Cytological Studies of Genus *Petunia**

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Introduction

The crossing between diploid and tetraploid has been attempted by a number of investigators, since it is of interest in elucidating the origin of triploidy and related phenomena. In *Petunia*, Ried (1930), Dermen (1931) and Steere (1932) reported the results of their experiments along this line. The present author has also carried out similar experiments during a period of several years. This paper is to report these results.

Material and Method

The tetraploid *Petunia* used in this experiment was selected from Sutton's Leviathan, the cytology of which has already been studied by the present author (1934a). As the diploid material Sutton's New Blue Bedding which has small flowers of purple color was used. This latter variety has been grown for several years in the garden of the Gifu Agricultural College and has been proved to breed true, at least in respect of the flower color. This strain is characterized by the presence

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of a trabant at each of the free ends of one of the bivalents (Fig. 1). The letter L before the plant number represents the Leviathan and N the New Blue Bedding. L16 and L6 belong to approximately the same phenotype, both having the same solid pink flower of large size.

The observation was made by the modified method of Belling's smear method using acetocarmine without iron. Smears at the heterotypic as well as homotypic metaphase gave us the somatic count. For the detailed study of chromosome associations in the heterotypic metaphase and anaphase, smears were boiled or pressed to such an extent that the cell contents were squeezed out from the cell wall. Permanent preparations made by the usual paraffin method and stained with Heidenhain's iron-alum haematoxylin were sometimes used. Figures were drawn with the aid of camera lucida at the level of the desk.

Fig. 1. Heterotypic metaphase of a diploid variety, New Blue Bedding. A trabant is seen at each free end of one of the bivalents. From a permanent preparation. ca. $\times 1150$.

Results of Experiments

1. Tetraploid selfed

In a plant L16 the self pollination was made which proved very successful especially when the stigma was used in its premature stage. Among 196 seeds sown 122 germinated and grew to maturity. The flower color and the leaf shape of the F_1 progeny displayed a considerable variation. Among 31 plants examined 29 were tetraploid. Of the remaining two plants, one had 29 chromosomes and the other 25. All these plants showed little morphological difference, each resembling the tetraploid parent.

2. Tetraploid $\times$ diploid

(1) Experimental results

The tetraploid parents were selected from the F_1 population of $L15 \times L8$ which showed little variation in flower color. They all had pink flowers, the same as their parents. The number and behavior of chromosomes in these plants were typical of the tetraploid. Crossing was carried out in three ways such as $L5 \times N1$, $L16 \times N1$ and $L6 \times N1$, all of which were successful, capsules of considerable size having been obtained. The germinating rate of the seeds, however, was very small. For instance, out of 107 $L16 \times N1$ seeds only three plants were obtained, and out of 384 $L6 \times N1$ seeds only 38 germinated and grew to maturity, while the selfed seeds of L16, as was stated above were very fertile showing the germinating rate of 61%.

Table 1. Frequency of the F_1 progenies having different number of chromosomes.

Mating	2n	3n-1	3n	3n+1	3n+2	4n
L5×N1	1	1	5			
L16×N1	1				1	1
L6×N1	1	1	23	1		

The result of somatic count of the F_1 progenies is represented in table 1. As is seen from the table, a majority of individuals in the F_1 progenies of L5 × N1 and L6 × N1 were

triploids while in the that of L16 × N1 no triploid appeared. The triploid plants all displayed intermediate characters with major trend toward the tetraploid parent. They could hardly be distinguished from the tetraploid in respect of growth habit, vigor, flower shape, leaf shape and leaf color. The size of flower was intermediate. The flower color was a mosaic of pink and purple. Though the quantitative proportion of both colors was varying, the greater area of each flower was pink in color.

Two 3n-1 chromosome plants and a 3n+1 chromosome plant were hardly distinguishable from the triploid. The 3n+2 chromosome plant showed high analogy to the tetraploid, except in flower color which consisted of pink and purple mosaically arranged. The 4n chromosome plant was apparently analogous to the tetraploid parent. A very interesting and striking result is the production of diploid progenies in all three matings. Two of them, L5.N1-10 and L6.N1-14, were complete replicas of the diploid parent. One from the crossing L16 × N1 showed a striking difference from both parents, that is, it was dwarf and bushy with many small branches and flowers. The flowers were very small and pink in color. The leaves were also small and roundish. All of these F_1 progenies were self fertile to a greater or less extent. The triploid progeny, in contrast to a majority of the results of previous investigators, proved to be fertile to a considerable extent, though the capsules were a little smaller than those of the tetraploid parents. The 23 chromosome plant was least fertile, all the capsules selfed having been very small with a small number of seeds.

(2) Cytological observations of triploid hybrids

The association of chromosomes in autotriploids may be classified into the following two types: (a) whole chromosome complement is formed into complete sets of trivalents, (b) there are some irregularities in a number of trivalents, with varying number of bivalents and univalents. *Canna* (Belling, 1925), *Datura* (Belling, 1925, 1927), *Hemerocalis* (Belling, 1925) and *Hyacinthus* (Belling, 1925; Darlington, 1929) are found to belong to the first type. More cases have been reported belonging to the second type, for instance, in *Morus* (Tahara, 1910; Osawa.

1920), *Campanula* (Gairdner, 1926), *Prunus* (Darlington, 1928), etc. It seems to me, however, that this distinction is not very clear. Dermen (1931) described that, in triploid hybrids of *Petunia*, chromosomes only rarely form trivalents; in the majority of cases, 9–11 small and large irregular chromosomes and sometimes 18–21 chromosomes are counted in the heterotypic metaphase. Streere (1932), on the other hand, found that in the triploid *Petunia* all chromosomes are regularly associated into complete sets of trivalents. The discrepancy between the observation of the two authors may be due to difference in the material.

In the present material there are 21 chromosomes in the heterotypic metaphase showing a considerable tendency toward the formation of trivalents. The complete conjugation into 7 sets of trivalents of all the chromosomes was observed, though not very frequently (Fig. 2). In many cases some of the trivalents are replaced by a bivalent and two univalents, or by three univalents (Fig. 3). This is probably due to the disruption in the metaphase or in the diakinesis of some of the prophaseic associations. All cases of chromosome combinations observed in the two triploid plants are tabulated in table 2. As is seen from the table the metaphase plate with four trivalents was found most frequently, and that with seven, four times out of a total of twenty four. A plate with only one trivalent or with no trivalents could not be found.

Table 2. All cases of chromosome combinations observed in two triploid hybrids.

Plant number	Chromosome combination	Frequency
L6.N1–11	7III	1
	6III+1II+1I	1
	5III+1II+4I	1
	5III+2II+2I	1
L6.N1–13	7III	3
	6III+1II+1I	1
	5III+2II+2I	1
	5III+1II+4I	1
	4III+4II+1I	4
	4III+3II+3I	2
	4III+2II+5I	1
	3III+5II+5I	1
	3III+4II+4I	2
	2III+5II+5I	1
	2III+4II+7I	1
	1V+4III+1II+2I	1

Figs. 2, 3. Heterotypic metaphase of triploid hybrids. Fig. 2 with 7III and Fig. 3 with 4III+1I. Acetocarmine smears. ca. $\times 2000$. **Fig. 4.** Trivalent and multivalent configurations of triploid hybrids. b, c, d, the ring of three; e, f, the chain of three; g, the V; h, the L; a, i, j, the double arc and rod; k, l, m, the Y; n, o, the chain of three showing a trabant; p, q, r, the multivalent. Acetocarmine smears. ca. $\times 2000$.

Belling (1927) pointed out that there are five possible types of configurations for trivalents in autotriploids: the triple arc, the double arc and rod, the triple rod united at one end (Y), the V, and the chain of three rods. In the present case all these types except the first are observed (Fig. 4). The rod in the double arc and rod is usually united at one end to the junction of the double arc. But those in which one end of a rod is joined to one of the partners of a bivalent ring interstitially are often found (Fig. 4, a). Not infrequently a chromosome is attached perpendicularly to a partner of a bivalent rod thus showing the L shape (Fig. 4, h). This may be a modification of the V. Very often a partner of a trivalent is found connected loosely by a fine thread to the other while it shows a very close affinity for the remaining one. The trabant which is found at both ends of one of the bivalents in the diploid parent, is also identified at one end of one of the chromosomes of the triploid hybrids (Fig. 4, n, o). It appears either at a free end or at an associated end of a trivalent. In Fig. 4 it must be noted that the chromosome with the trabant derived from the diploid parent is united very closely to the other one derived from the tetraploid parent, while the latter is loosely associated with the third one which is of the same tetraploid origin. This suggests that the intimacy of conjugation does not necessarily afford the criterion for the identity of the origin of chromosomes. Besides five fundamental types of trivalent configurations there are found occasional figures of the ring of three or triangle configuration (Fig. 4, b, c, d).

Among all the trivalent configurations the chain of three and the V are found most frequently. The double arc and rod occurs less frequently. The triple arc was not found at all. Rough counts of each configuration in 125 pollen mother cells are as follows: the chain of three 40, the V 46, the double arc and rod 8, the Y 11, the ring of three 8, the triple arc 0, and the L 12. It must be noted that in the ring and rod and the Y which occur infrequently, three ends are connected, while in the chain of three and the V only two ends are connected. Belling (1927) counted the different forms of attachment of the trisomics in primary $2n+1$ plants of *Datura* and for 108 trivalents he observed that there were 198 free ends, 149 junctions of two ends and 52 junctions of three ends. From this fact he concluded that "if the junctions of three ends were as stable as that of two, or if two chromosomes having combined by the ends in the thin threads stage, there still remained as much free attraction in this double end as there was in the single end; then there should be, in almost all trivalents, three ends combined (as two ends are nearly always combined in the diploid *Daturas* and *Cannas*). Therefore, the union of two ends hinders union with another homologous end. Hence, also, arises the frequency of the occurrence of separate

single chromosomes in the triploids as compared with the diploids where they are rare." This conclusion may also fit the present case where the Y, the ring and rod, and the triple arc, which are composed of the union of three ends, happen more rarely. The triple arc having double the triple union occurs most rarely so that in the present count its frequency was 0.

Belling and Blakeslee (1926) first established the hypothesis that chromosomes are associated only at their homologous ends. This was supported by numerous workers and has been applied to the explanation of the association of chromosomes. In the present case out of the 5 trivalent associations observed 4 are explained by this hypothesis but the ring of three or the triangle can never be deduced from this assumption when all three component chromosomes had different attraction at both ends. Huskins (1928) in wheat and Meurman (1929) in *Prunus* found similar figures in which, however, one of the chromosomes lay transversely and was connected at the same end to the two adjacent ones. Darlington (1928) in *Prunus* also observed the closed ring or the triangle configuration of three chromosomes which he interpreted to have been associated by the mutual attraction of the chromosomes, since the juxtaposition of homologous ends of chromosomes to make the three sides of a triangle is impossible. Belling and Blakeslee (1926, 1927) reported the appearance of the ring of three association in secondary trisomic *Datura* which they explained by the segmental interchange hypothesis. (c.f. Matsuda, 1934a). In the present material close observation shows that all three chromosomes are clearly connected end to end, so that it is quite different from Huskin's case. It seems very likely that the reversed crossing over has taken place in an ancestor between homologous chromosomes, so that one of the chromosomes attained the same attraction at both ends. The occurrence in the tetraploid parent of the configuration of the similar sort (c.f. Matsuda 1934a), leads us to greater certainty with regard to the occurrence of the reversed crossing over.

Some multivalents were found though rarely. They often consisted of five components (Fig. 4, q) though one composed of 6 chromosomes was once discovered (Fig. 4, r). As to the mechanism of the formation of multivalents one might first consider the presence of more than three members in any one of the homologous complexes. Such a condition was reported by Darlington (1928) and Meurman in *Prunus*, by Lawrence (1929) in *Dahlia* and by Darlington and Moffet (1930) in *Pyrus*. According to Darlington the diploid material of *Pyrus* with 34 chromosomes being phylogenetically, quadruply tetrasomic and trebly hexasomic, often shows every multiple valency up to the sexavalent. In *Petunia* it is indubitable that the basic number is 7. Consequently such

a condition as just described is improbable. Furthermore, the complete grouping of all the chromosomes into the seven sets of trivalents is inconsistent with this situation. In fact, the chromosome combination 7_{III} was found three times among 19 pollen mother cells of one and the same plant in which the multivalents occurred. This explanation, therefore, does not fit the present case. A more likely explanation at present is the segmental interchange hypothesis. If we postulate one interchanged chromosome in the triploid the pentavalent and hexavalent must naturally take place.

Among chromosome combinations represented in table 2 the majority are explained by the assumption that the chromosomes can only be associated by homologous ends. Only the combination $4_{III} + 4_{II} + 1_I$ seems to be inconsistent with this assumption, since four trivalents can not exist in one and the same pollen mother cell at the same time, together with more than three bivalents, as is seen from the following formula where a letter of alphabet is given to each homologue :

In fact, as is seen from table 2, such the combination appears four times among twenty three pollen mother cells examined. The idea that first comes to our mind as an explanation of this inconsistency is that any one of the chromosome complexes might consists of four members instead of three. If this assumption were true the above combination would be possible as the following formula shows :

The combination 7_{III} , however, would be impossible since four members in one chromosome complex can only be attained at the cost of a member of another complex (for example F in the above formula), as the whole number of chromosomes is definite, so that the presence of at least one bivalent is inevitable in any cases. On the other hand, the simultaneous occurrence of 7_{III} (Fig. 2) and $4_{III} + 4_{II} + 1_I$ (Fig. 4) in one and the same individual is the fact actually observed. This manner of explanation therefore can not be possible.

The segmental interchange hypothesis will also fit all the cases found in the present investigation. If we assume that the segmental interchange happened to take place in an ancestor between a non-homologous pair of chromosomes, each of two non-homologous chromosomes in the present material can possess an end homologous to the other; for instance in the following formula (i), E and F can each have

one end homologous to each other by which both chromosomes can be associated.

Consequently both the combinations are made possible at the same time, as is shown in the above formulae (i) and (ii).

In the anaphase the trivalents segregate at random. Consequently, the resultant gametes must have varying number of chromosomes ranging from 7 to 14. The majority of the homotypic metaphase counts were

Table 3. Frequency of homotypic metaphase plates with varying number of chromosomes in the triploid hybrid.

Chromosome number	7	8	9	10	11	12	13	14
Frequency	1	4	12	32	30	14	3	1

10 + 11 (Fig. 5). The frequency of the plates with different chromosome numbers observed is shown in table 3.

Figs. 5-8. Homotypic metaphase. Fig. 5. A triploid hybrid. 10+11. Acetocarmine smear. 1150. Fig. 6. A $3n+1$ hybrid. 10+12. Permanent preparation. ca. $\times 1800$. Fig. 7. $3n+1$ hybrid. 13+9. Two subsequent section of a permanent preparation. ca. $\times 1800$. Fig. 8. $3n+1$ hybrid. 11+(11+f). Acetocarmine smear. ca. $\times 1150$.

(3) Cytological observations of aneuploid hybrids

Among the aneuploid progenies a $3n+2$ plant could be more closely studied cytologically. In the heterotypic metaphase 23 chromosomes are always present, in one of which a trabant is identified at only one end (Fig. 9). The two extra chromosomes often are associated respectively with trivalents, thus forming the two tetravalents or tetrasomics. In many cases, therefore, the metaphase plate contains two tetravalents besides a varying number of trivalents, bivalents and univalents. Fig. 10 shows an ideal case with 5 trivalents and two tetravalents. The

disruption of the prophasic association of chromosomes is very common as is the case with the triploid and the tetraploid, thus giving rise to the presence of some bivalents or univalents. There are occasional figures of the ring of three (Fig. 9), which indicate the occurrence of reversed crossing over in an ancestor. A tetravalent with a peculiar type of configuration was discovered (Fig. 10, a). This figure is nothing but the ring of three and rod, the latter uniting with one of the component chromosomes of the former interstitially.

$3n+1$ plants derived from $L6 \times N1$ were examined. Detailed study was not possible on account of the scarcity of material. It has invariably a fragment besides 22 chromosomes. Its behavior in the heterotypic division could not be studied. Of three cells in which two homotypic metaphase plates could be counted, two were $10+12$ (Fig. 6) and $9+13$ (Fig. 7) respectively, while the remaining one was $11+(11+f)$ (Fig. 8). In the first two no fragment could be identified probably owing to the fact that it was attached to one of the chromosomes.

$3n-1$ plant derived from $L6 \times N1$ was studied. In the heterotypic metaphase figures were discovered with $4_{III} + 2_{II} + 4_I$ (Fig. 12), and

Figs. 9-10. Heterotypic metaphase of $3n+2$ plant. Fig. 9, $5_{III}+4_{II}$. Fig. 10, $2_{IV}+5_{II}$. Acetocarmine smears. ca. $\times 2000$. Figs. 11-12. Heterotypic metaphase of $3n-1$ plant. Acetocarmine smears. ca. $\times 2000$. Fig. 11, $3_{III}+3_{II}+5_I$. Fig. 12, $4_{III}+2_{II}+4_I$. Figs. 13-15. Homotypic metaphase of $3n-1$ plant. Acetocarmine smears. Fig. 13, $10+10$. ca. $\times 1150$. Fig. 14, $11+9$. ca. $\times 1000$. Fig. 15, $12+8$. ca. $\times 1000$.

$3_{III} + 3_{II} + 5_I$ (Fig. 11) respectively. The distribution of chromosomes in the heterotypic anaphase

Table 4. Distribution of chromosomes in the heterotypic anaphase in $3n-1$ plant.

Number of chromosomes	8	9	10	11	12
Frequency	3	16	24	12	5

observed is shown in table 4. Fig. 13, 14 and 15 show homotypic metaphase plates with 8-12 chromosomes.

(4) Cytological observation of diploid hybrids.

It has already been stated that one can distinguish two different kinds among the diploid progenies. The plant L6.N1-14 coming from the mating $L6 \times N1$ showed no difference from the diploid parent either in number or behavior of chromosomes. The other kind of diploid progenies L16.N1-2 could not be studied cytologically. Both kinds of the progenies, however, were selfed and produced some seeds. From the seeds of the former 20 plants were raised all of which revealed the same characters as the mother plant. In the heterotypic metaphase 14 chromosomes are present forming 7 bivalents. A trabant is invariably identified at each of the free ends of one of the bivalents (Fig. 16). The selfed seeds of L16.N1-2 were not very viable; only 5 plants were grown from 225 grains of seed. These plants all displayed almost homogeneous appearance with delicate stems and small leaves as was the case with their mother plants. The flowers were also small and pink colored with a few exceptions which showed very pale color. In the heterotypic metaphase no bivalents had trabants.

Fig. 16. Heterotypic metaphase of the diploid F_2 progeny of $L6 \times N1$, showing two trabants. Permanent preparation. ca. $\times 1400$.

3. Diploid $\times$ tetraploid

Two matings $N1 \times L6$ and $N1 \times L16$ afforded strikingly different results. 137 seeds from the latter were sown of which 85 germinated and grew to maturity. They all showed exactly the same phenotype as the diploid parent. Counts were made in 29 plants taken at random among the F_1 population of $N1 \times L16$. All of them had 14 chromosomes which, as a rule, appear as 7 bivalents in the heterotypic metaphase and behave as regularly as in the diploid parent.

Fig. 17. Homotypic metaphase of $27 + f$ chromosome plant. Acetocarmine smears. ca. $\times 1600$.

The $N1 \times L6$ crossing gave a considerable number of seeds which were less viable. Among fourteen plants obtained from these seeds few differences were recognized, all

having the intermediate characters between both parents in general, and rather resembling the tetraploid parent in stature, vigor, stems and leaves. The flower was a mosaic of pink and purple, rather tending to purple. The cytological examination of four plants selected at random proved that three were tetraploid and one had $27+f$ chromosomes (Fig. 17).

4. Triploid selfed

Among 291 seeds obtained from the triploid selfed, 74 seedlings were raised. They revealed complicated segregation with respect to flower color, shape of leaves, vigor and maturity. In the flower color they were almost the same as the mother plant, except that one had nearly the same flower as the diploid ancestor. Detailed cytological study of this latter plant could not be made, since it died accidentally before we could obtain the material. There is little doubt judging from its morphological character, however, that it is diploid. The appearance of diploid plants among F_1 progenies of triploid selfed is not rare, as is shown by Navashin's datum mentioned below.

Somatic counts were made at the homotypic as well as the heterotypic metaphase in 35 plants. The result is represented in table 5.

Table 5. Somatic counts in the progeny of the triploid selfed.

Chromosome number	26+f	26+f+f	27	27+f	28+f	29
Frequency	2	1	5	18	5	4

Most of triploid plants found heretofore were self sterile except minor cases. M. Navashin (1929) by open pollination

of triploid *Crepis capillaris* obtained $2n$, $3n$ and $2n+1$ plants with respective frequencies of 70, 33 and 2. Dermen (1932) obtained one 26 chromosome plant and three 27 chromosome plants from a triploid *Petunia* selfed. As is seen in the table 5 the present result is generally in agreement with Dermen's in that the 27 chromosome plant shows the highest frequency, without taking into account the fragment, but differs in that 28 and 29 chromosome plants appear, though with less frequency, instead of the 26 chromosome plant. Dermen also pointed out that the most successful gametic combinations in this selfing have been $13+13$ or $12+14$ or $13+14$. In the present author's experiments, besides these combinations $13+15$, $14+14$ or $15+14$ must have been successful since 28 and 29 chromosome plants were produced. The gametes with 10 or 11 chromosomes might have been produced in high percentage, as the chromosome distribution in the heterotypic division of the triploid plants suggests (table 3). It is not very probable, however, that these gametes function, since the unbalanced condition of the chromosome constitution in such gametes (being denoted, for instance, as AA BB CC DD E F G or AA BB CC D E F G) should react fatally upon their viability. More-

over, if such gametes functioned, such combinations as 11+11, 10+11 or 10+10 would result. Consequently, progenies with 22, 21 or 20 chromosomes would have appeared, which however, have never been found in either Dermen's or the author's data. Gametes with the chromosome number less than 10 possibly do not function, because a plant with chromosomes less than 19 has not been met with either in this experiment or throughout my *Petunia* investigations covering several years. All these facts lead us to the conclusion that, of the gametes of the triploid *Petunia*, those with 12, 13, 14 and 15 chromosomes are functional.

It is a peculiar characteristics of the F_1 progenies of triploid *Petunia* selfed that a great number of them have a fragment of chromosome (Fig. 18, 19, 20). In table 5 it is seen that out of 35 plants only 9 are

Fig. 18-20. Chromosome complement in the heterotypic metaphase of the progeny of a triploid selfed. Acetocarmine smears. Fig. 18, 26+2f plant. ca. $\times 1850$. Fig. 19, 26+f plant. ca. $\times 1850$. Fig. 20, 28+f plant. ca. $\times 2000$.

without fragments. More plants may have contained the fragment, since its observation is often difficult when it is attached to one of the chromosomes by which it is masked. In one plant two fragments were found (Fig. 18). They are either distributed into both daughter nuclei after heterotypic division, or sometimes are included in one and the same nucleus. The fragment may be divided in the heterotypic division as is seen in Fig. 19.

There are occasional figures of heterotypic metaphase without any trabants, although in many cases one trabant is identified. It is very likely that the chromosomes with the trabant has been completely excluded from the gametes in the course of the divisions. On the contrary, in a plant L6.N1-12-11 two chromosomes were found carrying the trabant. This is supposed to have originated from the fusion of both gametes each containing a trabant.

The heterotypic metaphase figures shown in Fig. 18 and 19 were found in pollen mother cells of a plant with 26 chromosomes, the former having two fragments and the latter one. The chromosome combinations are respectively $1_{IV} + 2_{III} + 8_{II} + 2f$ and $1_{IV} + 5_{III} + 3_{II} + 1_I + f$. The latter of these combinations can hardly be explained unless we postulate same

attraction at one end of each of two chromosomes non-homologous to each other, and the establishment of such attraction is most favorably interpreted by the segmental interchange hypothesis.

5. Triploid $\times$ diploid

A triploid plant derived from the crossing between the tetraploid and the diploid, was back crossed to the diploid plant. The crossing was successful, but among 250 seeds sown only 3 plants germinated. Each plant revealed a marked peculiarity, morphologically as well as physiologically. Plant 1 had small leaves, almost analogous in shape to the diploid parent, and small pink flowers. Stems and branches were a little slender. It lacked vigor and matured very late. Plant 2 grew better and matured rather early, attaining a considerable height. The leaves were of the Leviathan type with very short petioles but not so large as the Leviathan. The flowers were small and pink colored. Plant 3 was a dwarf with very small and elliptical leaves. They all seemed sterile or at any rate lacking in fertility, judging from the natural result, though they could not be experimentally tested.

Somatic counts were 21 in plant 1, 19 in plant 2, and 20 in plant 3. Detailed study was not possible on account of very scanty material. Five good metaphase figures of plant 3 showed the chromosome combination, $4_{III} + 3_{II} + 2_I$ (Fig. 21), $1_{III} + 6_{II} + 5_I$ (Fig. 22) and $2_{III} + 5_{II} + 4_I$ (Fig. 23), the last appearing three times. A trivalent was identified at both ends of one of the bivalents. In four good metaphase plates of plant 1 the counts were $5_{III} + (3_{II} + f)$, $1_{III} + 6_{II} + 6_I$, $6_{III} + 3_I$ and $4_{III} + 4_{II} + 1_I$. Plant 2 unfortunately died before we could obtain any material.

Figs. 21-23. Heterotypic metaphase of $3n-1$ plant derived from $3n \times 2n$ crossing. Acetocarmine smears. ca. $\times 2200$.

It is almost certain that in these crossings seven chromosomes have invariably been contributed by the diploid parent, because no aneuploid plant such as $2n+1$ or $2n-1$ has ever been discovered in all my investigations, among the diploid populations. Among the gametes of the triploid, therefore, those with 12, 13 and 14 chromosomes must be most functional. This also well corroborates the results of the selfing of the triploid.

The chromosome combinations $5_{III}+3_{II}$ and $4_{III}+4_{II}+1_I$ can also be adequately explained only by the segmental interchange hypothesis.

6. Diploid $\times$ triploid

From the reciprocal crossing of the proceeding, 441 seeds were obtained among which only three germinated. All these three plants showed rather less variation, being analogous to the primary triploid hybrids and died before maturing from unknown causes.

7. Tetraploid $\times$ triploid

A plant L6.N1-12 selected at random from the primary triploid progeny of the mating $L6 \times N1$, was crossed with a tetraploid plant originating from L6 selfed. Fifty six seedlings were raised from 222 seeds sown. They showed a considerable variation morphologically as well as physiologically. The flowers were a mosaic of purple and pink in color and intermediate in size, more or less tending to the diploid ancestor, N1. A certain number of plants were weak and dwarfed and could not survive to maturity. Among 31 plants examined there were one 24 chromosome plant, one 26 chromosome plant, six 27 chromosome plants, seventeen 28 chromosome plants and six 29 chromosome plants. In a metaphase plate of one 28 chromosome plant $1_{VI}+1_{III}+6_{II}+7_I$ were found (Fig. 24).

Fig. 24. Heterotypic metaphase of a 28 chromosome plant found among the progeny of $4n \times 3n$. The chromosome combination is $1_{VI}+1_{III}+6_{II}+7_I$. The cell was pressed. Acetocarmine smear. ca. $\times 2000$.

8. Triploid $\times$ tetraploid

From 134 seeds obtained 21 plants were raised. They showed a very wide range of variation as was the case with the tetraploid $\times$ triploid. The flower color was, as a rule, purple and pink mosaically arranged with two exceptions of which one had white flowers with deep shade in the center and the other had pale purple flowers with deep red veins in the center. Among 21 individuals studied there were twenty 28 chromosome plants and one 29 chromosome plant. In a good metaphase plate of one plant there was found a 7_{IV} combination (Fig. 25.)

Fig. 25. Heterotypic metaphase of a 28 chromosome plant found among the progeny of $3n \times 4n$. There exist 7_{IV} . Acetocarmine smear. ca. $\times 2200$.

Discussion

Many investigators have attempted to produce triploid by crossing tetraploid and diploid. In the majority of cases they unexpectedly secured diploid progenies from $2n \times 4n$ crossing, whereas triploids were produced from the reciprocal crossing. In *Petunia*, Steere (1932) recently carried out experimental crossings between *P. axillaris* ($2n = 14$) and a tetraploid giant variety, California Giant ($2n = 28$). Half of the progeny from $2n \times 4n$ were intermediate in size between the parents, while the other half resembled the diploid almost exactly in stature and habit. As the result of microscopical observation, some plant selected at random in this culture proved to be normal diploids. He explains this result as follows: "They can not have arisen parthenogenetically, but must have contained a certain number of chromosomes contributed by the pollen parent as the resemblance in habit and flower color showed. Different proportions of each set of parental chromosomes were apparently present in these F_1 plants, since there was such a wide range of variation, especially as one of the parents (*P. axillaris*) is known to be a pure strain." On the other hand, the reciprocal crossing $4n \times 2n$ gave a quite different result. The germinating rate of the seeds was very much lower than that in the reciprocal crossing. In a culture of F_1 progeny all plants were triploid, showing great variation in color and size of flowers and also growth habit and stature, ranging from those resembling the tetraploid to those almost similar to the diploid parent. Another culture consisting of triploid plants showed less variation than the preceding culture, the plants rather resembling the tetraploid parent in size and general habit.

Dermen (1931) reared four triploid plants from the diploid $\times$ tetraploid crossing in *Petunia*. Three of them were large and one small flowered. Later he carried out another crossing $4n \times 2n$, of which the F_1 progeny was triploid with the exception of one $3n-1$ plant.

Kostoff and Kendall (1931) secured the following four kinds of progeny with respect to the chromosome number as the result of crossing the tetraploid mutant found among the progeny of a variety of diploid *Petunia violacea*, with a variety of diploid *Petunia*: sixteen tetraploids, two $4n-1$ plants, one triploid and one $3n-1$ plant.

The present results are agreed with those of Steere in that the greater part of the F_1 progeny of $4n \times 2n$ was triploid, whereas from the reciprocal crossing $2n \times 4n$ no triploids were raised, the progeny being diploid maternal. The process by which the diploid progeny were produced in this latter crossing, however, must be different from Steere's case, since no significant variations were found among the progeny, all resembling the diploid parent and breeding true when selfed. Again,

there is little doubt that they resulted by diploid parthenogenesis or apogamy, since no genic influence of pollen parent could be seen upon them. Cases have been reported by various investigators where diploid parthenogenesis or apogamy was induced by pollinating a plant with pollen from distant species or genera. East (1930) obtained a number of diploid progeny by pollinating heterozygous *Fragaria vesca* ($2n=14$) with pollen from *F. virginiana* or *F. chiloensis* ($2n=56$). Basing upon the gene analysis most of this progeny were proved to have been produced by parthenogenesis. Recently (1934) Terao reported the result of his extensive experiments in which many different species of *Brassica* were pollinated with pollen from species with different chromosome numbers and also from different genera such as *Raphanus*, *Mattiola* and *Eruca*. Among the F_1 progenies a considerable number of diploid materials were obtained. Ichijima (1930) obtained F_1 progenies identical in morphological characters as well as in chromosome number with the female parent in crossing between different species of *Fragaria* with different chromosome number. Longley (1926) in crossing $2n \times 8n$ in *Fragaria* observed that all the F_1 progenies were diploid maternal in one crossing, while among F_1 progenies from other crossing using other species one plant was diploid and the other octoploid.

Jørgensen (1928) in *Solanum* and Noguchi (1928) in *Brassica* gave the cytological verification that when distant species are crossed the male nuclei do not fuse with the egg nucleus, and are disintegrated into fragments. Both cases account for the haploid maternal. How then does the haploid egg cell develop into the diploid embryo? East gave an account of his result stating that the beginning of the development must have been haploid but the diploidy was brought about through mitotic division of chromosomes without nuclear division. The present author also thinks that for the present this is the most probable explanation of the production of diploid progeny from $2n \times 4n$ crossings, though it lacks experimental evidence. Another hypothesis for the explanation of this phenomenon is that the egg nucleus may fuse with one of the synergids. We must wait for further detailed studies to determine which of these explanations is really true.

Now the question must be raised as to the reason why the nuclei from both parents do not fuse together in the embryo-sac. Some cases where both parents are of very distant species or genera will be explained by a lack of affinity between paternal and maternal chromosomes, but in the present case where the chromosomes of both parents are almost similar presumably belonging to the same genom, this account will not fit.

Lesley (1930) ascribes the cause of the difficulty of crossing tetraploid and diploid tomatoes to the differences in the pollen tube growth of haploid and diploid pollen. Namely he states. "It is possible that, as a rule,

diploid eggs are immature when reached by rapid growing haploid pollen tubes, and that the diploid pollen tubes grow too slowly to reach haploid eggs before they are too old." This explanation may seem to be applicable to the present case, but not only does it lack experimental basis, but it encounters difficulty in the fact that tetraploid or hypotetraploid progenies are produced in another F_1 population of the same mating.

The present author adopted the assumption of a karyo-plasma ratio for the explanation of the fact that diploid plants which were pollinated with the giant pollen grains gave diploid F_1 progenies (1934b). This assumption seems to fit the present case as well. In $2n \times 4n$ crossing the male nucleus probably enters the egg cell in which, however, the amount of cytoplasmic content is too small for the $2n$ number of paternal chromosomes, so that only the maternal nucleus develops into the embryo, the chromosomes duplicating later in some way, and the paternal nucleus degenerates. The fact that the progenies of another mating $N1 \times L16$ are all tetraploids and hypotetraploids, affords further support to the above assumption. In this case presumably the doubling of chromosomes has taken place in the course of maturation division of the embryo-sac mother cell, so that not only the chromatin content but the cytoplasm must have been duplicated, namely the ultimate egg cell must have attained double the normal volume. It is then very probable that 14 paternal and 14 duplicated maternal chromosomes can survive and develop, the karyo-plasmic ratio being normal.

In $4n \times 2n$ crossings, $L16 \times N1$ gave no triploid but one diploid, one $3n+2$ plant and one tetraploid, while $L6 \times N1$ gave 24 triploids besides one diploid and two heteroploids: and on the other hand, $N1 \times L6$ led to a result different from $N1 \times L16$. This fact indicates that $L6$ and $L16$ have different constitutions, either genic or chromosomal.

It is surprising that among each of three F_1 populations from the crossing $4n \times 2n$ is found one diploid plant. As for the process by which these diploid individuals have been produced, the following three cases can be considered: (1) a haploid female gamete which may have been produced in the tetraploid may give a diploid plant, fusing with a normal haploid pollen from the diploid parent. (2) Parthenogenesis may have been caused by the male substance, giving rise to a diploid progeny. (3) Diploid male gametes which may have arisen from a restitution nucleus in the diploid parent, may have entered the egg cells of the tetraploid parent, and developed into an embryo, the maternal chromosomes not fusing with the paternal ones but degenerating.

It is to be noted that among the three diploid hybrids one from the mating $L16 \times N1$ is phenotypically different from the others. No purple color is identified upon the flowers either of this F_1 plant or of the plants

in the F_2 progeny. This fact affords evidence that this plant was not produced by the process (i) mentioned above, since, if it did, some purple color would appear on the flowers of the progeny at least in the F_2 generation. Moreover, the production of haploid gametes in the tetraploid plant appears to be improbable on cytological grounds, since such a segregation of chromosomes in the heterotypic division as $21+7$ has scarcely been met with in tetraploid plants throughout my extensive observations. It is beyond question that the merogony mentioned in (3) is not the adequate explanation of the production of this plant, since not only is the flower color in this plant quite different from that in the diploid parent but other conspicuous characters such as of leaves, branches, etc. are strikingly different. We may then be justified in concluding that this plant was produced by the diploid parthenogenesis.

The two other diploid plants found among the progenies of other kinds of $4n \times 2n$ crossings suggest the occurrence of merogony. The phenomenon of merogony has been reported by a number of investigators in animal materials since the classical case of sea urchin, studied by O. and R. Hertwig (1887). In plant materials the data are very rare. Clausen and Lammerts (1929) crossed *Nicotiana digluta*, a synthetic amphidiploid species with 36 chromosomes (consisting of 24_{II} of *N. tabacum* + 12_{II} of *N. glutinosa*), with *N. tabacum* ($2n=48$) which is one of the original species. Among the F_1 progeny there was found a haploid *N. tabacum* with the chromosome number 24. This plant was morphologically identical with the paternal and was thought to have been produced through merogony. Furthermore, obtained the occasional pure *sylvestris* plants ($2n=12_{II}$) in back cross of F_1 *sylvestris-tabacum* ($12_{II}+12_I$) $\times$ *sylvestris* (12_{II}). They ascribed the origin of these plants to diploid merogony. As to the ground of this conclusion, they point out that "assuming random distribution of bivalents in F_1 *sylvestris-tabacum*, the chance of obtaining a 12-chromosome gametes is very small, only one in 4,096 and the chance in turn that such a gamete will contain nothing but *sylvestris* chromosome is again only one in 4,096." In the present case we can point out much fuller evidence for the occurrence of diploid merogony, as follows: (i) The maternal plant is not such a synthetic amphidiploid with evident origin as *N. digluta*. Accordingly, it is improbable that the whole genom in the paternal parent is contained in the maternal parent and in turn that gametes with only the chromosomes of the diploid parent are produced in the maternal parent. Nevertheless, the two diploid plants L5.N1-10 and L6.N1-14 are identical with the paternal parent either in morphology or cytology. (2) The F_2 progenies of these plants revealed no segregation. (3) In one of these F_2 plants which was cytologically examined, one bivalent was found invariably carrying a trivalent at each of its free

ends. From these facts, there seems to be no escape from the conclusion that these represent a true case of diploid merogony.

Kostoff and Kendall (1932) pointed out that there are two ways of explaining the origin of the tetraploid progeny of $4n \times 2n$ crossing, namely, (1) "There is the possibility that the egg nucleus ($n=14$) of the tetraploid mother plant may have been fertilized by both the generative nucleus and the vegetative one from the pollen grain of the diploid father plant, so that the resultant zygote contained $14+7+7=28$ chromosomes which, by subsequent division, developed the tetraploid progeny." (2) A 14 chromosome egg of the tetraploid mother plant may have been fertilized by a generative nucleus with 14 chromosomes originating from a dyad pollen grain. These probably cover all the possible explanations for the production of tetraploid plant among the offspring of $4n \times 2n$ crossing. It is not possible to determine which of the above alternatives is a real truth. However, due to the fact that its phenotypic characters resemble the tetraploid parent and also that the diploid pollen grains are not infrequently found in the diploid parent, the present author is rather inclined to accept the second of Kostoff's and Kendall's explanations.

Summary

1. The tetraploid plant of giant variety of *Petunia* was selfed. Among 31 plants of the F_1 progeny 29 were tetraploid, one $4n-1$ and one $4n-3$. No significant morphological differences were noticed among them.

2. The tetraploid plants of the giant variety and the diploid plants of small variety were crossed reciprocally. Among the populations of F_1 progenies two gave a high percent of triploids besides each one plant of heteroploids and a diploid. The third gave no triploid but respectively one plant of $2n$, $3n+2$ and $4n$. Among these three diploid plants, one was completely different from both the parents, while the other two were complete replicas of the diploid parent. The triploid plants showed approximately intermediate characters more or less tending toward the tetraploid parent.

3. The triploid progeny was cytologically studied. In the heterotypic metaphase 7 sets of trivalent were not infrequently found. Almost all the trivalent configurations possible in auto-triploids were observed excepting one. Besides these the ring of three or triangle, and the multivalents were sometimes met with. These may be interpreted by the segmental interchange hypothesis.

4. The $2n \times 4n$ crossing gave only the diploid progeny in one case. These progeny exhibited exactly the same characters morphologically as well as physiologically as the diploid parent. Another mating resulted in three tetraploids and one $4n-1$ plant.

5. The triploid hybrids selfed gave 26, 27, 28 and 29 chromosome plants in the F_1 progeny among which 27 chromosome plants were of the highest frequency. The majority of these progenies had one or two fragments of chromosomes. A trivalent derived from the diploid ancestor is identified at one end of one of the chromosomes.

6. The triploid hybrid was back crossed to the diploid parent. Three plants were obtained with the somatic number respectively of 19, 20 and 21. This shows that the gametes of the triploid with the chromosome numbers of 12, 13 and 14 function.

7. The tetraploid-triploid crossings gave the F_1 progenies of 24, 26, 27, 28 and 29 chromosomes with respective frequencies of 1, 6, 17 and 29.

8. The reciprocals of the above crossings gave one 29 chromosome plant and twenty 28 chromosome plants.

9. The appearance of diploid progenies in the crossings between $2n$ and $4n$ plants may be explained by the diploid parthenogenesis.

10. As to the appearance of diploid progenies in $4n \times 2n$ crossings, two different cases must be distinguished: (1) diploid parthenogenesis and (2) merogony. Both of these are supposed to occur.

11. The occurrence of tetraploid plants among the F_1 progeny of $4n \times 2n$ crossings probably was induced by the fertilization of an egg nucleus by a generative nucleus with double number of chromosomes.

12. The tetraploid and triploid plants of *Petunia* must be auto-tetraploid and auto-triploid.

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