CHROMOSOME BEHAVIOR IN FERTILE TRIPLOID ASTER HYBRIDS[1]

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THE sterility of triploids and other unbalanced polyploids is generally conceded to be a consequence of irregularities in the distribution of univalents and multivalents at meiosis. Occasional viable gametes are produced, however, because of nonrandom orientation and segregation of chromosome associations. Thus, SATINA and BLAKESLEE (1937a, b) found a higher frequency of n and 2n chromosome numbers in gametes produced by triploid Datura than was expected on the basis of random orientation of trivalents.

The Canina roses furnish a striking exception to the general rule of sterility of unbalanced polyploids (FAGERLIND 1940). There are also several reports of fertile triploid and aneuploid individuals in other species. DEMOL (1921) found that triploid *Hyacinthus orientalis* L. ($3n = 24$) was fertile, as were various aneuploid strains in this cultivated species. Microspore chromosome numbers of 8 to 14 were found (BELLING 1924) and the fertile progeny contained somatic chromosome numbers of 16 to 30. A triploid form of *Crepis artificialis* ($3n = 36$) was obtained in which 17 bivalents and 2 univalents regularly formed (COLLINS et al. 1929). This behavior resulted in 62 to 70 percent pollen fertility in the triploids.

In the course of a biosystematic study of several species of Aster (AVERS 1953), three fertile triploid F_1 hybrids were obtained. Their unusual chromosome behavior has furnished the basis for the present report.

MATERIALS AND METHODS

Twenty separate crosses between *Aster shortii* Lindl. ssp. *shortii* ($n = 18$) and *A. cordifolius* L. ($n = 9$) were attempted in 1950, and three of these yielded one F_1 hybrid each. One of these three hybrids was successfully backcrossed to the tetraploid parent species in 1951, and nine backcross plants were raised to maturity. In addition to the experimentally obtained individuals, one natural triploid also has been studied cytologically. All plants were grown in the greenhouse at Indiana University.

Buds of the 13 plants studied were fixed in chloroform-ethanol-acetic acid, 4:3:1, for one week, and squash preparations of pollen mother-cells were made in aceto-orcein. Pollen fertility was determined by staining with cotton blue.

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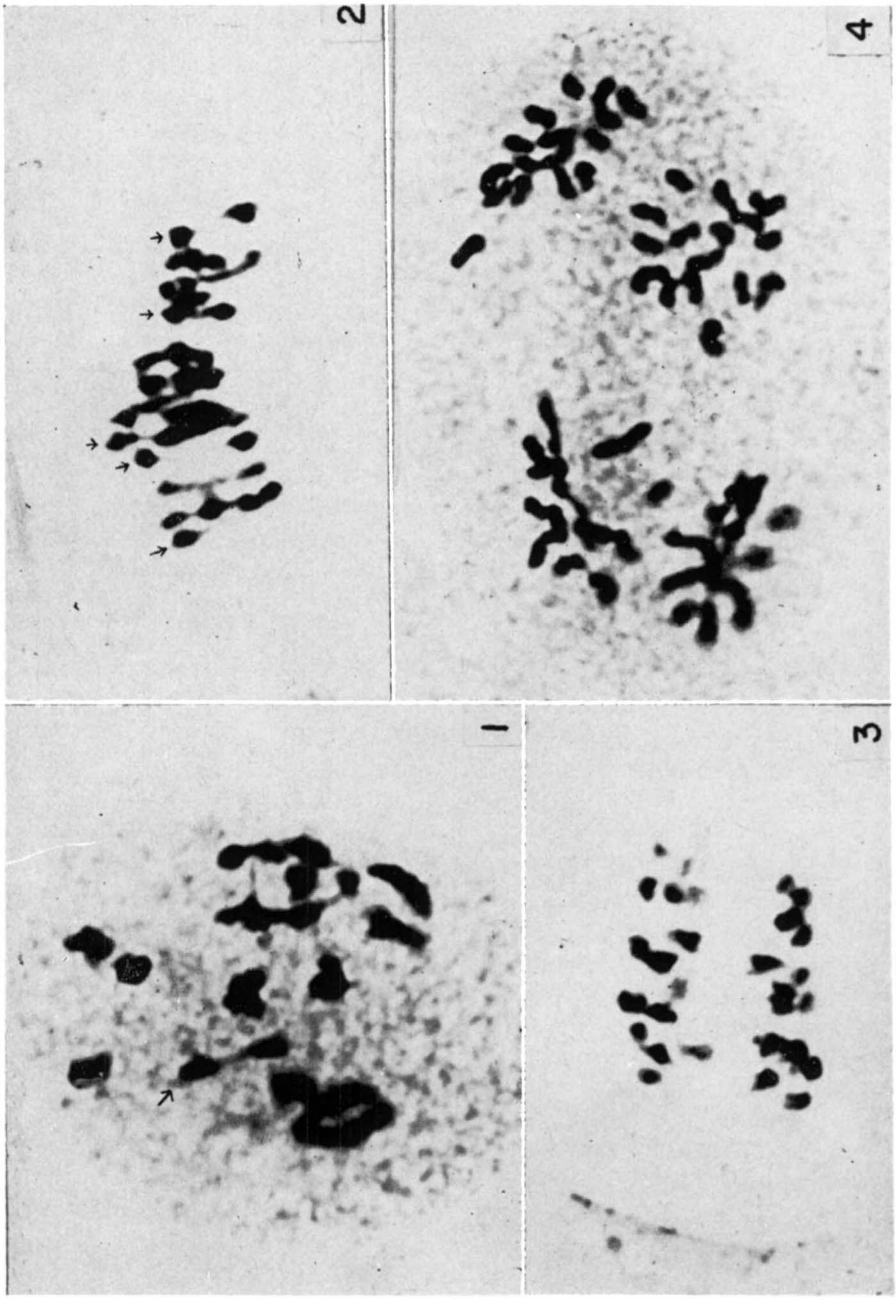


PLATE I.—Meiosis in fertile triploid Asters ($3n = 27$). × 2000.

TABLE 1

Chromosome constitution of three triploid F_1 Aster hybrids ($3n = 27$).

No. cells	Configurations			Expected gametic chromosome numbers[a]	
	Trivalents	Bivalents	Univalents	1 division of univalents	2 divisions of univalents
1	4	7	1	11–16	12–16
1	4	5	5	9–18	14–18
2	3	8	2	11–16	13–16
1	3	7	4	10–17	14–17
1	3	6	6	9–18	15–18
2	2	10	1	12–15	13–15
3	2	9	3	11–16	14–16
11	2	8	5	10–17	15–17
7	2	7	7	9–18	16–18
1	2	6	9	8–19	17–19
1	1	11	2	12–15	14–15
10	1	10	4	11–16	15–16
19	1	9	6	10–17	16–17
11	1	8	8	9–18	17–18
4	0	12	3	12–15	15
10	0	11	5	11–16	16
8	0	10	7	10–17	17
7	0	9	9	9–18	18
100	1.7	8.5	4.8	13–14	16

[a] Chromosome numbers are based on the following assumptions: (1) random distribution of univalents; (2) random orientation of trivalents; and (3) regular disjunction of all trivalents 2-by-1. The ranges of gamete chromosome numbers were determined empirically.

F_1 hybrids. All three hybrids were 90 percent pollen-fertile and contained 27 chromosomes in varying configurations at meiosis (table 1). In many microsporocytes it was seen that some univalents had begun to divide at diakinesis (fig. 1), and that all univalents divided at metaphase I (fig. 2). Although the univalent halves appear to disjoin precociously, their actual separation occurs synchronously with bivalent and trivalent segregation (figs. 3 and 5). Infrequently first anaphase cells were seen in which a lagging univalent was in the process of division. The nuclei enter a protracted interphase and at metaphase II the chromosome number exceeds 27. Sister nuclei contain 14 to 18 chromosomes, thus confirming the direct observations of MI

FIGURE 1.—Diakinesis showing 2 trivalents, 8 bivalents and 5 univalents. Arrow indicates precocious separation of univalent halves.

FIGURE 2.—Metaphase I showing dividing univalents.

FIGURE 3.—Anaphase I with 14 chromosomes going to one pole and 15 to the other, showing simultaneous separation of all chromosome associations.

FIGURE 4.—Anaphase II showing 16 chromosomes in each of two sister nuclei and 14 chromosomes in another nucleus of the tetrad. The fourth nucleus, with 14 chromosomes, is obscured. This indicates that the univalents have completed two successive divisions since the combined chromosome number exceeds 54, which would have been expected if the univalents had divided only once.

TABLE 2

Comparison of expected and observed gametic chromosome numbers in three F_1 triploids.[a]

Chromosome number	12	13	14	15	16	17	18	19
Expected[b]								
No. cells (103)	0.06 ± 3.1	1.03 ± 1.0	3.67 ± 3.3	16.48 ± 4.1	34.96 ± 4.9	31.16 ± 4.1	15.39 ± 3.0	0.26 ± 3.1
Per cent cells	0.06	1.00	3.56	16.00	33.94	30.25	14.94	0.25
Observed[c]								
No. cells (103)	0	1	12	21	37	22	10	0
Per cent cells	0.0	1.0	11.6	20.4	35.9	21.4	9.7	0.0
Dev./S.E.	0.0	0.3	2.5[d]	1.1	0.4	2.2[d]	1.8	0.1

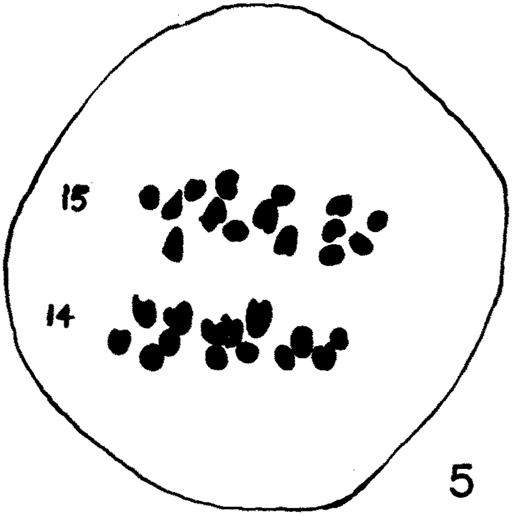
[a] Calculations are based on data presented in table 1.

[b] Determined by expansion of the binomial for each configuration listed in table 1.

[c] Counts were made of individual second anaphase nuclei.

[d] Indicates statistical significance.

univalent divisions. There is great difficulty in obtaining adequate preparations of anaphase II for purposes of counting potential gamete chromosome numbers since the meiotic products are arranged tetrahedrally. However, counts were made of 103 nuclei in 31 cells, and in every case the chromosome number fell within the range expected on the basis of two divisions of the univalents (table 2). In most cases in which sister nuclei could be counted at anaphase II, it was seen that they contained the same chromosome number. Figure 4 is a photomicrograph of a second anaphase cell in which 16 chromosomes are visible in each of two sister nuclei and 14 chromosomes can be seen in a third



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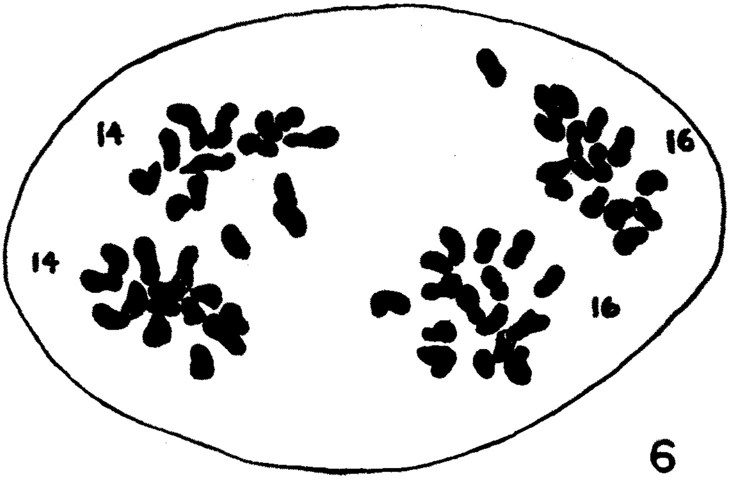

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FIGURE 5.—Diagram of figure 3. × 2000.
FIGURE 6.—Diagram of figure 4. × 2000.

nucleus. The fourth nucleus in this cell also contains 14 chromosomes although this is obscured since they were in another focal plane (see fig. 6). No micronuclei were observed in any of the material. Rarely a chromosome was seen to be excluded from a telophase nucleus.

Backcross hybrids. Crosses of the triploid to the diploid parent species were unsuccessful. This behavior is typical of heteroploid crosses in Aster where it has been shown that the diploid x tetraploid cross usually fails (AVERS 1953).

Nine backcross hybrids were raised to maturity. Four of the backcross plants were obtained from the cross *A. shortii* ($2n = 36$) x F_1 *A. shortii* x *cordifolius* ($3n = 27$) and the remaining five plants were obtained from the reciprocal cross. The chromosome numbers of the backcross progeny ranged from $2n = 33$ to $2n = 35$. It is significant that the chromosome numbers of this random sample are within the range expected on the basis of two divisions of the univalents in the triploid parent. Phenotypically the backcross individuals are very similar to one another and almost indistinguishable from the recurrent parent. Pollen fertilities of the backcross hybrids ranged from 80 to 90 percent.

The seed set in the backcrosses was 70 percent when the triploid was female parent as well as when it was used as male parent. These equivalent fertilities, and the chromosome numbers of reciprocal backcross hybrids, indicate that meiosis is probably similar in megasporogenesis and microsporogenesis.

In Aster as in Hyacinthus, aneuploidy does not result in increased sterility, nor in phenotypic change. ROTHWELL (unpublished data) has found a similar situation in *Claytonia virginica* L. in that aneuploid and polyploid Claytonias are usually highly fertile. Moreover, aneuploids are not morphologically distinct despite extreme variation in chromosome number.

Natural triploid. A natural triploid was collected in 1949 at Yellowwood State Forest in Brown County, Indiana. It is not completely certain whether it is a hybrid between *A. shortii* and *A. cordifolius* or whether it represents a cross between diploid and tetraploid *A. shortii.* That the former situation probably obtains is suggested by the absence of diploid *A. shortii* in southern Indiana as well as the fact that the plant was collected in a mixed population of *A. shortii* and *A. cordifolius.* Configurations at MI were within the range of chromosome associations found in the artificial triploids. Only 21 second anaphase nuclei were counted but the lowest chromosome number found was 14. If the univalents divided once and were distributed at random at the other division, lower gametic chromosome numbers would be expected. The natural triploid is 80 percent fertile, and morphologically similar to *A. shortii.*

DISCUSSION

The unexpectedly high fertility of the triploid hybrids may be due to any one, or some combination, of a number of factors.

1. Viable gametes would contain between 9 to 18 chromosomes if the univalents and trivalents were distributed at random. This situation obtains in *Hyacinthus orientalis* (DARLINGTON 1929), and to a certain extent in Aster.
2. Viable gametes would contain the haploid or diploid number of chromosomes if there were a constant pattern of univalent distribution. This situation

applies to the Canina roses in which the univalents are absent from the functional male gametes but are retained in the functional eggs (FAGERLIND 1940). In triploid Datura, it is believed that nonrandom orientation of trivalents leads to a significant increase in the frequency of n and 2n gametes (SATINA and BLAKESLEE 1937a, b). SEARS (1953) has also noted a tendency for most or all univalents to go together in Triticum-Haynaldia hybrids which, with elimination of gametes with low chromosome numbers, yields an increased frequency in higher chromosome numbers in later generations.

3. The univalents might divide twice at meiosis thus increasing the frequency of higher chromosome numbers, and virtually eliminating gametic chromosome numbers which approximate the haploid condition. The chromosome complement would vary, however, depending upon the number of univalents and trivalents.

There have been observations in various materials which indicate that the univalents may undergo two successive divisions at meiosis. CLAUSEN (1926) reported this situation in certain Viola hybrids although cytological studies did not extend beyond metaphase II. Other *sporadic* occurrences of two successive univalent divisions have been cited by DARLINGTON (1930) in the triploid cherry *Prunus avium nana,* by ERLANSON (1929) in the triploid hybrid *Rosa blanda* × *carolina,* in Raphanobrassica hybrids by KARPECHENKO (1927), in Brassica hybrids by MORINAGA (1929), in Saccharum hybrids by BREMER (1923), and in Ribes hybrids by MEURMAN (1928).

FEDERLEY (1913, 1931) reported the *regular* occurrence of this phenomenon in Pygaera hybrids. Meiosis in these moth hybrids is unique in several respects. There is little chromosome pairing at spermatogenesis and regularity of pairing at oogenesis of F_1 hybrids. Univalents may be eliminated at meiosis in the oocytes of backcross hybrids but not at meiosis of the F_1 hybrid females. Although some univalents may be eliminated at spermatogenesis, they will usually undergo two successive divisions in male hybrids. The hybrid derivatives are partially fertile regardless of variation in chromosome number. Later studies of Dicranura and Drepana hybrids failed to reveal the Pygaera-type meiosis (FEDERLEY 1943, 1949).

Calculations of the frequencies of various gametic chromosome numbers were made on several assumptions, including whether there are one or two univalent divisions, and these were compared with the observed frequency of chromosome numbers in anaphase II nuclei of the three triploid F_1 hybrids (fig. 7). It is quite apparent that the observed data fit with the frequencies calculated on the basis of two successive univalent divisions. The absence of classes of gametes containing fewer than 13 chromosomes immediately suggests a mechanism whereby the chromosome number is increased. However, from table 2 it is seen that there is a significant increase in the frequency of 14-chromosome gametes and a concurrent decrease in the 17-chromosome class. The same tendency is noted for the 15- and 18-chromosome classes. These results may be due to several factors: (1) not all the univalents divide twice; (2) there is a greater error in counting the higher chromosome numbers; (3) some univalents lag and are lost; and (4) more nuclei with fewer chromosomes are counted since there is less doubt in interpretation. If nonrandom orientation of trivalents were a contributing factor, then one would expect a concurrent increase in both the higher and lower numbers. Since the frequency distributions calculated on the basis of one division of univalents show a peak at 13- and 14-chromosomes, it is conceivable that the observed high frequency of 14-chromosome gamete nuclei is due to occasional univalents which divide but once. However, the other factors cannot be eliminated from consideration.

Although it seems quite certain that univalents undergo two successive divisions at microsporogenesis (and probably megasporogenesis) in Aster triploids, it has not definitely been established that these are both equational divisions. SEARS (1952) found that univalents in hexaploid wheat usually misdivide and never separate equationally at both meiotic divisions. The observations of two divisions of the univalents in the species cited above, do not necessarily imply that both divisions are equational.

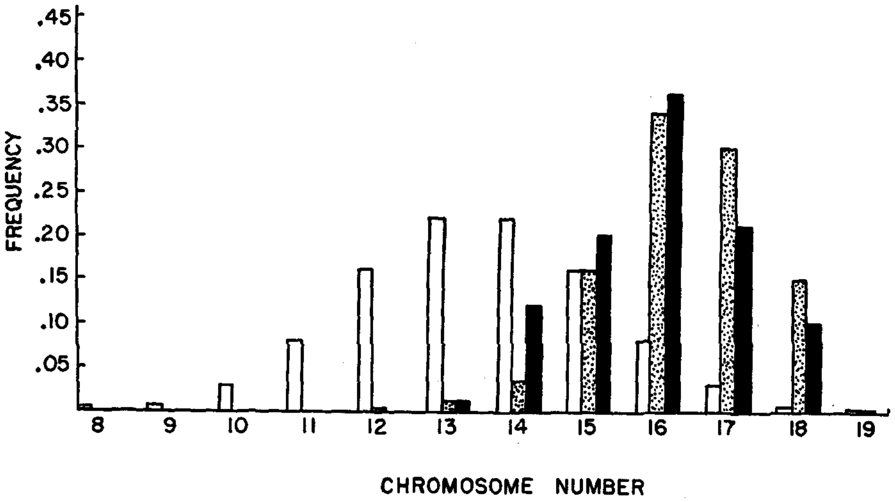

FIGURE 7.—Comparison of the expected frequency of gamete chromosome numbers with (a) only one division of univalents and random distribution (open bar), or (b) two divisions of all univalents (stippled bar), and (c) the observed frequencies (black bar).

The presence of a tertiary split in the meiotic metaphase chromosome has been claimed from cytological evidence by NEBEL (1941), HUGHES-SCHRADER (1948), and others. If cytological observations are supplemented with genetic data concerning gamete viability, then substantial evidence of a functional tertiary split is possible. However, fertility data in Aster, as in Pygaera, cannot be used unequivocally since there is no apparent phenotypic effect as a consequence of the presence of extra chromosomes between the triploid and tetraploid levels. All Aster hybrids are fertile regardless of the number of univalents and multivalents present. The same situation in Hyacinthus has been interpreted to mean that little chromosome differentiation has occurred with increase in the number of genomes (DARLINGTON 1929). However, the high fertility of Aster triploids and backcross hybrids strongly suggests that there is little genic unbalance. If the univalents were to misdivide one might expect greater genic unbalance, as a consequence of the production of telocentrics and isochromosomes, than would result with an increase in the number of whole chromosomes. The high fertility of the hybrids, therefore, suggests equational divisions rather than misdivisions of the univalents present. The fact that lagging chromosomes are rarely seen would also imply infrequency of misdivision.

On the evidence presented, it seems likely that the univalents in the F_1 Aster triploids studied do undergo two successive equational divisions. This is believed to be a cytogenetic demonstration of a functional tertiary split in the meiotic metaphase chromosome.

SUMMARY

Data have been presented which indicate that the univalent chromosomes regularly undergo two successive divisions at meiosis. This phenomenon accounts for the increased frequency of higher chromosome numbers in the resultant gametes of the Aster triploids studied, and consequent increased fertility. The chromosome numbers of nine backcross individuals studied were within the range expected on the basis of two divisions of the univalents in the triploid parent. One natural triploid studied exhibited the same behavior as the artificial triploids.

An attempt was made to correlate these findings with the probability of a functional tertiary split in the meiotic metaphase chromosome. The following observations strongly suggest that the univalents do not misdivide, but rather, undergo successive equational divisions.

1. Lagging univalents were rarely seen. The regularity of disjunction of univalent halves implies a regularity in centromere and chromonema separation, which is not necessarily expected with misdivision.

2. The high fertility of the triploids, which implies genic balance, is most likely the result of the increase in number of whole chromosomes to approximate the diploid complement in the gametes, as a consequence of equational divisions of univalents. Misdivisions result in the production of telocentrics and isochromosomes, which would yield greater genic unbalance and decreased fertility.

Thus, it seems likely that the univalents in the F_1 Aster triploids studied complete two successive equational divisions, which is a cytogenetic demonstration of a functional tertiary split in the meiotic metaphase chromosome.

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