual plants examined (Table 11).



Figs. 25-36. Meiotic metaphase-I figures of PMCs in the eu-hexaploid F_2 plants. x 1300.
Fig. 25. 27_{II}. Fig. 26. $26_{II}+2_{I}$. Fig. 27. $1_{III}+25_{II}+1_{I}$. Fig. 28. $1_{III}+24_{II}+3_{I}$. Fig. 29. $1_{IV}+25_{II}$. Fig. 30. $1_{IV}+24_{II}+2_{I}$. Fig. 31. $1_{IV}+31_{II}+4_{I}$. Fig. 32. $1_{IV}+22_{II}+6_{I}$. Fig. 33. $2_{IV}+23_{II}$. Fig. 34. $3_{IV}+21_{I}$. Fig. 33. $4_{IV}+18_{II}+2_{I}$. Fig. 36. $1_{IV}+1_{III}+22_{II}+3_{I}$.

The number of chromosomes in each metaphase-II plates was just 27 in 46.5 per cent of the plates examined and it varied between 23 and 30 in the rest (Table 12, Figs. 38-42).

Table 12. Distribution of chromosomes at meiotic metaphase-I in F_2 eu-hexaploid plants.

Plant exp. No.	Number of chromosomes								Total	Average number of chromosomes per plate
	23	24	25	26	27	28	29	30		
4— 1	—	2	—	8	22	4	—	—	36	
3	-	-	5	8	28	7	3	—	51	
4	—	2	1	15	26	12	3	—	59	
8	-	-	2	4	11	3	—	—	20	
9	1	2	4	10	15	6	1	1	40	
11	—	1	1	10	19	13	—	1	45	
13	—	1	5	11	20	14	1	—	52	
Total	1	8	18	66	141	59	8	2	303	26.8
Percentage	0.3	2.6	5.9	21.5	46.5	19.5	2.6	0.7	100	

The F_3, F_4 and F_5 plants described below were obtained by the selfing of the most fertile parent plants in the F_2, F_3 and F_4. The meiotic irregularities encountered at metaphase-I and -11 of PMCs in the eu-hexaploid plants obtained in the generations, from F_1 through F_5, are given in Tables 13, 14 and 15. The frequency appearance of the 27_{II} configuration was around 20 per cent in those plants (Table 13).

Table 13. Meiotic chromosome behaviors at metaphase-I in PMCs of eu-hexaploid plants.

Generation	Number of PMCs without uni- and multivalents (%)	Number of PMCs with multivalents (%)	Configurations with maximum number of multivalents	Total number of PMCs observed
F_1 1950	8 (36.4)	11 (50.0)	$3_{IV}+20_{II}+2_{I}$	22
F_2 1952	65 (24.5)	164 (61.9)	$4_{IV}+2_{III}+15_{II}+2_{I}$	265
F_3 1953	35 (22.2)	95 (60.1)	$5_{IV}+16_{II}+2_{I}$	158
F_4 1954	30 (25.0)	89 (74.2)	$4_{IV}+1_{III}+16_{II}+3_{I}$	120
F_5 1955	40 (22.9)	108 (61.7)	$4_{IV}+2_{III}+15_{II}+2_{I}$	175

The multivalents occurred in ca. 60 per cent of the cells examined in those F_2—F_5 plants, showing variation from 0 to 6 per cell. The number of univalents occurring simultaneously, as shown in the Table

14, attained to 5 or 6 at its maximum in every generations, while the average number per cell showed definite increase towards F_2 and the successive generations as compared with the F,. The chromosome number composing each nuclear plate of metaphase-II, varying between 23 and 30, was 27 in about 40 per cent of the plates examined in the progenies from F_2 to F,, and tended to increase its variation with the progress of generations (Table 15).

Table 14. Frequency appearance of univalents at metaphase-I in PMCs of eu-hexaploid plants.

Generation	Frequency of univalents per PMC (average)	Number of PMCs without univalents (%)	with univalents (%)	Total number of PMCs observed
F_1 1950	0—5 (0.71)	37 (66.1)	19 (33.9)	56
F_2 1952	O-6 (0.82)	133 (57.7)	112 (42.3)	265
F_3 1953	0—6 (0.80)	85 (53.8)	73 (46.2)	158
F_4 1954	o-5 (0.88)	66 (55.0)	54 (45.0)	120
F_5 1955	O-6 (0.85)	91 (52.0)	84 (48.0)	175

Table 15. Distribution of chromosomes at metaphase-II in PMCs of eu-hexaploid plants.

Generation	Number of plates with 27 chromosomes (%)	Chromosome numbers distributed in each plate min.-average-max.	Total number of plates observed
F_1 1950	21 (48.8)	25——27.0——29	43
F_2 1952	141 (46.5)	23 ——26.8——30	303
F_3 1953	105 (41.8)	23 -26.9 — 30	251
F_4 1954	42 (40.0)	24——26.5——29	105
F_5 1955	91 (41.4)	24 ——26.6——30	220

Those euploid plants produced by selfing certain aneuploids along with euploids among the progeny. Aneuploids thus induced were observed only in 23.1 per cent of the F_2 progeny examined, indicating presumably that, irrespective of its meiotic irregularities, the present trigenomic hexaploid was fairly tolerant of its pedigree culture. However, as shown in Table 16, the frequency appearance of aneuploids became higher in the F_3 and its successive generations than in the F,. The variation of chromosome number of aneuploid forms was 54 ± 1 in the F_2 and became greater progressively towards the F_3 and the later generations.

The pollen-fertility in the F_2 plants varied remarkably from plant to plant, but its extent of variation did not keep step with that of seed-fertility in the same plants. The fertility, the meiotic irregularity, and the aneuploid chromosome structure did not show any noticeable interrelationship among them with the individual plants in F_2 (Table 17). **As** stated elsewhere in the preceding page, the F_2 and subsequent

Table 16. Frequency appearance of euploid plants in the progeny of abc-trigenomic hexaploid plants.

Generation	chromosome number (2n) 47	51	52	53	54	55	56	Total number of plants examined	Percentage of euploid plants
F_1	—	—	—	—	4	—	—	4	100
F_2	—	—	—	1	10	2	--	13	76.9
F_3	1	—	2	2	6	1	—	12	50.0
F_4	—	1	2	2	4	2	1	12	33.3
F_5	—	1	1	5	6	2	--	15	40.0
Total	1	2	5	10	30	7	1	56	

Table 17. Pollen- and seed-fertilities of the F_2 progeny.

Plant exp. No.	Pollen fertility (%)	Number of viable seeds per silique	Viable seeds developed per ovule (%)	Normal configuration and its frequency at MI (%)		Chromosome number (2n)
4— 1	30.3	6.4	39.0	27_{II}	10.7	54
2	34.3	6.9	42.1	27_{II}	20.0	54
3	79.7	6.0	36.6	27_{II}*	33.3	54
4	31.7	5.2	31.8	27_{II}	29.2	54
5	63.5	7.0	42.7	$27_{II}+1_I$	26.1	55
7	67.1	3.6	21.8	$26_{II}+1_I$	21.2	53
8	13.0	8.1	49.4	27_{II}	30.0	54
9	70.5	5.8	35.4	27_{II}	20.0	54
11	79.4	4.2	25.6	27_{II}	32.1	54
12	54.8	6.1	37.2	$27_{II}+1_I$	22.7	55
13	27.9	6.5	39.6	27_{II}	17.4	54
15	39.6	4.5	27.4	27_{II}	29.4	54
16	27.7	5.5	33.5	27_{II}	31.3	54

* In this plant only 3 PMCs could be observed; $27_{II}, 1_{IV}+25_{II}, 1_{IV}+24_{II}+2_I$

progeny could be obtained by selfing of the most fertile parental plants having regular chromosome number. The fertility of the euploid and aneuploid plants in F_1—F_5 generations is shown in Tables

18 and 19. As Table 18 shows, the euploid plants in each generation were subject to remarkable individual variations in their pollen-fertility, but were relatively stable in their seed-fertility. In consequence, the continuous selecting of form for realizing the higher fertility to its offspring was of no avail. On the other hand, the pollen-fertility in aneuploid plants was definitely higher than the euploid forms and also subjected to rather slight variation in its grade (Table 19). The seed-fertility of the aneuploid plants was about equal to that of the euploid. In fact, all comparative evaluations with the fertility in

Table 18. Pollen- and seed-fertilities of the euploid progeny of trigenomic hexaploid plants.

Generation	Pollen-fertility min.-mean-max. (%)	Seed-fertility: Number of viable seeds per silique min.-mean-max.	Seed-fertility: Viable seeds developed per ovule min.-mean-max. (%)	Total number of plants examined	Year
F_1	33.5-38.8-44.1	6.0-6.1-6.1	36.0-36.3-36.5	2	1951
F_2	13.0-43.4-79.7	4.2-5.9-8.1	25.6-36.0-49.4	10	1953
F_3	20.6-37.0-68.1	3.5-5.0-6.9	21.3-30.2-42.1	6	1954
F_4	24.2-35.2-70.5	7.2-8.4-8.8	43.9-51.5-59.8	4	1955
F_5	10.0-41.6-62.9	3.8-5.7-7.2	23.2-34.8-43.9	6	1956

Table 19. Pollen- and seed-fertilities of the aneuploid progeny of trigenomic hexaploid plants.

Generation	Pollen-fertility min.-mean-max. (%)	seed-fertility: Number of viable seeds per silique min.-mean-max.	seed-fertility: Viable seeds developed per ovule min.-mean-max. (%)	Total number of plants examined	Year
F_2	54.8-61.8-67.1	3.6-5.6-7.0	21.8-33.9-42.7	3	1953
F_3	67.2-76.0-82.5	3.8-5.3-7.0	23.0-32.1-43.8	6	1954
F_4	62.3-73.0-80.5	3.6-5.5-6.2	22.5-31.1-40.5	8	1955
F_5	52.9-68.6-75.3	2.9-5.0-6.4	19.0-33.3-39.0	9	1956

F_2—F_5 generations showed clearly that as regards fertility phenomenon there was nothing to distinguish between the aneuploid and the euploid plants.

b. Progeny obtained by successive open-pollination

Among the F_2 plants produced under open-pollination, unlike those produced by selfing, there appeared a certain number of morphologically different plants which could readily be identified as those derived from the F_1 plant cross-pollinated with some other ***Brassica*** forms. By their morphological features most of these hybrid-type F_2 plants gave the impression that they might have the 4x-B. *cernua* (Yamashiona) as their pollen provider, which had been cultivated extensively in the experimental farm quite adjacent to the F_1 plants. The chromosome pairings at metaphase-I were complicated in these plants. The frequent appearance of multivalents, trivalents in particular, in their PMCs was likely to verify the foregoing impression. Each individual belonging to this group was vigorous in its growth, with none of their stamens retrogressing, and it was not inferior in its seed-fertility as compared with any F_2 eu-hexaploid individuals. It was revealed, moreover, that the progeny of these hybrid-type F_2 plants became more and more fertile through the generations, with a subsequent gradual reduction in size and uniformity of their seeds, and that those individuals became, in turn, to show definite resemblance to B. *cernua* in

Table 20. Frequency occurrence of various chromosome associations at metaphase-I of PMCs in F_2 aneuploid plants.

A) 6x-t-l plants

Configuration	Plants examined		Total	Percentage
	4–5	4–12		
$27_{II}+1_I$	12	10	22	24.4
$26_{II}+3_I$	5	6	11	12.2
$1_{III}+26_{II}$	4	3	7	7.8
$1_{III}+25_{II}+2_I$	3	2	5	5.6
$1_{III}+24_{II}+4_I$	—	1	1	1.1
$1_{IV}+25_{II}+1_I$	7	9	16	17.8
$1_{IV}+24_{II}+3_I$	4	4	8	8.9
$1_{IV}+23_{II}+5_I$	—	1	1	1.1
$2_{IV}+23_{II}+1_I$	3	1	4	4.4
$2_{IV}+22_{II}+3_I$	3	—	3	3.3
$3_{IV}+21_{II}+1_I$	2	2	4	4.4
$4_{IV}+19_{II}+1_I$	—	1	1	1.1
$1_{IV}+1_{III}+23_{II}+2_I$	2	1	3	3.3
$2_{IV}+1_{III}+22_{II}$	1	1	2	2.2
$2_{IV}+1_{III}+21_{II}+2_I$	—	1	1	1.1
$3_{IV}+1_{III}+19_{II}+2_I$	—	1	1	1.1
Total	46	44	90	100

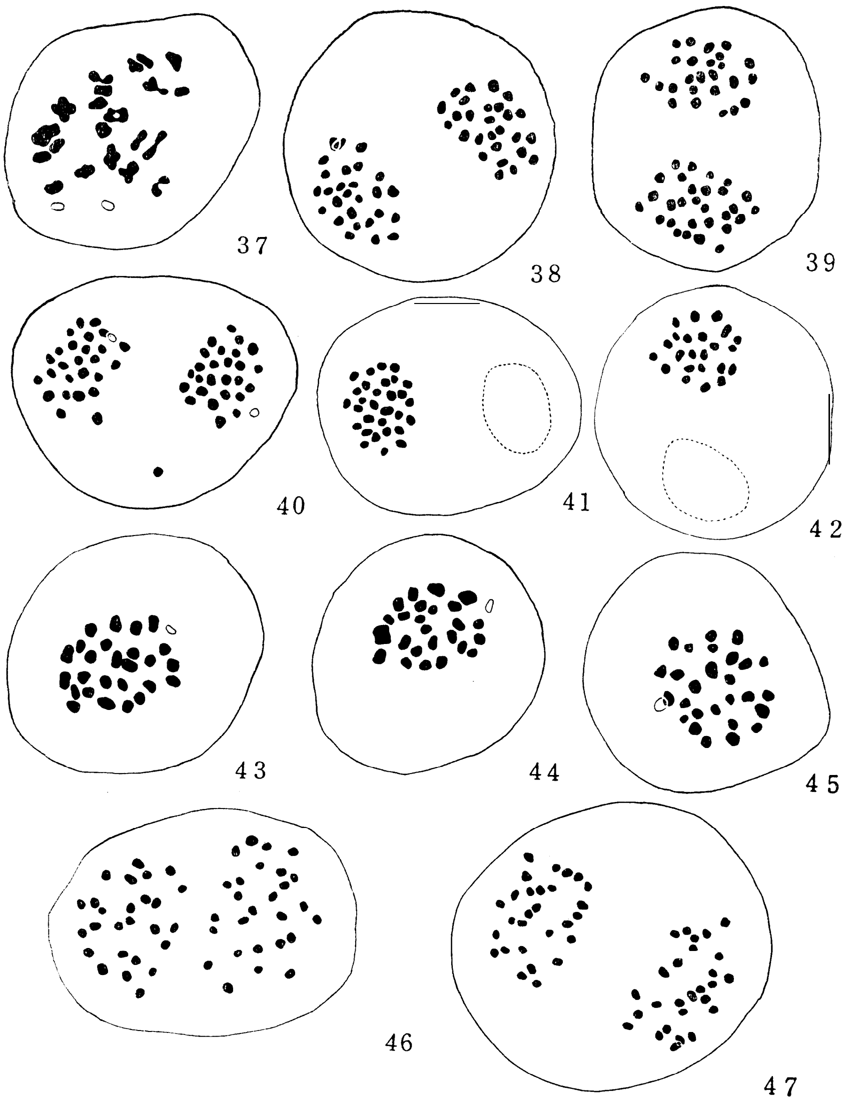
B) 6x-l plant

Configuration	Plant examined 4-7	Percentage
$26_{II}+1_{I}$	14	21.2
$25_{II}+3_{I}$	6	9.1
$1_{III}+25_{II}$	1	1.5
$1_{III}+24_{II}+2_{I}$	2	3.0
$2_{III}+22_{II}+3_{I}$	1	3.0
$1_{IV}+24_{II}+1_{I}$	12	18.2
$2_{IV}+22_{II}+1_{I}$	10	15.2
$3_{IV}+20_{II}+1_{I}$	6	9.1
$3_{IV}+19_{II}+3_{I}$	5	7.6
$5_{IV}+16_{II}+1_{I}$	1	1.5
$1_{IV}+1_{III}$ -t-23,,	1	1.5
$1_{IV}+1_{III}+22_{II}+2_{I}$	1	1.5
$2_{IV}+1_{III}+21_{II}$	1	1.5
$2_{IV}+1_{III}+20_{II}+2_{I}$	2	3.0
$2_{IV}+1_{III}+19_{II}+4_{I}$	2	3.0
$3_{IV}+1_{III}+18_{II}+2_{I}$	1	1.5
Total	66	100

their morphological features. Though the F_4 was the latest of generations of which the author could examine, and cytological observations could not carry out with all the individuals belonging to the F_3 and F_4, from their morphologic and fertility characters observed it could be duly suggested that the above F_4 generation produced through thrice-repeated open-pollinations was composed of a group of hyper-aneuploid forms, each of which was quite similar in its genome constitution to *aabb* of B. *cernua* or nearly so.

5. Progeny of trigenomic aneuploid ($6x\pm 1$) F_2 plants

The F_2 progeny by a selfed F_1 plant was composed of a number of euploid plants and certain aneuploid, i.e., 6x+ 1 or 6x- 1, ones (Table 16). These aneuploids were hardly distinguishable from the euploids in their morphological and fertility characters. The frequent appearance of aneuploids in the next selfed generation of the eu-hexaploids (Table 16) made it necessary to examine and determine with the progeny raised by the selfing of aneuploids the detailed meiotic behaviors and the fertility phenomena, and, in consequence, to follow up the cytogenetical procedures with which a certain aneuploid



Figs. 37–42. Meiotic divisions of PMCs in the eu-hexaploid F_2 plants. × 1300. Fig. 37. metaphase-I, $4_{IV}+2_{III}+15_{II}+2_{I}$. Fig. 38. metaphase-II, 27-27 segregation. Fig. 39. metaphase-II, 29-25 segregation. Fig. 40. metaphase-II, 27-l-27 segregation with 2 monad chromosomes. Fig. 41. metaphase-II, daughter nuclear plate having 30 chromosomes. Fig. 42. metaphase-II, daughter nuclear plate having 23 chromosomes.

Figs. 43-47. Meiotic divisions of PMCs in the 6x-l (F_2 4–7) and the 6x-k 1 (F_2 4-5) plants. x 3.300.

Figs. 43 and 44. metaphase-I in 6x-l plant, $26_{II}+1_{I}$ and $1_{IV}+24_{II}+1_{I}$ respectively. Fig. 45. metaphase-I in 6x-t- 1 plant, $27_{II}+1_{I}$. Fig. 46. metaphase-II in 6x-l plant, 26-27 segregation. Fig. 47. metaphase-II in 6x+ 1 plant, 27-28 segregation.

could return to the eu-hexaploidy and the others not.

The meiotic irregularity shown by the F_2 aneuploids was no more conspicuous as compared with those of the F_2 eu-hexaploids (Table 20, 21; Figs. 43-47).

Table 21. Distribution of chromosomes at meiotic metaphase-II of PMCs in F_2 aneuploid plants.

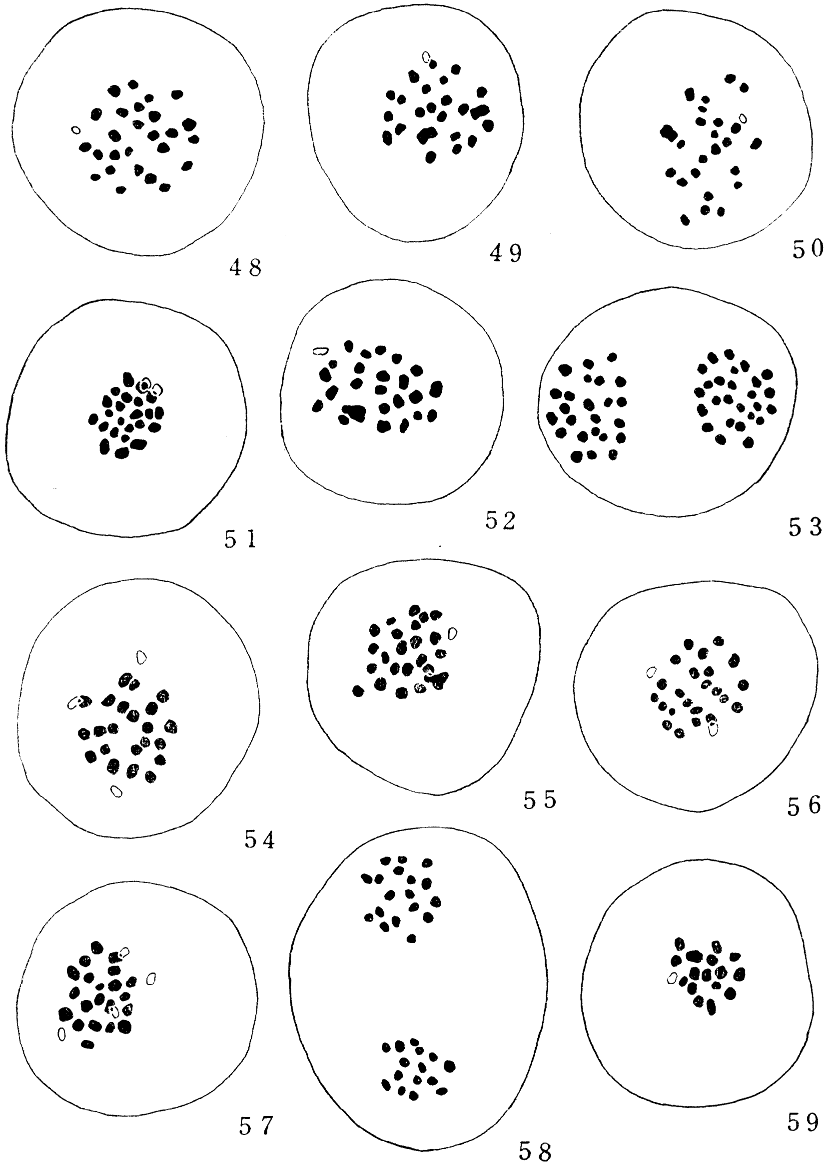
Plant No.	Chromo-some number	Number of chromosomes 24	25	26	27	28	29	30	Total number of plates	Average number of chromosome
4—5	55	2	5	6	9	5	2	1	30	
12	55	—	2	3	14	8	3	—	30	
Total		2	7	9	23	13	5	1	60	27.0
Percentage		3.3	11.7	15.0	38.3	21.7	8.3	1.7	100	
4—7	53	—	4	22	18	4	—	1	49	26.5
Percentage		—	8.2	44.9	36.7	8.2	—	2.0	100	

a. The progeny raised by the selfing of 6x+1 plant

Observation of the meiosis in 5 F_3 individuals in the progeny of a 6x+1 plant (F_2 4-5) disclosed, as shown in Table 22-A, that 2 plants were 6x and the remaining ones were 6x+ 1, 6x-1, and 6x-3, respectively, The usual occurrence of 3 univalents at metaphase-I in F_3 1A–3 (6x-3) was likely to indicate that in this plant there occurred a triple chromosome deficiency with each of 3 different pairs out of 27. All the aneuploids treated showed the higher pollen-fertility than the 6x form, but the seed-fertility was about equal to the 6x, excepting 6x-3 plant (Table 22-A).

b. The progeny raised by the selfing of 6x-l plant

(i) F_3 generation: The F_3 seeds obtained from F_2 4-7 (6x-l) were sown in two separate groups, i.e., the larger-sized seeds (group A) and smaller-sized ones (group B). Three individuals (F_3 l-A3, A6, A13) chosen at random among the plants grown up from group A seeds proved to be 6x-3, 6x-3, and 6x-1, respectively, and another three (F_3 1–B1, B3, B6) chosen at random among those group B seeds were 6x-4, 6x-3, and 6x-6, respectively (Table 22-B). It was thus found that a seed of aneuploid composition dwindles in size, as a rule, corresponding to the reduced somatic chromosome number. It may be of particular interest that any one exact 6x plant could not be obtained among the F_3 plants examined.



Figs. 48-59. Meiotic divisions of PMCs in the trigenomic aneuploid progeny raised by self -pollinations. × 1300.
Fig. 48. metaphase-I in F_3 1-A13, $26_{II}+1_I$. Fig. 49. metaphase-I in F_3 1-A3, 2_{IV} -r-21,, + 1_I. Fig. 50. metaphase-I in F_3 1-A6, $1_{IV}+23_{II}+1_I$. Fig. 51. metaphase-I in F_3 1-B6, $23_{II}+2_I$. Fig. 52. metaphase-I in F_3 1-B1, $1_{III}+23_{II}+1_I$. Fig. 53. metaphase-II in F_3 1-Bl, 24-26 segregation. Fig. 54. metaphase-I in F_4 13-9, $22_{II}+3_I$. Fig. 55. metaphase-I in F_4 13-10, $1_{III}+22_{II}+1_I$. Fig. 56. metaphase-I in F_4 14-2, $23_{II}+2_I$. Fig. 57. metaphase-I in F_4 14-7, 21_{II} t-4,. Fig. 58. meta-phase-II in F_5 5II-4, 15-18 segregation. Fig. 59. metaphase-I in F_5 5II-4, 16_{II}+ 1.

Table 22. Meiotic irregularity of PMCs and fertility in the

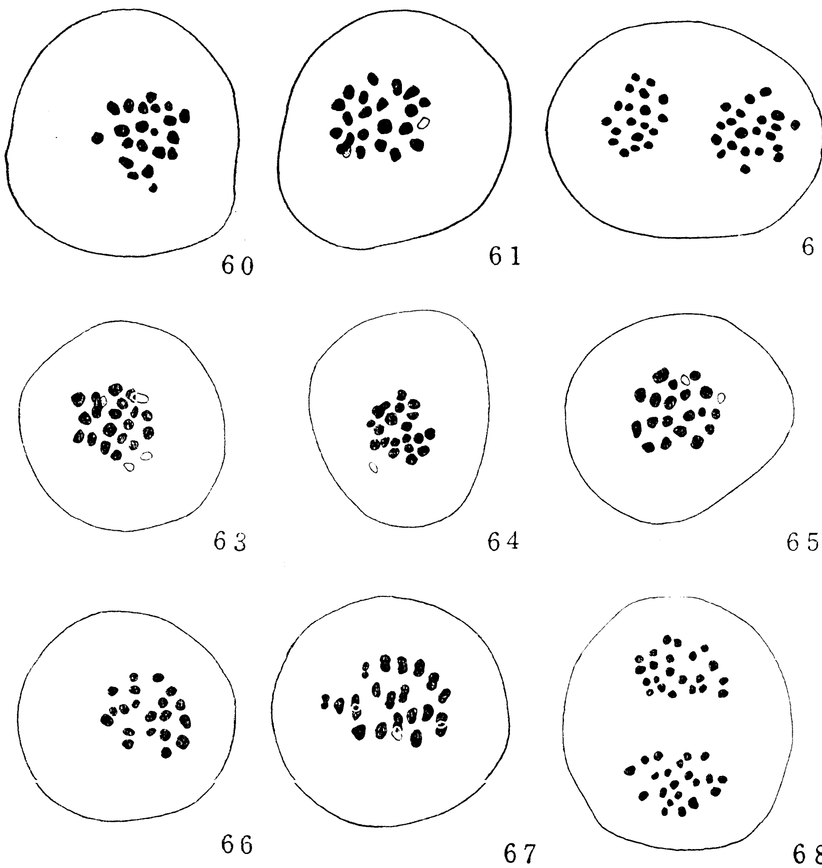
Plant exp. No.	Chromosome number (2n)	Most frequent configuration at MI (%)		Most pairing configuration at MI	Maximum number of univalent appeared in a PMC
A) Progeny of F_2 4-5 (6x+ 1) ;					
F_3 1A- 2	55	$27_{II}+1_{I}$	23.1	$5_{IV}+1_{III}+15_{II}+2_{I}$	6
1	54	27_{II}	25.0	$4_{IV}+1_{III}+17_{II}+1_{I}$	5
4	54	27_{II}	21.1	$5_{IV}+1_{III}+14_{II}+3_{I}$	5
5	53	$26_{II}+1_{I}$	31.3	$4_{IV}+1_{III}+17_{II}$	5
3	51	$24_{II}+3_{I}$	27.8	$3_{IV}+18_{II}+3_{I}$	3
B) Progeny of F_2 4-7 (6x-1) ;					
$F_3$1-A13	53	$26_{II}+1_{I}$	21.7	$4_{IV}+1_{III}+16_{II}+2_{I}$	3
A 3	51	$2_{IV}+21_{II}+1_{I}$	30.8	$4_{IV}+17_{II}+1_{I}$	5
A 6	51	$1_{IV}+23_{II}+1_{I}$	41.7	$4_{IV}+17_{II}+1_{I}$	1
B 3	51	$25_{II}+1_{I}$	33.3	$3_{IV}+19_{II}+1_{I}$	3
B 1	50	$1_{III}+23_{II}+1_{I}$	40.0	$3_{IV}+18_{II}+2_{I}$	4
B 6	48	$23_{II}+2_{I}$	50.0	$2_{IV}+1_{III}+18_{II}+1_{I}$	2
F_4 13-10	48	$23_{II}+2_{I}$	60.0	$1_{III}+22_{II}+1_{I}$	2
14- 2	48	$23_{II}+2_{I}$	50.0	$1_{IV}+21_{II}+2_{I}$	2
13- 9	47	$23_{II}+1_{I}$	24.0	$1_{IV}+1_{III}+20_{I}$	5
14- 7	46	$21_{II}+4_{I}$	35.0	$1_{IV}+19_{II}+4_{I}$	4
$F_5$5II- 3	42	$1_{III}+19_{II}+1_{I}$	61.5	$2_{IV}+16_{II}+2_{I}$	2
5III- 6	42	21_{II}	50.0	$2_{IV}+17_{II}$	2
5II - 4	33	$16_{II}+1_{I}$	50.0	$2_{IV}+12_{II}+1_{I}$	3
F_6 23-10	43	$21_{II}+1_{I}$	54.5	$1_{III}+20_{II}$	1
21- 1	40	$19_{II}+2_{I}$	51.7	$1_{III}+18_{II}+1_{I}$	3
21- 2	40	$19_{II}+2_{I}$	66.7	$1_{III}+18_{II}+1_{I}$	2

The mode of chromosome pairings at metaphase-I was specific to each of those three 6x--3 plants examined: in plant $F_3$1–B3 the chromosome configuration observed in 33.3 per cent of its PMCs was $25_{II}+1_{I}$, indicating presumably that this plant was lacking in 3 chromosomes, of which 2 composing a homologous pair; in plant $F_3$1–A3 the configuration $2_{IV}+21_{II}+1_{I}$ (Fig. 49) was observed in 30.8 per cent of its PMCs, and in plant $F_3$1–A6 the configuration $1_{IV}+23_{II}+1_{I}$ (Fig. 50) self-pollinated progenies of 6x+ 1 and 6x- 1 plants.

Total number of PMCs observed	Chromosome numbers distributed in each plate at MII min.-mean-max.	Total number of plates observed	Pollen-fertility (%)	Number of viable seeds per silique	Year
13	24 – 27.1 -30	30	71.0	5.4	1954
20	25 – 27.0 – 29	27	25.4	6.1	〃
19	24 – 26.9 – 29	31	58.3	4.9	〃
16	25 – 26.3 – 29	28	60.0	5.5	〃
18	21 – 25.6 – 27	30	65.1	3.0	〃
23	24 – 26.3 – 28	45	71.5	2.4	1954
26	23 – 25.4 – 28	30	71.6	1.4	〃
12	20 – 24.7 – 27	22	77.4	3.9	〃
6	22 – 25.2 – 27	52	70.8	3.6	〃
10	22 – 24.9 – 26	30	82.3	2.2	〃
14	21 – 24.1 – 27	56	80.6	2.9	〃
15	22 – 23.7 – 26	23	80.1	4.8	1955
14	22 – 23.9 – 26	10	81.2	4.3	〃
25	22 – 23.3 – 25	41	73.9	4.5	〃
20	21 – 22.9 – 26	25	74.4	5.6	〃
13	19 – 20.9-23	34	83.7	5.8	1956
10	20 – 21.3 – 23	25	83.3	5.9	〃
10	14 – 16.8 – 19	44	69.2	1.0	〃
11	20 – 21.4 – 23	34	79.2	—	1957
29	19 – 20.0 – 21	21	83.7	—	〃
27	18 – 19.9 – 22	31	79.9		〃

was observed in 41.7 per cent of its PMCs. These latter two aneuploid plants show in all probability that they have experienced a certain chromosomal changes such as the multiplication of some homologous chromosomes and as some structural changes in their chromosomes. The 6x-4 ($F_3$1–B1) plant, with the configuration I,,, i- $23_{II}+1_I$, occurring in 40.0 per cent of its PMCs, appeared, like the foregoing two 6x--3 plants, somewhat aberrant in its chromosome constitution (Fig. 52). The 6x-6 (F_3 1-B6) plant, with the configuration $23_{II}+2$, (Fig. 51) occurring in 50.0 per cent of its PMCs, was considered to have lost 6 chromosomes, of which 4 composing 2 pairs and the remaining 2 belonging to different 2 pairs.

The 6 plants examined were similar to show rather high pollen-fertility of 70-80 per cent, but were considerably lower than the original 6x plants in their seed-fertility (Table 22-B). There was no definite interrelation to be seen between the number of chromosome



Figs. 60-68. Meiotic divisions of PMCs in the trigenomic aneuploid progeny raised by self-pollinations. x 1300.

Fig. 60. metaphase-I in F_5 5III-6, 21_{II}. Fig. 61. metaphase-I in F_5 5II-3, $1_{III}+19_{II}+1_I$. Fig. 62. metaphase-II in F_6 21-1, 19-21 segregation. Fig. 63. metaphase-I in F_6 21-1, $1_{III}+17_{II}+3_I$. Fig. 64. metaphase-I in F_6 21-1, $1_{III}+18_{II}+1_I$. Fig. 65. metaphase-I in F_6 21-1, $19_{II}+2_I$. Fig. 66. metaphase-I in F_6 21-1, 20_{II}. Fig. 67. metaphase-I in F_6 23-10, $21_{II}+1_I$. Fig. 68. metaphase-II in F_6 23-10, 21-22 segregation.

and the grade of fertility in the F_3 aneuploid individuals examined.

(ii) F_4 generation : Both of the 2 F_4 strains, the strain F_4 13 produced by a selfing of 6x--4 (F_3 1–B1) plant and the strain F_4 14 by a selfing of 6x-6 (F_3 1–B6) plant, were undersized in plant heights and were positively distinct morphologically from any eu-hexaploid plants. Examination of these F_4 plants revealed that their chromosome numbers were 6x-6 (F_4 13–10), 6x-7 (F_4 13–9), 6x-6 (F_4 14–2), and 6x-8 (F_4 14–7), respectively. The configuration $23_{II}+2_{I}$ (Fig. 56) was found in 60.0 per cent of the PMCs in F_4 13-10 plant and in 50.0 per cent of those in F_4 14–2 plant ; and there observed infrequent occurrence of multivalents and of univalents, and thus rather regular pairings were noticed to occur in these plants. In the 6x-7 plant multivalents were of rather frequent occurrence and the configuration $23_{II}+1_{I}$ was found only in 24.0 per cent of the cells examined. And in the 6x-8 plant, there usually appeared 4 univalents in each cell and the configuration $21_{II}+4_{I}$ was found in 35.0 per cent of the PMCs examined. It appeared likely that the two 6x-6 plants were each deficient of 6 chromosomes of which 4 composed 2 pairs and the rest 2 belonged to 2 different pairs, and that the 6x-7 plant was deficient of 7 chromosomes of which 6 composing 3 different pairs and the rest one belonging to another pair, and that the 6x-8 plant was deficient of 8 chromosomes of which composing 2 pairs and the rest 4 belonging to 4 different pairs.

Those 4 F_4 plants behaved similarly to the F_3 plants in the pollen-fertility, they showed rather higher seed-fertility than the F_3 plants. Moreover, those 4 F_4 plants were about equal to each other in the pollen-fertility, but the 6x-8 plant, having the lowest number of chromosomes among them, showed, in contrast, the highest seed-fertility.

(iii) F_5 generation : Both of the 2 F_5 strains, the strain F_5 511 produced by a selfing of 6x-6 (F_4 13-10) plant and the strain F_5 5111 produced by a selfing of 6x-8 (F_4 14-7) plant, were morphologically akin to the *B. carinata* than the original hexaploids. Examination of 3 of the F_5 plants revealed that their chromosome constitutions were 6x–12 (F_5 5II–3), 6x-12 (F, 5III–6), and 6x-21 (F_5 5II–4), respectively. In plant F_5 5II–3 the configuration $1_{III}+19_{II}+1_{I}$ (Fig. 61) was found in 61.5 per cent of its PMCs examined, making it conceivable that this plant had a set of trisome occurring in its chromosome constitution. Plant F_5 5111-6, with the configuration 21_{II} (Fig. 60) occurring in 50.0 per cent of its PMCs, appeared to have lost 6 pairs of chromosomes. Plant F_5 5II–4, which was likely to lack 20 chromosomes composing 10 pairs and one belonging to another pair, revealed the configuration $16_{II}+1_{I}$ in 50.0 per cent of its PMCs and had no serious meiotic irregularity occurring in it.

The two 6x-12 plants, having different chromosome constitution each other, were both remarkably high in pollen- and seed-fertility. On the other hand, the 6x-21 plant was extremely low in fertility.

(iv) F_6 generation : The 3 F_6 plants examined were Plant F_6 21-1 and F_6 21-2, each with Plant F_5 511-3 as its parent, and other Plant F_6 23-10, with Plant F_5 5III-6 as its parent. The two 6x-14 (F_6 21-l and F_6 21-2) plants were alike in their morphological features, in the grade of their fertility, and in the meiotic behavior of chromosomes. At metaphase-I of these plants the configuration $19_{II}+2_I$ (Fig. 653 was found in more than 50 per cent of their PMCs, indicating presumably that 12 out of their 14 chromosomes lost composed 6 pairs and the rest 2 belonged 2 different pairs. In the two 6x-14 plants no quadrivalent was observed at metaphase-I of PMCs, but a single trivalent made its occasional appearance (Figs. 63, 64). The 6x-11 (F, 23-10) plant, with the configuration $21_{II}+1_I$ (Fig. 67) occurring in 54.5 per cent of its PMCs, was considered to have lost 11 chromosomes, of which 10 composing 5 pairs an.d the rest 1 in another pair. In this plant, as in the two 6x-14 plants, only a single trivalent appeared occasionally along with regular bivalents at metaphase-I.

The 3 F_6 plants examined were all about 80 per cent in their pollen-fertility (their seed-fertility remained undetermined).

Discussion

1. Meiosis in the trigenomic tri- and hexaploid plants

a. Trigenomic triploid plants

As stated elsewhere in this report, a natural or an artificial digenomic species crossed with a monogenomic species gave birth to a trigenomic triploid F_1 hybrid. Such F_1 hybrids so far obtained between a natural digenomic species and a monogenomic species were as follows : *B. chinensis-B. carinata* F_1 (Morinaga, 1932; Howard, 1942), *B. carinata-B. pekinensis* F_1 (Iwasa, 1951), *B. carinata-B. campestris* F_1 (Mizushima, 1952). But the F_1 hybrids so far obtained between an artificial digenomic species and a monogenomic species were produced only from the cross combination, artificial *B. napus-B. nigra* (Mizushima, 1952).

The genomes *a, b,* and c were partially homologous among each others, as has been pointed out by several workers (Morinaga and Fukushima, 1933; U, 1935 ;Haga, 1938 ; Sikka, 1940 ; Fukushima, 1945 ; Mizushima, 1952, etc.). The maximum numbers of bivalents formed in F_1 hybrids between two monogenomic species and those in the spontaneous haploid plants of certain digenomic species have been reported by some workers (see Table 23). According to these facts, the trigenomic triploid, a *b* c, are likely, if the quite rare occurrence of autosyndetic chromosome pairings is to be taken into account, to produce allosyndetic pairings not infrequently. Observations given by

Table 23. The maximum numbers of bivalents formed in F_1 hybrids between two monogenomic species and those in the spontaneous haploids of digenomic species.

	Genomes	Chromosome pairing in meiosis	Authors
A) F_1 hybrid	*ab*	$(0-5)_{II}$ -t- (18-Q	Olsson (1960)
	bc	$(0-4)_{II}+(17-9)_{I}$	Mizushima (1952)
	ac	$(0-8)_{II}+(19-3)_{I}$	u (1935)
B) Haploid	*ab*	$(0-2)_{II}+(18-14)_{I}$	Mizushima (1944)
	bc	$(0-3)_{II}+(17-11)_{I}$	Kriyama and Watanabe (1955)
	ac	$(0-7)_{II}+(19-5)_{I}$	Morinaga and Fukushima (1933)

some workers on the chromosome pairings in such abc-trigenomic triploid forms will be summarized in the following:

Authors		MI configurations
Morinaga (1938)		$(1-9)_{II}+(25-9)_{I}$
Howard (1940)		"Trivalents and quadrivalents were found in addition to bivalents and univalents."
Mizushima (1952)	(1)	$(0-9)_{II}+(27-9)_{I}$
	(2)	$(7-10)_{II}+(13-7)_{I}$
The present author		$(2-9)_{II}+(23-9)_{I}$

These results are revealing that they are practically similar, excepting the cases of Mizushima (2) and Howard. It is particularly noteworthy in the case of Mizushima (2) that a F_1 hybrid synthesized from 3 monogenomic species formed larger number of bivalents at metaphase--I. Trivalents and quadrivalents appeared in the F_1 hybrids in the case of Howard show clearly that allosyndetic pairings are usually accompanied by certain autosyndetic pairings in these hybrids. The difference in the pairing quantity of chromosomes among those trigenomic F_1 hybrids may naturally be attributed to certain environmental conditions and as well as to the genetic factors differently affecting the meiotic chromosome behaviors in different plants (cf. Michaelis, 1929, in *Epilobium*; Nakamura, 1936, in *Impatiens*; Straub, 1937, in *Gasteria*; Sax, 1937, in *Tradescantea*; Kostoff, 1930, in *Nicotiana*; Beadle and McClintock, 1928, in *Zea*; Clausen, 1930, in ***Viola*** ;

Okamoto, 1957, in *Triticum*; Riley, 1958, in *Triticum*), but here, in advance, the main causal factor appears to be that there is definite differences between the extent of differentiation induced in *b* and *c* genomes constituting *B. carinata*, and that in *b* genome of *B. nigra* and c genome of *B. oleracea* (cf. Fukushima, 1945; Mizushima, 1952). The difference appeared in the pairing quantity of chromosomes among F_1 hybrids having the same genome constitution has been reported already by several workers with several plant species: Kihara and Lilienfeld (1932, 1935) in *Triticum, Aegilops* and some alied forms; Skovsted (1937) in *Gossypium*; Emsweller and Jones (1938) in *Allium*; Fukushima (1945) in *Raphano-Brassica*.

b. Trigenomic hexaploid plants

Excepting Mizushima's synthetic *carinata* plant, whose chromosome pairings are found to be $(0-4)_{IV}+(17-9)_{II}$ at metaphase-I, all the synthetic digenomic species so far produced showed the occurrence of a large number of bivalents accompanying only a few univalents at metaphase-I, so that the later meiotic processes were nearly normal in all of those species (Ramanujam and Srinivasachar, 1943; Frandsen, 1944, 1947 ; Mizushirna, 1952; Olsson, 1960). In the synthetic trigenomic hexaploid, on the contrary, the selective pairings of chromosomes were far from perfect; as shown in Tables 13 and 14, the appearance of multi- and univalents was quite common, the normal configuration 27_{II} being formed in 36.4 per cent of the PMCs examined in the F_1 hybrids and only in 22.2—25.0 per cent of those in the F_2—F_5 plants. The chromosome pairings of $(0-7)_{IV}+(27-13)_{II}$, taking place at metaphase-I in PMCs having no univalents, were observed by Mizushima (1952) in his hexaploid F_1 hybrids. The author's observation, that the number of multivalents appearing in the PMCs of his hexaploid F_1 hybrid and in those of its F_2—F_5 progeny did not exceed 6 per cell, was found to coincide in outline with Mizushima's (Table 13). In the present hexaploid F_1 hybrid and its F_2—F_5 descendants kept under observation there was seen a set of 27 chromosomes occurring at metaphase-II in 40.0-48.8 per cent of the daughter nuclear plates examined and the frequency of their occurrence was likely to decline progressively from generation to generation with the original hexaploid form, implying a concurrent rise in the frequency appearance of aneuploidal gametes in those descendants (Table 15). Howard (1942) obtained a $6x+1$ plant in the next generation of his hexaploid and Mizushima (1952), who obtained only 8 aneuploids among 28 plants examined in the F_2 progeny, was led, in turn, to the conclusion that his trigenomic hexaploids are quite tolerant of their practical pedigree cultures. The aneuploid plants educed by the author's hexaploid and found occurring among its progeny showed progressive increase in the frequency towards the later generations; i.e., 3 aneuploids among 13 F_2 descendants and became far more numerous among the F_3—F_5 descendants. Moreover, this gradual increase in the frequency occurrence of aneuploids among the F_2—F_5 plants was intimately accompanied by a corresponding rise in the variation of chromosome numbers in those aneuploids and appeared to be closely related to the meiotic irregularity, more particularly to the appearance of univalents at metaphase-I, in those parental eu-hexaploid plants produced in each successive generation of the hexaploid, as it is evident from the data given in Tables 14, 15 and 16.

Through what mechanism becomes a trigenomic hexaploid to have univalents in PMCs ? On the fact that univalents appear occasionally in the tetraploid *Primula sinensis*, but never in the diploid form, Darlington (1937) explained that the number of chromosomes is far greater in the former than in the latter plant, so that in the former the pairing of chromosomes may be intervened, the exchange of partner among them at pachytene stage will be hindered, so that their pachytene associations become to be incomplate. Such explanation implies in brief that in the tetraploid *Primula* the time-limit placed on the chromosome pairings results in the appearance of univalents. Mizushima (1952) examined several synthetic di- and trigenomic hexaploids in *Brassiceae* and found that, as a general rule, the univalents occurring in any hexaploid plant vary in their maximum number and in the frequency of their appearance with the particular degree of affinity among the concurrent genomes in the plant. He layed emphasis on the Darlington's " time-limit" hypothesis by adding that the presence of too many chromosomes retards their pairings and promoted the pairings of those semi-homologous chromosomes which are placed in a position convenient for the pairing and that a consequent reduction in the frequency of formation of chiasmata in the paired part of the homologous chromosomes increases the number of unpaired chromosomes at metaphase-I.

It is conceivable that the progressive increase in the frequency occurrence of univalents in the eu-hexaploid progeny may be ascribable in part to the structural changes produced in the chromosomes by some meiotic irregularity in these plants, and mainly to the unbalanced disjunction of quadrivalents and trivalents and also even to the formation of bivalents among semi-homologous chromosomes. If the gametes unbalanced in their chromosome constitution are concerned in fertilization by selfing, the duplication of homologous chromosomes and the inevitable loss of partner chromosomes in the zygotes will result in the appearance of quadri-, tri-, or univalents at metaphase-I in the eu-hexaploid progeny. The foregoing assumption may be supported by the fact that the eu-hexaploid F_2 plants examined were somewhat different with each other in the frequency occurrence of trivalents in their PMCs (see Table 11). This aberrant formation of gametes appeared progressively to increase in all the three successive generations (F_3—F_4) of the eu-hexaploids examined, as shown in Table 13, 14 and 15. It is probable that as a result of the frequent appearance of univafents and multivalents in the eu-hexaploids the frequency of formation of aneuploids rises in each successive progeny of the eu-hexaploids. As described above, the conspicuous meiotic irregularity of the trigenomic hexaploid and the frequent appearance of aneuploids among its progeny are indicative of the extreme difficulty for preserving the hexaploid original forms by the pedigree culture.

2. Fertility of the trigenomic hexaploid

Any artificially raised polyploid is generally low in its fertility as demonstrated in many species. This low fertility is supposed to result mainly from the meiotic irregularity in polyploids. On the other hand, the milder the meiotic irregularity of the original diploid hybrid, the severer is that of its tetraploid form produced, and vice ***uersa.*** Such an antagonistic relationship of the meiotic irregularity between an original diploid hybrid and its tetraploid form has been clearly detected in several species (see Darlington and Mather, 1949).

It has recently been discovered that the hexaploid wheat has in its chromosome 5B (5) a gene or genes which prevent the pairing of homoeologous, but not homologous, chromosomes (Okamoto, 1957, **1962;** Sears and Okamoto, 1958; Riley and Chapman, 1958). The fact, that the amphidiploid hybrids raised by the crosses between the diploid species usually show higher frequent occurrence of multivalents than do the natural allopolyploids, makes it probable that the gene for preventing the pairing of homoeologous chromosomes may be taken as a product of mutations at the level of the allopolyploidy.

However, as the frequent appearance of multivalents is not invariably followed by reduced fertility (e.g., Brix and Quadt, 1953, in *Dactylis*), and as the retrogression of stamens appeares in the present trigenomic hexaploid, the low fertility of the polyploids may be attributed to the variety of causal factors, such as genic, cytoplasmic, or environmental (cf., Newcomer, 1941; Greenleaf, 1942; Beaseley and Brown, 1942 ; Shifriss, 1942; Atwood, 1944).

It has been reported that an experimental attempt to raise the fertility grade in several kinds of artificial polyploids was made with some success (Randolph, 1941, in ***Zea;*** Mashima and Uchiyamada, 1955, in *Oriza*; Shimotsuma, 1961, in *Citrullus*; etc.). The tetraploids derived from certain monogenomic species of the genus *Brassica* are relatively high in fertility and could be rendered still more fertile by through the selective breeding. Kadota and Ito (1952) have succeeded by the selection procedure in raising the desirable high level of fertility in the tetraploid form of *B. pekinensis*. Swaminathan and Sulbha (1959) also succeeded through the 19 consecutive generations of the mass pedigree culture in selecting out a highly improved fertile tetraploid form of *B. campestris* and obtained the following fact that the number of chiasmata per cell was the same in the first and in the 19th generation of the tetraploid, while the number of bivalents per cell was greater and that of multivalents smaller in the 19th than in the original generation. Parthasarathy and Rajan (1953) succeeded in making the tetraploid *B. campestris* to become highly fertile as in the diploid form through a number of generations under open-pollination.

On the other hand, the synthetic amphidiploid forms in the genus *Brassica* did not show high fertility, though the meiotic division of their PMCs was nearly normal and the appearance of multivalents or univalents at metaphase--1 was also of rare occurrence, and they were often self-incompatible (Frandsen, 1947 ; Mizushima, 1952 ; Olsson, 1960 a, b). Some offsprings of synthetic *juncea* and *napus* which have been obtained by the mass selection for fertility during several generations were quite fertile, showing the stabilized meiosis and the self-compatibility (Olsson, 1960 a, b). Several digenomic hexaploid forms produced by the crosses between monogenomic and digenomic species had usually multivalents and univalents formed at metaphase-I and behaved rather infertile (Karpechenko and Bogdanova, 1937 ; Mizushima. 1952 ; Iwasa, unpublished). As stated elsewhere in a preceding page, the formation of multivalents and univalents was of frequent occurrence in the present trigenomic hexaploid, and such meiotic irregularity was progressively increased in the subsequent selfed generations. Moreover, the selections have been repeated in vain during five successive generations of this hexaploid strain for the purpose of raising their seed-fertility. All these facts in combination are likely to point out the extreme difficulty of increasing the fertility with such trigenomic hexaploid form.

3. Peculiarities on the aneuploidal progeny

Of the selfed progenies of 6x+ 1 and 6x-l plants only 18.2 per cent individuals, i.e., an unexpectedly small proportion, were 6x forms, and of the selfed progenies of 6x plants about 50 per cent or less were 6x forms, and these facts definitely indicated that in the descendants of such hexaploid strain the original genotype could hardly be maintained or inferred without the examination of chromosome counting with each individual. Moreover, as regard to the fertility grade, there was nothing to distinguish between the aneuploid and the euploid plants in the progeny of the hexaploid. These facts may imply that in the successively selfed progeny of the hexaploid the aneuploids are apt to increase in number from generation to generation and are prevented from returning to the eu-hexaploidy. Table 24 will

Table 29. Chromosome number ($2n$) of the progenies of 6x-t-l and 6x − 1 plants under self-pollination.

F_1	F_2	F_3	F_4	F_5	F_6
54	55	55			
		54,54			
		53			
		51			
	53	53	48	42	40,40
		51,51,51	47	33	
		50	48		
		48	46	42	43

show clearly that the chromosome number of the selfed progeny of a 6x-1 plant became reduced from generation to generation. In the successive progeny obtained by the open-pollination or selfing with a 6x-29 plant (Iwasa, 1963), which had been obtained from the trigenomic hexaploid plant through twice backcrossings with *B. pekinensis*, as pollen provider, the number of chromosomes became increased from generation to generation in the following way:

s
6x-22 plant (2n=32)→6x-19 plant (2n = 35)
o s↗
6x-29 plant (2n=25)→6x-22 plant (2n=32)
s↘
6x-18 plant (2n=36)

0 : open-pollination ; ***s*** : selfing.
(These aneuploids will be dealt with in detail in the another report.)

It may be presumable from these facts that in the selfed progeny of the trigenomic hexaploid the number of chromosomes may be reduced to 40 or thereabouts and that, if a more forced interpretation is to be allowed, those aneuploids may be expected to break up into the digenomic tetraploid forms such as very near to *aabb*, *bbcc*, or ***aacc*** in their genome constitution. It is duly conceivable, besides, that those descendants, if left in an aneuploidal condition through many successive generations, will have many genic and chromosomal changes produced and accumulated in them, with the probable result that they will have among their descendants a number of secondary balanced forms with a new genic balance established in them.

4. Utilization of trigenomic hexaploid

The information gained so far by the author about his trigenomic hexaploid and by several workers about the di- and trigenomic hexaplaids have led to the conclusion ; It is hopelessly difficult to meet the current need for the synthesis and breeding of new hexaploid species in the genus *Brassica*, as Mizushima (1952) and Olsson (1960) have already pointed out. However, these synthetic hexaploids may serve as valuable breeding materials because their conspicuous meiotic irregularity is very available for the new recombination of genes among different genomes, for the substitution of allochromosomes and as well as for the addition of allochromosomes (cf., Gerstel, 1945, 1946, in ***Nicotiana*** ; O'Mara, 1953, in *Triticum* and *Secale*; Hyde, 1953, in *Triticum* and *Haynaldia*).

Summary

1. The mode of meiosis, the grade of fertility, and certain cytogenetical features were observed in an artificially synthesized trigenomic hexaploid form, having all the genomes, ***a,*** *b*, and c, belonging to the genus ***Brassica,*** and in its progeny.

2. Synthesis of this trigenomic form was only possible by a cross between B. *carinata* and B. *pekinensis,* and not by a cross between any monogenomic and digenomic species, as shown in Table 2. The

crosses were made on the diploid and on the tetraploid levels. Some seeds obtained by these crosses were small-sized and the rest otherwise, showing clearly that the former were true F_1 and the latter matroclinous seeds.

3. The F_1 hybrid obtained, growing more vigorously than its parent species, were morphologically somewhat intermediate between its parent species.

4. In the trigenomic triploid F_1 hybrid, several bivalents were usually found at metaphase-I and their number per PMC ranged from 2 to 9, and various irregularities occurred continuously through the later meiotic stages.

5. With its meiotic irregularities, the trigenomic triploid F_1 hybrid was remarkably low in fertility, its pollen-fertility being 0.4 per cent in an average and the number of viable seeds attaining 27.8 per individual. The F_2 plants of this triploid hybrid showed by their morphological and chromosomal features that most of the fertilizable gametes produced in the triploid F_1 hybrid were each possessed of 27, 28, or 26 chromosomes.

6. The following were the main cytological aspects observed in the PMCs of the trigenomic hexaploid F_1 hybrid at metaphase-I, -II, and at the pollen-tetrad stage of their meioses: Rather frequent formation of multivalents and univalents at metaphase-I; appearance of the configuration 27_{II} in 36.4 per cent of the cells; occurrence of metaphase-II daughter nuclear plates, of which 48.8 per cent were normal containing 27 chromosomes, and the remaining 51.2 per cent abnormal, each plate containing 25, 26, 28, or 29 chromosomes in it; and the appearance of various kinds of sporads in addition to normal tetrads in less than 16 per cent of the cells examined.

7. The trigenomic hexaploid was not very fertile, provided with its stamens degenerating in different degree from individual to individual, and also in different periods of their growing. Such degeneration of the stamens was likely to develop from some genic interaction among the three different genomes, *a, b,* and c, constituting a hexaploid hybrid form, because the stamens in any of the aneuploids educed among the hexaploid progeny were quite normal in appearance. The average pollen- and the average seed-fertility of the hexaploid F_1 plants were 38.8 per cent and 36.3 per cent, respectively.

8. In the selfed F_2—F_5 progeny of the hexaploid hybrid, the frequency appearance of the configuration 27_{II} at metaphase-I was only 25 per cent or less and that of multivalents ca. 60 per cent of the cells examined ; the number of univalents appearing simultaneously was 0 to 6 per cell and the frequency of their appearance increased

gradually with the advance of generations in the progeny, so that only ca. 40 per cent of the metaphase-II daughter plates examined were each possessed of 27 chromosomes, and these normal plates were likely to decrease in number in the successive generations of the hexaploid strain.

The frequency appearance of aneuploid individuals was 23.1 per cent, being a relatively small in proportion, in the selfed F_2 progeny of the hexaploid and 50 per cent or more in the F_3—F_5 generations, and the variation in the number of chromosomes composing the aneuploids showed an increasing tendency towards the later generations.

The continuous selective breeding was undertaken to obtain any one hexaploid strain, which may be highly fertile, among the selfed F_2—F_5 generations has remained ineffective at all. And a comparative examination in the fertility of eu-hexaploid and aneuploid plants showed that the latter were far higher than the former in the pollen-fertility and also that the former were slightly higher or nearly equal to the latter in the seed-fertility.

9. Morphological observations of the F_2—F_4 progenies obtained by the successive open-pollinations from the hexaploid F_1 plant showed that the hexaploid F_1 plant could be easily fertilized by the pollen-grains of the tetraploid *B. cernua*, and that the hybrid-type F_2 plants thus produced, which had probably been *aaabbbc* genomes, seemed easy to collapse into the descendant plants having *aabb* or neighborhood in their genome constitutions.

10. A follow-up examination of the selfed F_3—F_6 progenies of a $6x+1$ and a $6x-1$ plants, each educed among the F_2 plants of the hexaploid F_1 hybrid, led to the following findings: Of 5 F_3 descendants from a $6x+1$ F_2 plant, 2 were 6x and the remaining 3 were $6x+1$, $6x-1$, or $6x-3$, whereas 6 F_3 descendants from a $6x-1$ F_2 plant were all $6x-(1-6)$ and not 6x. The number of chromosomes showed marked decreasing tendency towards the F_3—F_6 descendants raised by the successive selfing of $6x-1$ F_2 plant. In those aneuploidal F_3—F_6 descendants the meiotic divisions became gradually stabilized with those plants in good correspondence to the decreasing of the chromosome number, but such decrease in the number of chromosomes was not generally followed by any marked deterioration in the fertility with those plants.

11. As has been just stated above, the progeny of the trigenomic hexaploid was highly intolerant of the pedigree culture of sustaining its original hexaploid genotypic structure. But these individuals may serve as the promising breeding materials to be used in obtaining the intergenomic gene recombination and the substitution or addition of allochromosomes, if their conspicuous meiotic irregularity is to be

turned to good account.

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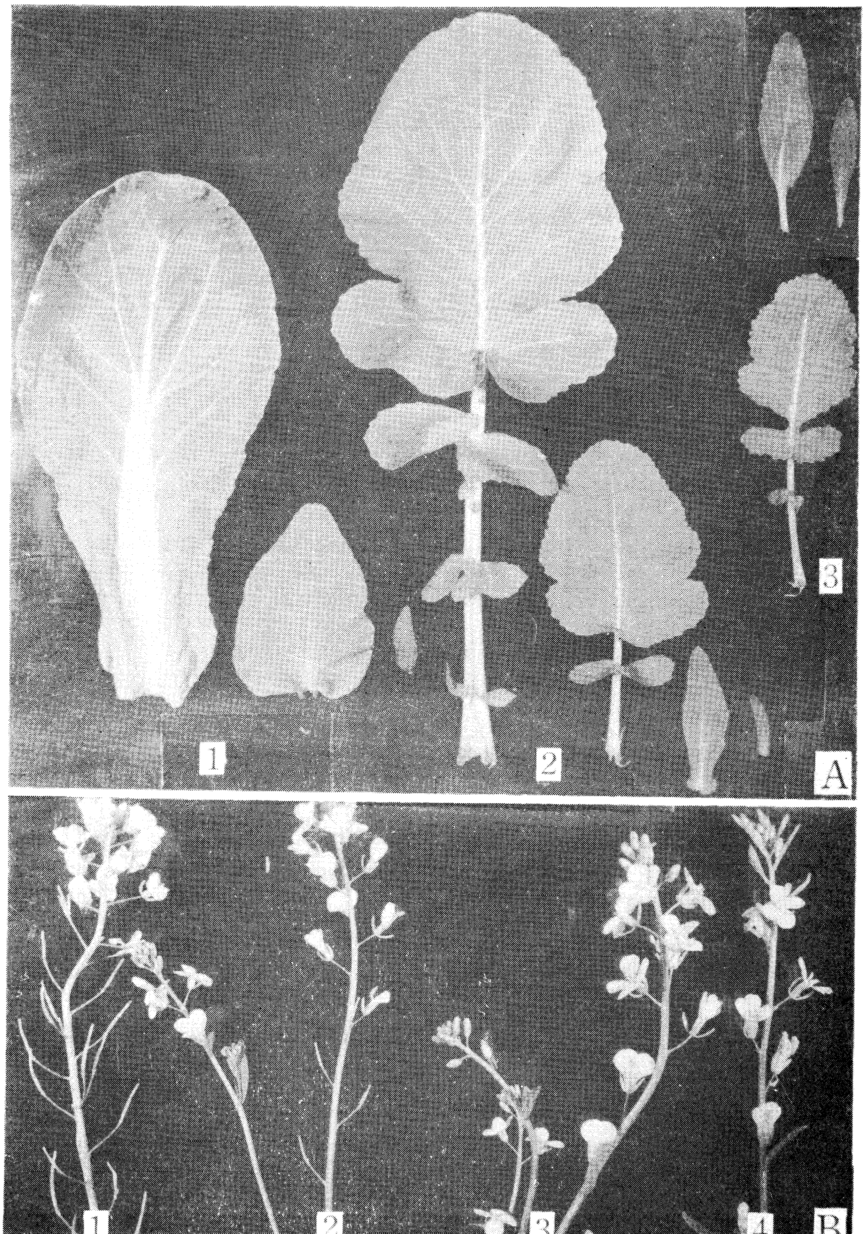
Explanation of Plate 1

Figs. A and B. Morphological characteristics of *B. pekinensis*, *B. carinata*, and of F_1 hybrid between them.

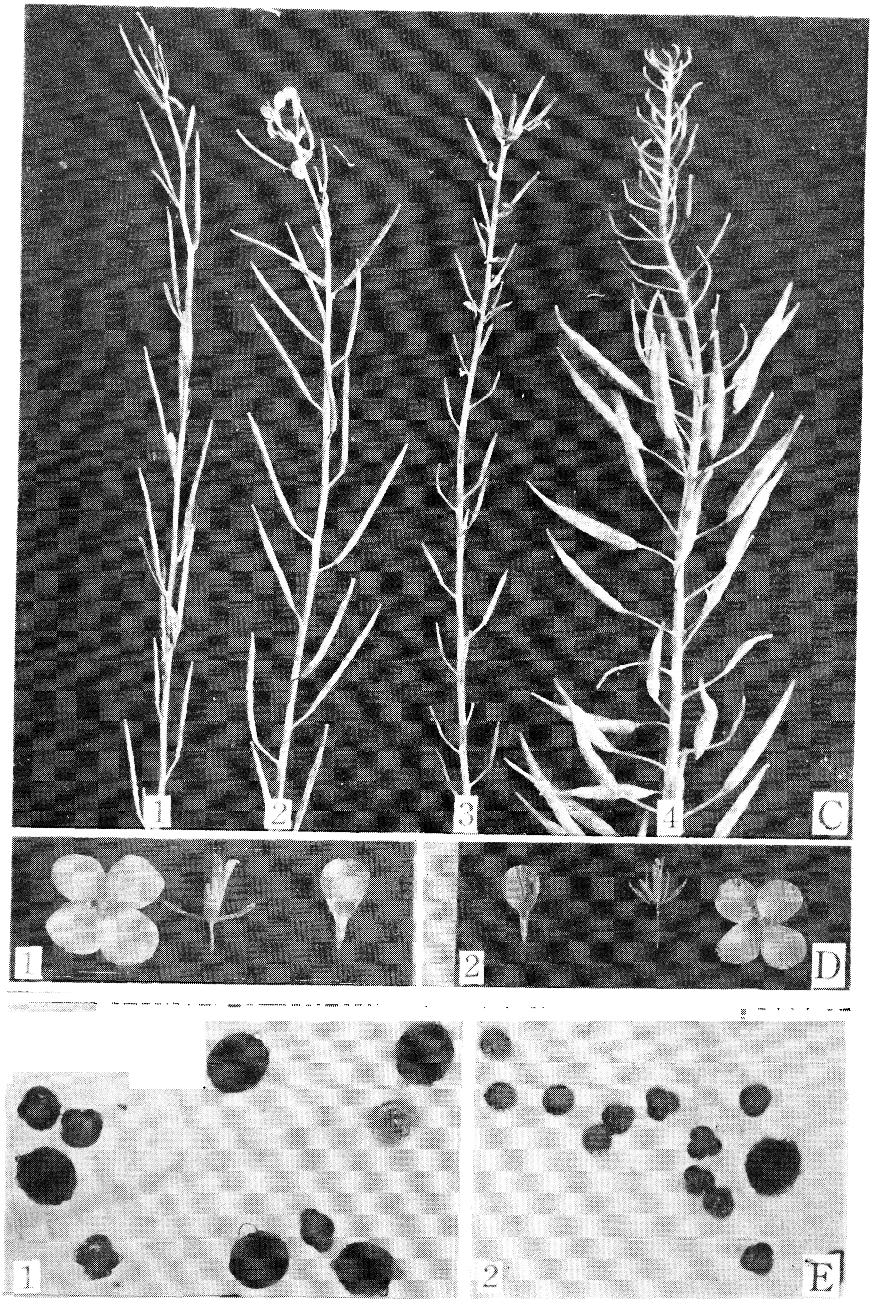
A : Leaves. 1, *B. pekinensis;* 2, F_1 hybrid ; 3, *B. carinata.*
B: Flower clusters. 1, *B. pekinensis*; 2, F_1 hybrid ; 3, amphidiploid F_1 hybrid ; 4, *B. carinata.*

Explanation of Plate 2

Fig. C. Comparison of siliques set among the parental and hybrid forms. 1, *B. carinata* ; 2, amphidiploid F_1 hybrid; 3, F_1 hybrid ; 4, *B. pekinensis.*
Fig. D. Comparison of flowers between amphidiploid F_1 hybrid and original F_1 hybrid. 1, amphidiploid F_1 hybrid; 2, original F_1 hybrid.
Fig. E. Pollen-grains stained by aceto-carmine. 1, amphidiploid F_1 hybrid; 2, original F_1 hybrid.



Trigenomic hexaploid hybrid forms in ***Brassica***



Trigenomic hexaploid hybrid forms in ***Brassica***