ASPECTS OF ASYNAPSIS IN PLANTS. I. RANDOM AND NON RANDOM CHROMOSOME ASSOCIATIONS

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DURING the course of a cytogenetic study of some derivatives of a cross between *Nicotiana tabacum* and *N. rustica,* the population was found to vary in the extent of chromosome synapsis occurring during meiosis in microsporocytes. A detailed cytological study was therefore undertaken in this population and plants showing normal pairing, partial asynapsis and complete asynapsis were isolated. In the progenies of partially asynaptic plants segregation occurred for the extent of synapsis, thereby indicating that the character is genetic. The data from the analysis of chromosome pairing were subjected to Poisson and binomial analyses to see whether any particular pattern of pairing occurs in this allotetraploid material. Such a study revealed some interesting information and hence the Poisson analysis was extended to all reported cases of asynapsis and desynapsis, where detailed data are available. The results are presented in this paper.

MATERIAL AND METHODS

Seeds belonging to the second segregating generation of the sixth backcross of F_1 *N. rustica* × *N. tabacum* to *N. rustica* were obtained through the kind courtesy of DR. HAROLD H. SMITH of the Cornell University. The material belonged to two families, 55379 and 55380, and were grown in the field during 1955–56, along with normal *N. rustica* and *N. tabacum*. F_3 generation was grown during 1956–57. For the study of microsporogenesis, preparations were made by the Propionocarmine schedule (SWAMINATHAN *et al.* 1954). Fixations were carried out from different plants on the same day and in many cases, several fixations were done from the same plant at regular intervals. Estimation of pollen fertility was based on stainability in acetocarmine as well as by functionability as revealed by data on seed setting. For testing for goodness of fit for Poisson or binomial distributions, classes with expected frequencies of less than five were grouped to make up this minimum number, Poisson analysis being applied to plants with low bivalent frequency and binomial analysis for those cases with a proportion 0.1 or more of the normal bivalent frequency.

The two fractions of the binomial distribution are the proportion (p) of the observed bivalent frequency to the potential frequency of bivalents and ($1-p$). Tests for truncated Poisson distribution were made as per the procedure of RAO and CHAKRAVARTHY (1956).

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RESULTS

Meiosis in asynaptic plants: A rapid examination of pollen fertility in the *rustica-tabacum* population showed that fertility ranged from three to 70 percent. Chromosome associations at diakinesis and metaphase I of meiosis were studied in 23 plants, which showed that the plants with nearly complete pollen sterility were asynaptic (0 to 4 bivalents per cell) and that the plants with 80 to 90 percent pollen sterility were partially asynaptic (5–18 bivalents). The number of plants observed under each category in the population is shown below.

Family	Normal (22–24 II)	Partially asynaptic (5–18 II)	Completely asynaptic	Total
55379	6	1	—	7
55380	11	2	3	16

As the occurrence of univalents at diakinesis and M I could be due to the failure of initial pachytene association (asynapsis) as well as to a precocious terminalization of chiasmata followed by slipping of chiasmata after a normal pachytene pairing (desynapsis), pachytene stages were studied in two plants in which there was no pairing at diakinesis and M I. No pairing occurred at this stage and consequently the plants fall into the asynaptic category. In partially asynaptic plants, however, it was difficult to ascertain precisely the frequency of univalents occurring at pachytene, though several unpaired chromosomes could be observed.

A detailed comparative study was made of meiosis in two plants of each of the three types, i.e., with normal pairing, partial asynapsis and complete asynapsis. All fixations were made on the same day. The data are given in Tables 1, 2 and 3 (see also Figures 1 and 2). There were no detectable morphological differences among these plants. Two bivalents were found to be attached to the nucleolus in the normal synaptic plants. In asynaptic plants, either one bivalent or one to three univalents were found to be associated with the nucleolus. The nucleolus was also comparatively weakly stained in the asynaptic plants. At metaphase I, there was a greater degree of stickiness among chromosomes in normal plants in

TABLE 1

Chromosome associations at diakinesis

Family and plant no.	No. of cells	Mean no. of configurations with Xta/cell				Mean no. of Xta per cell	Remarks
		0	1	2	3		
55379;							
28/1 and 2	45	0.27	1.18	19.17	3.55	50.07	Normal pairing
55380;							
22/1 and 30/1	30	0.61	1.98	19.33	2.32	48.86	Normal pairing
55379; 3/3	20	37.20	4.65	0.75	0	6.15	Partial asynapsis
55380; 5/2	25	46.32	0.84	0	0	0.84	Asynapsis
55380; 6/9	26	46.92	0.39	0.15	0	0.69	Asynapsis
55380; 7/2	15	14.16	11.69	5.23	0	22.15	Partial asynapsis

TABLE 2

Frequency of aberrant cells, pollen fertility and seed-setting in normal and asynaptic plants

Plant no.	Percentage of aberrant cells at			Pollen fertility (%)	Seed bearing capsules per plant (%)	No. of seeds per capsule	
	A I	A II	Sporad stage			Selfing	Open pollinated
30/1	21.43	25.42	30.51	70.31	21.54	82.3	90.6
5/2	97.61	69.32	69.31	3.80	6.85	0	71.6
6/9	96.10	94.34	78.18	5.44	7.50	0	36.0
7/2	86.32	78.72	69.93	11.61	17.39	31.5	77.0

comparison with those of the asynaptic ones. Most of the bivalents in asynaptic and partially asynaptic plants were of the rod type with chiasma in only one of the arms. During the subsequent stages of meiosis, there were several irregularities like sticky bridges and lagging chromosomes at A I and A II, unequal distribution of chromosomes at M II and the occurrence of dyads, triads, pentads and micronuclei at the sporad stage. The percentage of cells with such aberrations is given in Table 2. Pollen fertility was very low in the asynaptic plants. There were however some giant, well-stained grains which probably arise as a result of the formation of monads or dyads.

All the plants with complete or partial asynapsis were selfed and also crossed with normal *rustica* pollen. In completely asynaptic plants, no seeds were obtained on selfing. Seed setting was, however, obtained in asynaptic plants both in
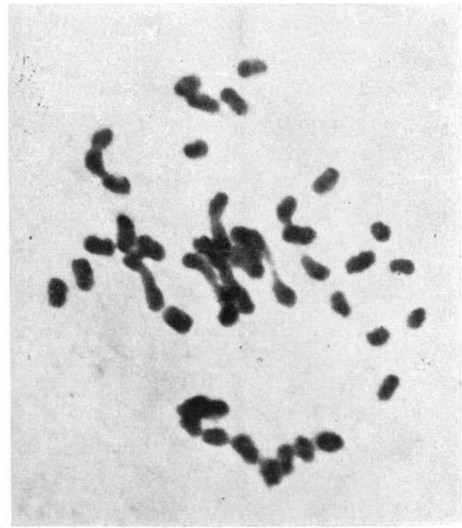
FIGURE 1.—Metaphase I in plant No. 5/2 with 48 univalents.

FIGURE 2.—Metaphase I in plant No. 7/2 with 7 bivalents + 34 univalents.

crosses with normal *rustica* pollen and under open pollinated conditions. Thus, the ovules of these plants were functional. Seed setting was observed in partially asynaptic plants on selfing, in crosses with normal plants and under open pollinated conditions. The total number of capsules formed under open pollinated conditions in the different plants is given in Table 2. Only six to seven percent of such capsules were, however, found to contain seeds in plants number 5/2 and 6/9.

Inheritance of asynapsis: Out of the 23 plants studied during 1955–56, seven plants were either partially or wholly asynaptic. Selfed seeds from the partially asynaptic plant No. 7/2 as well as open pollinated seeds from the others were sown during November 1956, along with *N. rustica* and progenies of normal plants of the *rustica-tabacum* derivative. All this material was, however, damaged severely by a hailstorm which hit Delhi in March, 1957. Only ten plants survived and flowered. Among them, two partially asynaptic and two completely asynaptic plants were found. Asynapsis in the *rustica-tabacum* hybrids therefore appears to be genetically controlled, though it is not possible at this stage to indicate precisely the number of factors that may be concerned.

Poisson analysis of bivalent frequencies in different cells: So far, no case of asynapsis has been recorded in *N. rustica*. In *N. tabacum*, CLAUSEN and CAMERON (1944) identified a "pale sterile" mutant which was also partially asynaptic. This mutant was used by them to develop the monosomic series of *tabacum*. SMITH (1950) who produced the *rustica-tabacum* hybrids used in the present study and who has critically investigated this material has not reported any asynaptic plant in them. From the data now available, their occurrence could only be explained on the assumption of a spontaneous mutation. In view of the hybrid nature of this material and also since *N. rustica* and *N. tabacum* are both allopolyploids, a Poisson analysis of the frequencies of cells with different numbers of bivalents was carried out to find out whether bivalent formation occurs wholly at random. The data are presented in Table 3 (see also Figure 3). Only data from metaphase

TABLE 3

Frequency of bivalents in asynaptic plants at Metaphase I

| Plant no. | Number of cells containing a bivalent frequency of | | | | | | | | | | | | | χ^2 |
	0	1	2	3	4	5	6	7	8	9	10	11	12	
5/2	29	17	5	5	....	....	....	....	....	....	....	....	....	0.661
	26.45	19.84	7.44	1.86										
6/9	32	4	1	0	1	....	....	....	....	....	....	....	....	2.752
	29.30	7.62		1.08										
3/3	0	2	2	1	8	29	6	5	....	....	....	....	....	69.13*
		5.67	8.75	11.22	10.80	8.32	5.34	5.43						
7/2		2					1	8	8	2	15		5	16.596*
		5.86					5.25	5.22	5.61	5.35	8.19		6.32	

The upper figure is the experimental number of cells with the stated number of bivalents; the lower figure is the number expected on the Poisson distribution.
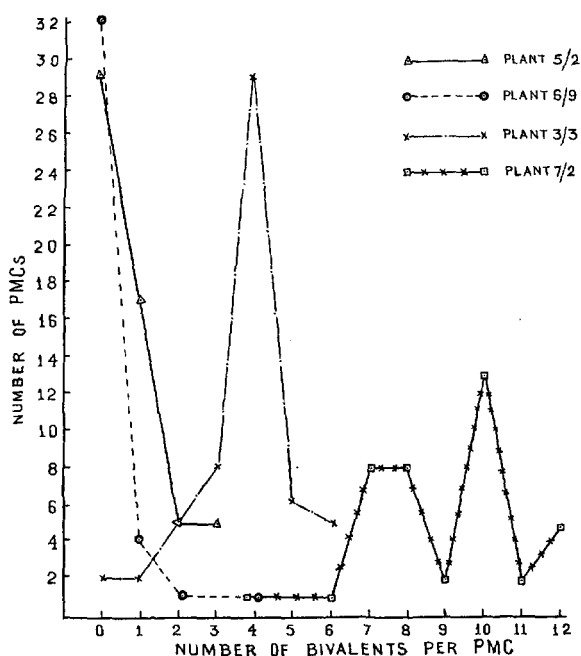* Significance at 5% level.

FIGURE 3.—Frequency of occurrence of cells with different bivalent numbers in asynaptic and partially asynaptic plants.

I plates were analyzed, since a large number of cells could be studied at this stage. It can be seen from Table 2 that plants 5/2 and 6/9 showed the lowest χ^2 with a good fit for Poisson distribution. In the other two plants, the deviations from the frequencies expected on the basis of Poisson distributions were significant. Tests for binominal distribution also revealed large deviations from those expected. The curve in the case of plant 7/2, looks bimodal in view of the very low frequency of cells with nine and 11 bivalents. This may, however, be due to the number of cells studied in each class being insufficient. From this data it may be concluded that the bivalents in plants 3/3 and 7/2 are not formed at random.

DISCUSSION

There are two important questions regarding the asynaptic condition in *rustica-tabacum* derivatives which need consideration. First, why is there a gradation in pairing frequency from regular bivalent formation to complete asynapsis? Secondly, what is the deviation from a binomial distribution in the mode of bivalent formation in partially asynaptic plants due to? Regarding the first question, a probable explanation is the existence of a gene dosage effect similar to that observed in Solanum (SWAMINATHAN 1952). Since partially asynaptic plants gave rise to completely asynaptic ones, it is not likely that altogether different genes control the types of asynapsis met with. Lack of chromosome homology cannot be used to explain either partial or complete asynapsis since some plants in the same

population showed regular bivalent formation. Nor can environmental conditions account for the observed differences in pairing since first, the plants were grown under similar conditions and fixations were made on the same day and secondly, the character was inherited. The possibility that asynapsis may result from the pleiotropic effect of the mammoth gene is rendered unlikely by the fact that the *rustica-tabacum* derivatives also segregated at Delhi for the mammoth character (MURTY and SWAMINATHAN 1957). Thus, the asynaptic plants isolated by us and the different degrees of pairing observed in them seem to owe their origin to spontaneous mutation and gene dosage effect respectively.

The nonconformity of bivalent formation in partially asynaptic plants to a Poisson or binomial distribution reveals some interesting possibilities. Certain homologous chromosomes appear to be capable of undergoing synapsis under cellular conditions which are not favorable for the pairing of other chromosomes. Thus, it appears that even within the chromosome complement of a plant, there may be differences among the different chromosomes concerning their require-ments for the initiation of pairing. In the genus Nicotiana, spontaneous asynapsis has so far been reported only in *N. tomentosa* (GOODSPEED 1954). In X-rayed progenies of *N. tabacum* and *N. sylvestris*, some asynaptic variants have been recorded. No asynaptic mutants have been reported in *N. rustica*. Whether the bivalents occurring in partially asynaptic *rustica-tabacum* derivatives arise from pairing within a particular genome of *N. rustica* or from pairing within some chromosomes of both the genomes (modified by the presence of some segments of *tabacum* chromosomes) cannot at present be ascertained.

Non randomness in the response of individual chromosomes and even segments of chromosomes with reference to the action of various mutagens and external agencies such as temperature is well-known. REES (1958) presented evidence that in some partially asynaptic plants of Scilla particular chromosomes respond to abnormal conditions within the cells in different ways. A specific chromosome response to temperature was inferred by JAIN (1957) in Lolium from the dis-proportionally high occurrence of nonsynchronized cells with one or two advanced bivalents. Such specificity may or may not be related to chromosome length. Localization effects have long been studied with references to the distribution of chiasmata. However, no attempt has been made to study the response of homol-ogous pairs of chromosomes to conditions unfavorable for the origin of pairing as in asynapsis, nor to the retention of chiasmata in paired homologues according to the time sequence normal to the species as in desynapsis. In view of the funda-mental interest aroused by this problem, the results of different authors, who have reported quantitative data on the chromosome associations found in asynaptic or desynaptic plants, were subjected to a Poisson and binomial analyses. The results are summarized in Tables 4 and 5. It should be mentioned that in all the cases where a significant deviation from Poisson or binomial distribution is reported in Tables 4 and 5, the deviation occurred in all classes and not merely in a single one, i.e., no case of compound or truncated Poisson distribution was found.

From the data in Tables 4 and 5, the following general conclusions can be

TABLE 4

Poisson analysis in desynaptic plants

Material and somatic chromosome number	Plant no.	Mean no. of bivalents per cell	Fit for Poisson and binomial distribution	Remarks
Zea mays (2n=20) *	3695–5	0.30	Very good	Nil
"	3694–5	0.50	Fair	Deviation in first two classes
"	3694–14	0.65	Very good	Nil
"	3693–1	1.34	Fair	Deviation in the first three classes
"	3696–24	2.40	Poor	High deviations in all classes
"	3699–14	4.86	Fair	Sample size small
"	3695–7	6.82	Fair	Sample size small
"	3701–1	8.15	Poor	Deviation in all classes
Pisum sativum (2n=14) †	A_3	3.80	Very poor	Deviation in all classes
"	B_4	3.90	Very poor	Deviation in all classes
"	B_7	3.90	Very poor	Deviation in all classes
"	B_1	4.50	Very poor	Deviation in all classes
Secale cereale (2n=14) ‡	100–2	2.38	Fair (P between 0.10 and 0.20)	High Deviation in middle classes
"	100–3	2.63	Very poor	Deviation in all classes
"	1–3	4.40	Very poor	Deviation in all classes
"	6–7	4.98	Very poor	Deviation in all classes
Ulmus glabra (2n=28) §	1	1.05	Very good	Nil
"	7	2.16	Very good	Nil
"	10	2.23	Good	Nil
Matricaria inodora (2n=36) ‖	1	5.50	Fair	Sample size only 20 cells
Triticum aestivum (2n=42) ¶	1703–1	1.90	Fair	Data pertaining to material
"	1704–1	2.40	Good	Fixed at 10:10 A.M. on 4/8
"	1705–1	2.60	Good	Fixed at 10:10 A.M. on 4/8
"	1695–2	11.50	Very poor	Fixed at 10:10 A.M. on 4/8

* Data from BEADLE (1933).
† Data from KOLLER (1938).
‡ Data from PRAKKEN (1943).
§ Data from EHRENBERG (1949).
‖ Data from VAARAMA (1950).
¶ Data from LI *et al.* (1945).

drawn. (1) In cases of nearly complete asynapsis or desynapsis, an occasional bivalent may be formed at random. (2) As the mean bivalent frequency approached the equivalent of half the potential number of bivalents, a gradual departure from Poisson distribution commences, ending in very wide deviations in those cases where n/2 or more bivalents are formed or where more than the number of bivalents constituting a genome in an alloploid species are formed. (3) The above trend holds good whether the plants concerned are diploids like Rye or *Pisum sativum,* allopolyploids like *Triticum aestivum,* autopolyploid like the Solanum hybrid, or probably secondary polyploids like tomato and maize, or even aneuploids. It thus appears that when conditions conducive to normal chromosome pairing are present only inadequately, there is a definite difference in the ability

TABLE 5

Poisson analysis in asynaptic plants

Material and somatic chromosome number	Plant no.	Mean no. of bivalents per cell	Fit for Poisson and binomial distribution	Remarks
Triticum aestivum[*]				
(2n=42; plants studied had 2n=40)	9 plants	5.77	Very good	Nil
Lycopersicon esculentum[†]				
(2n=24)	57–1	4.30	Fair	}Deviation in middle classes
"	57–7	5.03	Fair	
"	17–2	5.45	Poor	
"	59–4	7.03	Poor	}Deviations in all classes
"	3–3	8.04	Poor	
F_2 *Gossypium hirsutum*[‡]				
× *G. barbadense* (2n=52)	11	6.30	Good	Nil
"	7	6.40	Good	Nil
"	8	6.70	Fair	Sample size small
"	6	7.00	Fair	High deviation in one class
"	1	8.80	Good	Deviations in middle classes
"	3	11.60	Very poor	Deviations in all classes
F_2 and F_3 of amphidiploid[§]	358	3.50	Very good	Nil
(*Solanum parodii* × *S.*	190	4.54	Very good	Nil
saltense) 2n=48	362	14.00	Very poor	Deviations in all classes

[*] Data from HUSKINS and HEARNE (1933).
[†] Data from SOOST (1951).
[‡] Data from BEASLEY and BROWN (1942).
[§] Data from SWAMINATHAN (1952).

of the different chromosomes of a plant species to behave normally. As a consequence some chromosomes of a complement are unable to undergo synapsis, some others are able to do so, although with a reduced chiasma frequency per bivalent. In allopolyploids, this might be related to the differential requirements of the different genomes with reference to prerequisites for pairing; and in diploids such a situation would suggest that each pair of chromosomes might behave as an independent unit under such conditions. Various theories involving (1) physical changes in the chromosomes or in the cytoplasm, (2) disturbances in the normal time relations between pairing and division of the chromosomes, (3) genetically controlled enzyme deficiencies which exert an influence on the breakage and reunion of chromatids etc., have been proposed to explain the mechanism of asynapsis. DARLINGTON (1957) suggested that such abnormal cells can be considered as containing nuclei where cooperation was restricted over limited distances, while in normal cells the general cooperation within nuclei is permitted by the cytoplasm. However, he did not exclude the responsibility of cooperation between chromosomes in close association. Whatever may be the correct cause, it appears from the present study that genetically controlled threshold effects exist to which homologous chromosomes may respond in a specific and independent manner.

SUMMARY

During a cytogenetic study of the second segregating generation of the sixth backcross of F_1 N. rustica × N. tabacum to N. rustica, segregation was observed for different degrees of asynapsis during microsporogenesis. From a consideration of various alternatives, it is concluded that asynapsis in the population owes its origin to a spontaneous mutation. It is also presumed that gene dosage effects may be responsible for the different degrees of asynapsis found among different plants.

Analysis of the data for Poisson and binomial distribution revealed significant differences on the nature of association of chromosomes between the partially asynaptic and completely asynaptic segregants. Cases reported previously of asynaptic and desynaptic mutants in various plant genera were also subjected to Poisson and binomial analysis. From the results, it is concluded that (a) the odd bivalents found in completely asynaptic plants are formed at random; (b) as the mean bivalent frequency approaches the equivalent of one half the potential number of bivalents, a gradual departure from Poisson or binomial distribution ends in very wide deviations where $n/2$ or more bivalents are formed, and (c) this type of response wherein specific homologous pairs in a chromosome complement show ability to pair under conditions which lead to asynapsis in other pairs is prevalent among all types of plants, whether they be diploids, secondary polyploids or primary polyploids of various types.

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