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# STUDIES IN POLYPLOID VARIETIES OF SUGAR BEETS

## X. Chromosomal Variation in Progeny of Crosses Involving Diploid, Triploid and Tetraploid Plants<sup>1)</sup>

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### Introduction

The triploid sugar beets which are of great promise in hybrid varieties grown for sugar production are however of little value as a source of breeding materials from the point of genealogy. However, they are well suited not only for pure cytological studies on chromosomal behavior at the triploid level but also for research on the influence of different chromosome numbers on morphological qualities. The empirical reason for this is that when triploid plants are intercrossed or surrounded by plants of other ploidy levels such as diploid and tetraploid, not a few offsprings with aneuploid chromosome numbers are born from the said triploid plants.

In the present paper the descriptions are focussed on the frequency of seedlings with different chromosome numbers in the progenies of crosses involving diploid, triploid and tetraploid parental plants, and some considerations are made on possible causations of occurrence of a definite sequence of the aneuploid distribution in hybrids of crosses between different levels of polyploidy.

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### Materials and Methods

The following strains were used for making all possible combinations of crosses except crosses of  $4x \times 2x$  and its reciprocal crossing phase,  $2x \times 4x$ . The crosses between  $2x$  and  $4x$  have already been intensely studied by MOCHI-

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1) Contribution from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

ZUKI (5), KNAPP (2) and others, owing to their economic importance.

- $2x$  ( $2n=18$ ) : GW-359  
 „ : M-14 (a male sterile strain)  
 $4x$  ( $2n=36$ ) : H-4002 (an induced  $4x$  from GW-359)  
 $3x$  ( $2n=27$ ) : H-4002  $\times$  GW-359  
 „ : M-14  $\times$  H-4002

These were combined as given below.

- $2x \times 2x$  : intercrossing of GW-359  
 $2x \times 3x$  A : GW-359  $\times$  (H-4002  $\times$  GW-359)  
 B : GW-359  $\times$  (M-14  $\times$  H-4002)  
 $3x \times 2x$  A : (H-4002  $\times$  GW-359)  $\times$  GW-359  
 B : (M-14  $\times$  H-4002)  $\times$  GW-359  
 $3x \times 3x$  A : intercrossing of hybrids from H-4002  $\times$  GW-359  
 B : intercrossing of hybrids from M-14  $\times$  H-4002  
 $3x \times 4x$  A : (H-4002  $\times$  GW-359)  $\times$  H-4002  
 B : (M-14  $\times$  H-4002)  $\times$  H-4002  
 $4x \times 3x$  A : H-4002  $\times$  (H-4002  $\times$  GW-359)  
 B : H-4002  $\times$  (M-14  $\times$  H-4002)  
 $4x \times 4x$  : intercrossing of H-4002

Because of the high degree of self-sterility in sugar beets, crossed seeds are easily obtained by planting two individuals side by side, each of which comes from different ploidy levels, and keeping them in isolation from other plants. However, in securing 100 percent of hybrid seeds, in other words in retarding self-pollination that scarcely may happen under some circumstances, artificial emasculation followed by artificial pollination must be employed. This was done as the occasion arose.

The matured seeds in seed balls (clusters or glomerules in other names) attached on the branch of inflorescence axis of each plant were examined on their seed setting percentage, size and germination of seed ball, and chromosome numbers of seedlings arising from hybrid germs. Chromosome counts were made at the tip of primary roots of 2 to 3 cm length. They were treated with cold water at 3° to 5°C for 24 hrs., in such a way that the chromosomes became much shorter and broader rendering the identification of individual chromosomes much easier. FARMER'S fluid was employed as the fixative and FEULGEN'S reaction as the staining and maceration means. Preparations were made by the usual squash method, including final staining with aceto-carmin.

TABLE 1. Germination test of hybrid seeds from crosses involving 2x, 3x and 4x as parental materials.

Type of cross	Source of 3x <sup>1)</sup>	Size of seed ball <sup>2)</sup>	Seed fertility %	Germination % <sup>3)</sup>	Number of days in germination	
2x×2x		L	89.8	72.7	2.1	
		M	69.6	58.7	2.3	
		S	48.8	26.7	2.7	
2x×3x	A	L	—	21.0	3.6	
		M	31.0	18.2	3.4	
		S	—	9.5	4.1	
	B	L	40.0	20.3	3.0	
		M	20.5	18.9	2.7	
		S	4.7	2.1	3.3	
3x×2x	A	L	29.8	21.8	3.3	
		M	29.5	12.0	3.5	
		S	5.0	4.5	4.4	
	B	L	9.1	8.0	2.8	
		M	12.8	3.1	2.6	
		S	1.2	0.9	3.6	
3x×3x	A	L	40.9	28.3	3.3	
		M	41.0	18.2	4.5	
		S	12.5	3.7	5.4	
	B	L	24.5	23.1	3.5	
		M	24.4	13.2	3.2	
		S	5.0	2.8	3.5	
3x×4x	A	L	—	18.9	3.0	
		M	—	16.0	2.7	
		S	—	7.4	4.3	
	B	L	30.2	13.6	2.9	
		M	16.3	9.1	3.3	
		S	29.7	9.4	3.2	
4x×3x	A	M	—	40.6	2.3	
		B	L	—	23.9	3.2
			M	—	20.4	3.2
S	—		8.1	3.0		
4x×4x		L	52.4	41.3	3.3	
		M	23.1	16.5	3.4	
		S	3.3	0.6	3.0	

1) A; H-4002×GW-359 B; M-14×H-4002 2) L; above 5 mm in diameter  
M; 5-4 mm S; 4-3 mm 3) Germination % =  $\frac{\text{no. of germinated seeds}}{\text{no. of seeds in 100 seed balls}}$

## Experimental Results

### 1. Germination Test

The size of the seed ball, fertility of the seed, rate and number of days for germination, were examined in seed samples of progenies from crosses of different combinations. They are presented in Table 1.

As seen in this table, it was shown that the seeds from  $2x \times 2x$  gave especially better results in their germination and seed fertility, than seeds from all of the other cross combinations. Next to  $2x \times 2x$ ,  $4x \times 4x$  showed good results, while in the seeds from other crosses which involved  $3x$  as one of the parents, viz. crosses such as  $2x \times 3x$ ,  $3x \times 2x$ ,  $3x \times 3x$ ,  $3x \times 4x$  and  $4x \times 3x$ , values less than half of  $2x \times 2x$  were given.

As to the number of days from seeding to germination,  $2x \times 2x$  was shortest, and all of the others showed no noticeable differences among combinations.

### 2. Chromosome Counts

Fig. 1 gives some examples of microphotographs of chromosomes at the

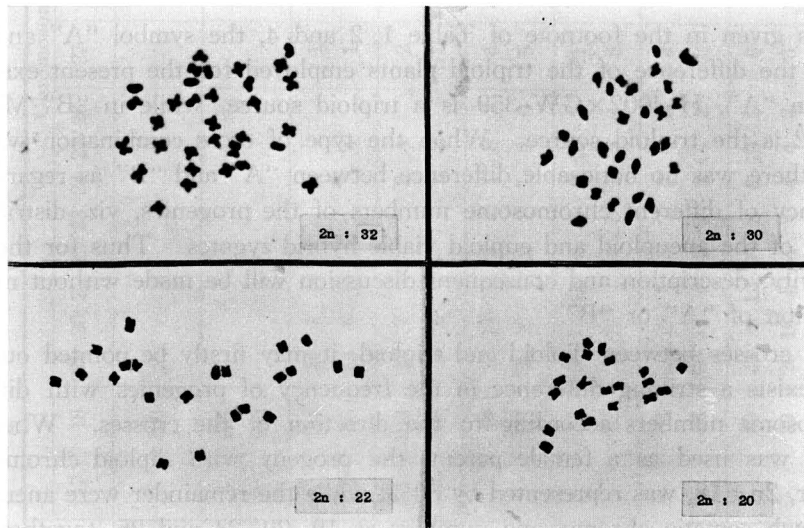


Fig. 1. Examples of aneuploid somatic chromosomes;  
 $2n=20, 22, 30$  &  $32$ .

mitotic metaphase in aneuploid root tips, giving  $2n=20, 22, 30$  and  $32$  of chromosome counts.

As shown in these pictures, the identification of the satellite—usually a single set is expected to exist in a diploid plant—was not so easy, however,

the presence of constrictions in each chromosome was easily recognized and there was no difficulty in interpretation as far as chromosome counts were concerned.

a:  $2x \times 2x$  and  $4x \times 4x$

All of the progenies in the intercrossed  $2x$  are diploid, giving zygotic chromosome numbers of  $2n=18$  without exception. This indicates a high constancy of chromosomes in the level of diploid, while in the progenies from  $4x \times 4x$ , besides the tetraploid zygote of  $2n=36$ , about 14% of the progenies are aneuploids, ranging from  $2n=33$  to 38. This is a relatively high percentage of occurrence of the aneuploid offsprings, even though the phenomenon of mixoploidy, going back to the diploid and giving rise to aneuploid has been noticed during the maintenance practices of some induced tetraploid strains. Since quite a few tetraploid strains, in the authors' experience, have had a strong tendency to remain constant (NAGAO, TAKAHASHI & KINOSHITA (7)).

The data obtained in  $2x \times 2x$  and  $4x \times 4x$  are presented in Table 2 and 4 respectively.

b:  $2x \times 3x$  and  $3x \times 2x$

As given in the footnote of Table 1, 2 and 4, the symbol "A" and "B" denote the difference of the triploid plants employed for the present examination; in "A", H-4002  $\times$  GW-359 is a triploid source, while in "B" M-14  $\times$  H-4002 is the triploid source. When the type of cross combination was the same, there was no noticeable difference between "A" and "B" as regards the frequency of different chromosome numbers of the progenies, viz. distribution pattern of the aneuploid and euploid viable hybrid zygotes. Thus for the sake of brevity, description and consequent discussion will be made without making distinction of "A" or "B".

In crosses between diploid and triploid, it may firstly be pointed out that there exists a striking difference in the frequency of progenies with different chromosome numbers according to the direction of the crosses. When the diploid was used as a female parent, the progeny with diploid chromosome number,  $2n=18$ , was represented by 80%, while the remainder were aneuploids with such somatic chromosome numbers as 19, 20, 24 and 25, together with a few plants (1%) of  $2n=27$ , the triploid. Four intermediate classes between 18 and 27, viz. the classes of 21, 22, 23 and 26 were missing.

In contrast with this, the condition was quite different in the reciprocal cross, where the triploid was used as the female parent. Here, all chromosome numbers between  $2x$  and  $3x$  were found, ranging from 18 to 27. Although  $2n=18$  was the class most numerously represented, which frequency being

TABLE 2. Number of somatic chromosomes in the progeny from crosses,  $2x \times 2x$ ,  $2x \times 3x$ ,  $3x \times 2x$  and  $3x \times 3x$ .

Type of cross	Source of parental triploid	Chromosome numbers																			Tot.					
		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		36	37	38	39	40 (44)
$2x \times 2x$	A		100																							100
	Total		100																							100
	%		100																							100
$2x \times 3x$	A		88	14	1			2			1															106
	B	1	75	13	6				4		1															100
	Total	1	163	27	7			2	4		2															206
	%	.5	79	13	3			1	2		1															100
$3x \times 2x$	A		38	23	14	11	5	4	1	8	1	6														111
	B		26	32	10	4	3	1	1	2	6	5					1									91
	Total		64	55	24	15	8	5	2	10	7	11					1									202
	%		32	27	12	7	4	3	1	5	4	5				.5										100
$3x \times 3x$	A		56	17	19	7	5	7	5	6	3	4														129
	B		33	28	17	5	5	1	3	4	2	2														100
	Total		89	45	36	12	10	8	8	10	5	6														229
	%		39	20	16	5	4	4	4	4	2	3														100

A ... GW-359 (2x), "H-4002 × GW-359" (3x) and H-4002 (4x)

B ... GW-359 (2x), "M-14 × H-4002" (3x) and H-4002 (4x)

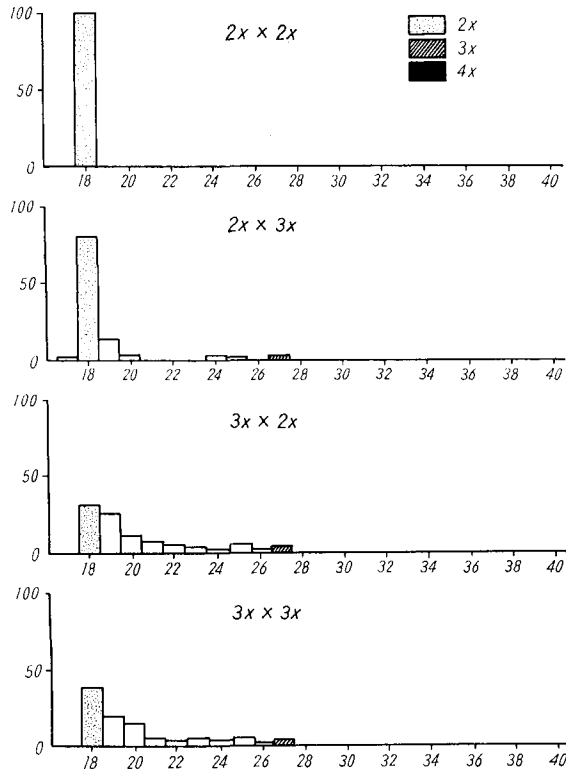


Fig. 2. Frequency distribution of the progenies with different chromosome numbers, in the crosses of  $2x \times 2x$ ,  $2x \times 3x$ ,  $3x \times 2x$  and  $3x \times 3x$ .

30% at the most, the numbers close to diploid such as 19 (trisomic) and 20 were relatively in abundance.

The distribution patterns are diagrammatically given in Fig. 2.

c:  $3x \times 3x$

In the progenies of intercrossed triploids, the consecutive series of chromosome numbers were turned out, ranging from 18 to 27. This is as shown in Table 2 and 3 and Fig. 2, in which it may be said that a similar distribution pattern to the progenies of  $3x \times 2x$  resulted, suggesting a great viability of the classes around the euploid numbers, though the curve falls a little more rapidly from the class 19, than in the progenies of  $3x \times 2x$ .

Table 3 is the distribution pattern in frequency of different chromosome numbers of progenies from crosses of  $2x \times 2x$ ,  $2x \times 3x$ ,  $3x \times 2x$  and  $3x \times 3x$ , when hybrid seed-balls in each crossing type are sorted into three groups of





seed-ball size. In the table "L", "M" and "S" stand for seed-ball diameter of "above 5 mm", "5-4 mm" and "4-3 mm" respectively. Throughout all combinations, no noticeable differences of distribution patterns were found among the three groups of ball size when the type of cross was the same. These phenomena holds true in progenies from  $3x \times 4x$ ,  $4x \times 3x$  and  $4x \times 4x$ , of which results are given in Table 5. Thus hanceforth, in the present paper, the seed size will be disregarded.

d:  $3x \times 4x$  and  $4x \times 3x$

The results obtained from crosses of  $3x \times 4x$  and their reciprocal ones are presented in Table 4, 5 and Fig. 3. In the progenies of  $3x \times 4x$ , nearly whole members of chromosome series, covering diploid to tetraploid, viz. 18 to 36, were found, though 95% of the progenies fell under classes between 27 (triploid) and 36 (tetraploid), and the classes below the triploid number had only

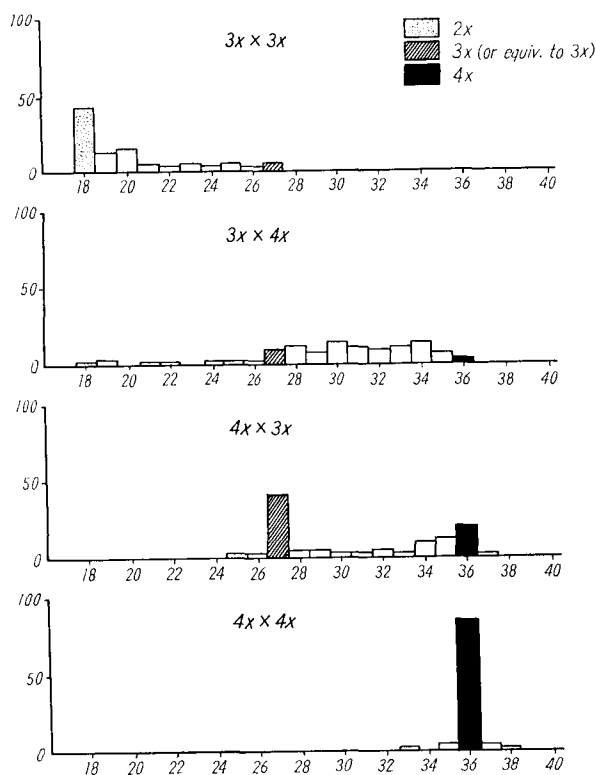


Fig. 3. Frequency distribution of the progenies with different chromosome numbers, in the crosses of  $3x \times 3x$ ,  $3x \times 4x$ ,  $4x \times 3x$  and  $4x \times 4x$ .

TABLE 4. Number of somatic chromosomes in the progeny from crosses,  
 $3x \times 3x$ ,  $3x \times 4x$ ,  $4x \times 3x$  and  $4x \times 4x$ .

Type of cross	Source of parental triploid	Chromosome numbers																	Tot.							
		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		34	35	36	37	38	39	40 (44)
$3x \times 3x$	A		56	17	19	7	5	7	5	6	3	4														129
	B		33	28	17	5	5	1	3	4	2	2														100
	Total		89	45	36	12	10	8	8	10	5	6														229
	%		39	20	16	5	4	4	4	4	2	3														100
$3x \times 4x$	A		1	2		1				1	1	6	4	5	14	8	4	9	11	5	1					73
	B						1		1			9	15	8	15	10	12	8	12	7	2					100
	Total		1	2		1	1		1	1	1	15	19	13	29	18	16	17	23	12	3					173
	%		.6	1		.6	.6		.6	.6	.6	9	11	8	17	10	9	10	13	7	2					100
$4x \times 3x$	A										5	1	1					2								9
	B									1	1	43	3	3	3	3	4	3	8	13	23	1		1		110
	Total									1	1	48	4	4	3	3	4	3	10	13	23	1		1		119
	%									.8	.8	40	3	3	3	3	3	3	8	11	19	.8		.8		100
$4x \times 4x$	A																2	5		86	5	2				100
	Total																	2	5		86	5	2			100
	%																	2	5		86	5	2			100

A ... GW-359 (2*x*), "H-4002 × GW-359" (3*x*) and H-4002 (4*x*)

B ... GW-359 (2*x*), "M-14 × H-4002" (3*x*) and H-4002 (4*x*)

TABLE 5. Number of somatic chromosomes in the progeny from crosses,  
 $3x \times 3x$ ,  $3x \times 4x$ ,  $4x \times 3x$  and  $4x \times 4x$ .

Type of cross	Size of seed ball	Chromosome numbers																			Total				
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36		37	38	39	40 (44)
$3x \times 3x$	L	38	24	10	3	4	2		4	1	4														90
	M	44	19	18	6	2	5	6	5	4	2														111
	S	7	2	8	3	4	1	2	1																28
	Total	89	45	36	12	10	8	8	10	5	6														229
	%	39	20	16	5	4	4	4	4	2	3														101
$3x \times 4x$	L		1		1	1		1	1	1	8	11	7	11	6	5	7	8	5	3					77
	M	1	1								6	7	4	17	9	9	7	13	5						79
	S										1	1	2	1	3	2	3	2	2						17
	Total	1	2	1	1	1	1	1	1	15	19	13	29	18	16	17	23	12	3	3					173
	%	.6	1	.6	.6	.6	.6	.6	9	11	8	17	10	9	10	13	7	2	2						103
$4x \times 3x$	L									22	1	1	1	4	3	7	9	15					1	64	
	M								1	1	26	3	4	2	2		3	4	7	1				54	
	S																	1						1	
	Total							1	1	48	4	4	3	3	4	3	10	13	23	1			1	119	
	%						.8	.8	40	3	3	3	3	3	3	8	11	19	.8			.8		100	
$4x \times 4x$	L																								
	M														2	5	86	5	2					100	
	S																								
	Total														2	5	86	5	2					100	
	%														2	5	86	5	2					100	

a few plants,  $2n=18\sim 26$  having together a little less than 5%. Between 27 and 36, no class or classes which were strikingly predominant existed. However 30 was rather well represented and included 27% of the progenies. Thus, on the whole, the type of distribution is more or less unimodal, without having a pronounced mode.

On the other hand, the frequency pattern in the progenies of  $4x \times 3x$  was different from that in  $3x \times 4x$ , in the reciprocal crossing phase of  $4x \times 3x$ . Here, the range of variation was from 25 to 37 (a supernumerary type of tetraploid), and 27 ( $3x$ ) and 36 ( $4x$ ) were the classes most numerously represented, giving dimodality of the curve as shown in Fig. 3. As to the percentage of  $3x$  and  $4x$  in the entire progenies, the former was 40% and the latter 19%.

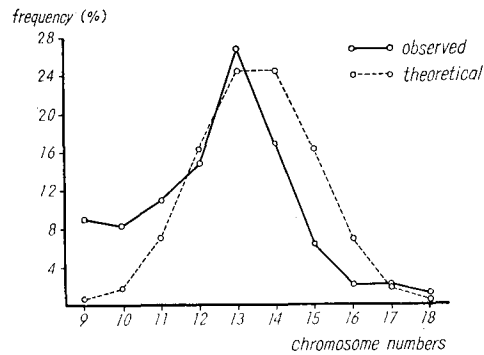
### Discussions and Conclusions

Reviewing the results obtained from  $2x \times 3x$  and  $3x \times 2x$ , it was noted that two types of gametes, male and female, in  $3x$  plants are quite different in their function of developing into viable zygotes. In female gametes, those with chromosome numbers between  $x+1$  and  $2x-1$  have such a function or stand a good chance of turning out viable aneuploid zygotes, although the gametes with  $x$  chromosomes have the highest function, providing large number of viable euploid zygotes in the progeny of  $3x \times 2x$ .

On the other hand, in male gametes of  $2x \times 3x$ , only the pollens with  $x$  or  $x+1$  chromosomes participate in producing viable zygotes, suggesting that the male gametes that have aneuploid chromosome complements other than  $x+1$  are subject to some kind of selections, including certation of pollen grains. The gametic selection may be less strict on the female side than on the pollen.

In order to proceed into the genetical background of gametes in triploid plants, the cytological aspects of meiosis in the triploid must be referred to. A detailed search is under way at present, however, the chromosome number of the first anaphase in PMCs was counted, as shown in Fig. 4.

As may be expected based on the assumption that all gametes will receive at least  $x$  chromosomes while the remaining  $x$  chromosomes, the "odd" or univalent chromosomes, will be distributed at random at the first meiotic division, the classes 13 and 14 are most numerous; however the actual distribution curve differs, to some extent, from this assumption. The right part of the observed values is low, while in the left part, all classes below 12 are highly represented. Especially, in the class 9, that is, the class equivalent to monoploid chromosome numbers, the observed and the theoretical frequencies



chromosome numbers	9	10	11	12	13	14	15	16	17	18	Total
observed	80	73	95	125	230	143	53	18	16	10	843
(%)	(9.5)	(8.3)	(11.3)	(14.8)	(27.3)	(17.0)	(6.3)	(2.1)	(1.9)	(1.2)	(99.7)
theoretical	1.6	15.2	59.9	138.3	207.4	207.4	138.3	59.9	15.2	1.6	844.8
(%)	(0.2)	(1.8)	(7.1)	(16.4)	(24.6)	(24.6)	(16.4)	(7.1)	(1.8)	(0.2)	(100.2)

Fig. 4. Chromosome numbers of dyads at first anaphase in a triploid plant

Theoretical frequency is based on random distribution of univalents.

are strikingly different from each other, showing values of 9.5% and 0.2% respectively. In triploid sugar beet it has not yet been ascertained whether or not the same type of chromosomal behavior as given in the PMC may take place in the EMC. It would, however, be reasonable to assume that there may be no essential and conspicuous differences between them.

As already mentioned, approximately 30% of the progenies of  $3x \times 2x$  was seemingly pure diploid,  $2n=18$ . The relative germination percentage—a measure based on germination percentage of intercrossed  $2x$  seeds is considered as 100%—in seed from female  $3x$  plant was calculated as 18% on the average. If this value, 18%, is multiplied by 0.3, the proportion of the pure diploid in the progeny of  $3x \times 2x$ , a value 5.4% will be obtained. This numerical estimate, 5.4%, means that at least approximately 5% of the all hybrid germs, in which several types of germs such as undeveloped, underdeveloped or degenerated may also be included, grow into zygotes in which chromosome complements are seemingly pure diploid and grow into young plants.

As pointed out, the theoretical frequency of  $x$  gametes is 0.2%, and the observed value is 9.5%, while the value calculated by the distribution frequency

of chromosome numbers in the progenies of  $3x \times 2x$  falls between the above two, giving a value of 5.4%. The discrepancies of these three values are too great to be ignored as mere variations which may happen in this type of examination.

In the consideration of one of the possible causations that give rise to an unconformity between the theoretical and the observational frequency distributions of chromosome numbers in the dyads of the triploid, it is firstly pointed out that an elimination of extra chromosomes may be brought about during meiosis, thus favoring gametes with lower chromosome numbers. Consequently the actual distribution curve is displaced to the left. The validity and significance of the above explanation are partly proved through cytological observation which is outlined in Table 6.

TABLE 6. Frequency of lagging chromosomes at meiosis in PMC of a triploid plant (GW-359  $\times$  H-4002).

Number of laggard	Anaphase-I	Telophase-I	Telophase-II
no laggard	394	953	488
1 laggard	59	351	136
2 laggards	24	246	78
3 laggards	2	68	12
4 laggards	2	17	4
Total	481	1653	718
<u>Number of cases with laggard</u> Total	18.1%	41.6%	32.1%

With this alone, however, it is difficult to explain the fact that the frequency distribution of chromosome numbers in the dyads of  $3x$  plant runs counter to the frequency of zygotic chromosome numbers in the progenies of  $3x \times 2x$ . Especially it is impossible to give a plausible explanation to account for the great difference between the frequency of progenies with  $2n=18$  in  $3x \times 2x$  and that of dyads with  $n=9$  in  $3x$  plants.

Before a discussion is attempted on the possible causation of the above, the following fact may be worthy of note. In the present examination no conspicuous differences were seen between  $2x$  and  $3x$  plants, when they were employed as female parents of crosses with  $2x$  male plants, with respect to either i) number of bracts which sustained sessile flowers or flower clusters in their axils for a certain length of stalk, or ii) mean number of flowers (germs) per single flower cluster. Hence the difference of the number of floral

organs can hardly be used as one of the probable causes of giving rise to the phenomenon mentioned above.

Thus, other factors which may play an important role in the causation must be considered. It may be natural to assume that some of them are connected with certation of pollens including selective fertilization, and zygotic selection involving divergent germination power of germs with different chromosome numbers.

In this connection, the authors wish to propose a hypothetical assumption, a certain type of selection which may take place during megasporogenesis.

From an embryological view point, female gametes of sugar beets fall under the so-called polygonum type, viz. monosporic-8-nucleate type, in which, as a rule, an inner most tetrad, the calazal one, out of the four linear tetrads, functions and gives rise to the embryo sac, while the remaining three tetrads degenerate and disappear (OKSIJUK (8), ARTSCHWAGER and STARRETT (1), and MAHESHWARI (4)).

In the megaspores of the triploid beet, it is reasonable to expect the occurrence of the numerical variation of chromosomes in dyads or tetrads which originate from an identical megaspore mother cell. In such a case, these sister spores might be in a state of competition with each other, because of their differences in chromosome numbers, and a spore with euploid or near-to-euploid chromosome complements might overcome other sister spores, and subsequently begin to grow a functioning embryo sac, regardless of, or having little connection with, whether the said spore originates from the calazal side or microphyllar side. If this will prove true, through scrupulous observation, this phenomenon may be accepted as one of the important factors of selection force that act during megasporogenesis. The verification of this phenomenon, however, has not yet been carried out.

Table 7 is a comparison of the two distribution patterns of zygotes with different chromosome numbers in progenies of  $3x \times 3x$ . One represents observational frequencies obtained from intercrossed  $3x$  plants and the other gives the estimated—or expected—frequencies of the same crossing type. In the latter a zygotic series was a priori constructed by other actual results obtained from two kind of crossing types,  $2x \times 3x$  and  $3x \times 2x$ . Observed zygotic frequencies of progenies from  $2x \times 3x$  and  $3x \times 2x$  would imply the frequencies of gametic series of  $3x$  parents; male gametes in  $2x \times 3x$  and female gametes in  $3x \times 2x$ . With these frequencies two types of gametes must function to produce a zygotic series such as is met with in the  $3x \times 3x$  progenies. As shown in the table the observational frequencies are fairly well concordant with the estimated ones, with one small exception, i.e. in the region above



TABLE 7. Observed frequency of chromosome numbers in progeny from  $3x \times 3x$ , in comparison with expected frequency estimated from other crosses,  $3x \times 2x$  and  $2x \times 3x$ .

Chromosome numbers	$2x \times 3x$	$3x \times 2x$	$3x \times 3x$	
			Estimated	Observed
18	79	32	25.1	38.9
19	13	27	25.3	19.7
20	3	12	13.8	15.7
21	0	7	7.8	5.2
22	0	4	4.4	4.4
23	0	3	3.0	3.5
24	1	1	1.6	3.5
25	2	5	5.0	4.4
26	0	4	4.5	2.2
27	1	5	6.0	2.6
28	0	0	1.2	0
29	0	0	0.4	0
30	0	0	0.1	0
31	0	0	0.1	0
32	0	0	0.2	0
33	0	1	0.9	0
34	0	0	0.3	0
35	0	0	0.07	0
36	0	0	0.05	0
37	0	0	0	0
38	0	0	0	0
39	0	0	0.01	0
40	0	0	0.02	0
41	0	0	0	0
42	0	0	0.01	0

$3x$  no plant appeared in the actual progenies.

In this connection, attention should be paid to the examinations done by LEVAN (3) and MOCHIZUKI (5). LEVAN made crosses of  $3x \times 3x$ , with the result that, in the progenies, whole series of chromosome numbers were found ranging from  $2x$  to  $4x$ . He stated that, though, the region above the triploid number had often been clearly represented, in some other cases the region

between  $2x$  and  $3x$  was not so completely represented. He also made crosses of  $3x \times 2x$  and  $2x \times 3x$ , from which he computed the frequencies of functioning gametes in producing viable zygotes when  $3x$  plants were intercrossed. Based on this he predicted the zygotic frequency of  $3x \times 3x$ , and compared it with the actual observational results of  $3x \times 3x$ . In his case, however, the goodness of fit between the observation and its expectation was not so high, as compared with the authors' case. The discrepancy in the results of the authors' and the LEVAN's, cannot be explained as yet.

The authors have next to discuss on the results of crosses when triploid plants are combined with tetraploid plants. As shown in Table 4 and 5, and Fig. 3, there are noticeable differences in the distribution of different chromosome numbers in progenies of two crosses,  $3x \times 4x$  and  $4x \times 3x$ , as well as in the range of chromosome numbers, according to the direction of crosses.

When the triploid was used as the female parent, a series of chromosome numbers between  $2x$  and  $4x$ , with missing classes of 20 and 23, were found, the number between 27 and 36 being in excess and represented by more than 95% of the progenies. The triploid number represents 9% while the tetraploid has 2%. About 60% of the progenies are found between the classes of 30 and 34, though there is no conspicuous class among them, suggesting that the gametic (and/or zygotic) selection is less strict in  $3x \times 4x$  than in  $3x \times 2x$ . Although the possible reason is inexplicable at present, the following fact should be worthy of note; in  $3x \times 2x$  the ratios of chromosome number between male and female gametes range between 1/1 and 1/2, while in  $3x \times 4x$  the ratios range between 2/1 and 1/1. In this connection LEVAN (3) also reported that the functioning proportion  $x : 2x$  pollen is different in  $3x \times 3x$  from that in  $2x \times 3x$ , while  $2x$  pollen grains are more prevalent in the cross  $3x \times 3x$ .

As to the frequency of the  $3x$  zygote, the observed frequency of 9% is very high as compared against the theoretical frequency of 0.2% which is based on the reason already mentioned. The so-called "relative germination percentage" of seeds raised in the parental triploid plant of the cross,  $3x \times 4x$ , was estimated as 27%. Therefore the percentage of triploid viable germs per total germs may be calculated as 2.43%. This falls between two numerals, 0.2% and 9.5%, concerning which the meaning has already been mentioned in the earlier part of this chapter, where results from  $3x \times 2x$  were the object of the discussion. Thus in  $3x \times 4x$ , the same inclination, with regard to the above three kinds of numerals, as in  $3x \times 2x$  is presented.

In the reciprocal cross, where the tetraploid was used as the female parent, the frequency pattern of zygotes with different chromosome numbers is quite different. As shown in the table and the figure, a suspension bridge type of

distribution is obtained, having two maxima of euploid classes, 18 and 36. This implies severe gametic (and/or zygotic) selection among male gametes of different chromosome numbers, gametes with euploid and thereabouts euploid being favored.

As to the proportion of frequencies of euploid progenies from two types of crosses,  $2x \times 3x$  and  $4x \times 3x$ , the following intriguing results must be pointed out. They are:

	frequency of euploid zygotes in progenies		
	$2x$	$3x$	$4x$
$2x \times 3x$	79%	1%	0
$4x \times 3x$	0	40%	19%

It must be borne in mind that male gametes or pollens are subjected to severe strains when disengaged from the nourishing tissue of the pollen sac. They germinate on the stigma and grow down through the style under strong competition. Here, when fertilization takes place between normal diploid plants, numerical chromosome ratio between stigma (or style) and pollen is kept at 2 : 1. In the crosses,  $2x \times 3x$  and  $4x \times 3x$ , this ratio is of special interest, in connection with the differences of frequencies of euploid zygotes in their progenies. That is:

type of zygote	frequency in progenies	ratio of chromosome number (female tissue : pollen)
$2x$ from $2x \times 3x$	79%	2 : 1
$3x$ from „	1%	1 : 1
$3x$ from $4x \times 3x$	40%	4 : 1
$4x$ from „	19%	2 : 1

The results that i) the frequency of  $3x$  zygotes from  $4x \times 3x$  which were given by functioning  $x$  pollens is relatively low in comparison with that of  $2x$  zygotes from  $2x \times 3x$ , and that ii) the frequency of  $4x$  zygotes from  $4x \times 3x$  is relatively high as compared against that of  $3x$  zygotes from  $2x \times 3x$ , may partly be explained by the ratio of chromosome set between female floral tissue and pollen. The ratio of 2 : 1 is seemingly most favored, the other two ratios 1 : 1 and 4 : 1 are rather incongruous.

In the tetraploid plant, a considerable amount of irregularity in meiotic division has been reported by many workers, and this would account for the observed inconsistency of chromosome numbers in the progeny of some tetraploid strains (MOCHIZUKI (5), NAGAO and TAKAHASHI (6)). However as shown in the progenies from  $4x \times 4x$  presented in Table 4, the variation of chro-

mosome number is limited within one or two chromosomes more or less than the typical number, 36, together with very low frequencies of appearance of these aneuploid zygotes. Thus the considerable production of aneuploid gametes, which sometimes occupy more than 50% of tetrads in tetraploid plant, seems to have little chance to participate in producing viable zygotes, when the tetraploid plant is employed as the male parent of the cross,  $3x \times 4x$ .

### Summary

1. A survey was made on the frequency distribution of the chromosome numbers in hybrid populations of sugar beets from all possible cross combinations at three ploidy levels,  $2x$ ,  $3x$  and  $4x$ , including the reciprocal crossing phase.

2. Four strains were used as crossing materials. They are H-4002 as the tetraploid source, GW-359 and M-14 as the diploid sources, and M-14  $\times$  H-4002 and GW-359  $\times$  H-4002 as the triploid sources. Hybrid seed samples from the following seven types of crosses were examined in their germination and chromosome numbers;  $2x \times 2x$ ,  $2x \times 3x$ ,  $3x \times 2x$ ,  $3x \times 3x$ ,  $3x \times 4x$ ,  $4x \times 3x$  and  $4x \times 4x$ .

3. The  $2x \times 2x$  gave especially better results in seed fertility and in germination of hybrid seeds, than all of the other cross combinations.

4. Chromosome counts were made for individual seedlings from all combinations. Summarizing the results obtained from the above crosses, the following may briefly be stated.

5. Though euploids are the classes most numerous represented in progenies of almost all the crosses, aneuploids with various chromosome numbers were also found. The class close to euploid, especially close to the trisomic class,  $2n=19$ , was in excess.

6. The frequency distribution of aneuploid plants was different according to the level of ploidy,  $2x$ ,  $3x$  or  $4x$ , and to the direction of crossing phase, i.e.  $2x \times 3x$  or  $3x \times 2x$ , and  $3x \times 4x$  or  $4x \times 3x$ .

7. A high frequency of aneuploids was found in all combinations when the triploid strain was employed as the female parent and was crossed with all ploidy parents, i.e.  $3x \times 2x$ ,  $3x \times 3x$  and  $3x \times 4x$ , all giving the frequency of 60% or more.

8. The aneuploid frequency of the remaining combination was always less than the former cases. It was ordered in a sequence of  $4x \times 3x > 2x \times 3x > 4x \times 4x > 2x \times 2x$ .

9. A causal reason as to why such a definite sequence of aneuploid distribution was obtained in the progenies of the crosses combined at the different

polyploid level was discussed.

10. It was suggested that the main causal reason is due to some differences, existing between the male and female side of both parents in the chromosomal abnormality at meiosis, and the subsequent occurrence of gametic and zygotic selection which result in the elimination of certain chromosome classes and the favoring of other classes in various stages of the development of the gametes and the zygotes.

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