NUMERICAL CHROMOSOME INSTABILITY IN NICOTIANA HYBRIDS. I. INTERPLANT VARIATION AMONG OFFSPRING OF AMPHIPLOIDS¹

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PREVIOUS studies of genetic segregation in several amphiploids synthesized from *Nicotiana tabacum* and closely related species, suggested that chromosome behavior may not have been regular. Particularly, amphiploid *N. tabacum* × *N. tomentosiformis* had given such high frequencies of recessive offspring that they could not be explained on any basis which assumed normal cytological behavior. CATCHESIDE (1956) had demonstrated earlier in tetraploid maize that nondisjunction of chromosomes at meiosis may increase the proportion of recessive segregants from that in autopolyploids. Therefore, it was decided to investigate the chromosome numbers found in male gametophytes (pollen grains) of the synthetic *Nicotiana* polyploids and among their progenies and to establish the relationships between chromosome imbalance and genetic results for this material.

During the course of the study it appeared that the chromosome numbers also varied in somatic cells of the investigated plants. A second publication will deal with that aspect (YANG, in preparation).

A similar cytological study was undertaken in the genus *Gossypium* by BERNARDO (1962; YANG and BERNARDO 1961). His work paralleled the genetical investigations of artificial amphiploids in that genus (GERSTEL and PHILLIPS 1958; PHILLIPS and GERSTEL 1959; PHILLIPS 1962). Comparisons will be drawn between the behavior of artificial amphiploids from the two genera.

MATERIALS AND METHODS

Crosses were made between cultivated tobacco, *N. tabacum* L., a natural tetraploid with $n=24$, and three wild diploid ($n=12$) species, *N. sylvestris* Speg. et Com., *N. otophora* Griseb., and *N. tomentosiformis* Goodspeed. These species are close relatives of *N. tabacum* since the ancestor of *N. tabacum* has been demonstrated to be an allopolyploid from a species closely resembling *N. sylvestris* and one related to the two other diploid species (GOODSPEED 1954). The hybrid seedlings were treated at the time of their germination with colchicine to produce hexaploids (3 hr; 0.12 percent aqueous solution). The polyploids were identified by morphological characters and male fertility, and confirmed cytologically by chromosome counts. Six hexaploid plants (with 72 chromosomes), two of each hybrid combination, served as the major material for this study.

Chromosome counts were made of microspores from these six hexaploids and from root tips

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of their pentaploid progenies obtained by backcrossing to tobacco. (Throughout this paper, the offspring of a backcross of a synthetic hexaploid to the *N. tabacum* parent will be referred to as pentaploids regardless of whether or not aneuploids were present in the population.) In addition, somatic counts were also made of progenies from two hexaploid plants of 6x (*N. tabacum* × *N. sylvestris*) which had not been confirmed to have 72 chromosomes. Cytological preparations of microspores were made according to the method of BURNS and YANG (1961). For mitotic counts, root tips were treated 3 hr with 0.003 percent aqueous 8-hydroxyquinoline at 18°C, hydrolysed in N HCl at 60°C for 5 to 10 min, and stained with aceto-orcein. Since within-plant variation of chromosome numbers was shown to be frequent at various levels of ploidy (YANG 1964), the "typical" somatic chromosome number of a plant was determined on the basis of at least five clear cells having the same number of chromosomes.

RESULTS

Variation among microspores: The hexaploids produced not only balanced triploid microspores (Table 1), but their chromosome numbers varied from 33 to 39 with modes at the expected number of 36. Results from different anthers of a bud and different buds of a plant were not kept separately owing to the small sample sizes. Only in one case where 33 counts were obtained from each of two anthers of the same bud was a statistical comparison between anthers possible; the t-test showed no significant difference. Data from different anthers were bulked in this case as well as in others where the t-test was not performed. Homogeneity chi-squares were nonsignificant for frequency distributions of spores from two different plants of the same amphiploid combination. Therefore, the data from similar hexaploids were combined in constructing Table 1.

As shown, the hexaploids involving *N. tomentosiformis* had a wider range of variation than the other two hexaploid types, but the homogeneity chi-square among the three frequency distributions indicated that the differences were not significant.

Variation among progeny plants: The somatic chromosome numbers of pentaploid progenies ranged from 56 to 64 with a mode at the expected number of 60 (Table 2). Since the microspore analyses indicated homogeneity between different plants of a particular hexaploid combination, data from offspring of the amphiploids of similar type were bulked. Because of the low male fertility of the hexaploids, only small progenies were obtained from backcrosses using the hexaploid as the male parent. The data were extended by also counting the chromosomes in progenies of backcrosses in which the hexaploids were maternal parents. The frequency distributions of the reciprocal crosses did not differ significantly; consequently, the data were combined for the analyses presented in Table 2.

The differences in range of variation were more pronounced in progenies than in microspores of the three hexaploid types. The amphidiploids with *N. tomentosiformis* produced again a wider range and a higher percentage of aneuploid chromosome numbers. However, the chi-square test of the frequency distributions demonstrated again no statistical difference among the three kinds of pentaploid populations.

Additional chromosome counts were obtained from progenies of two hexaploid

TABLE 1
Frequency distributions of microspores from hexaploids

Hexaploid parent	Gametic chromosome number								Off-type (percent)		
	33	34	35	36	37	38	39	Total	Hypo-	Hyper-	Total
6x(<i>tbc</i> × <i>sy1</i>)*	5	19	46	67	28	3	.	168	41.7	18.4	60.1
6x(<i>tbc</i> × <i>oto</i>)	4	23	60	112	47	11	.	257	33.8	22.6	56.4
6x(<i>tbc</i> × <i>tfr</i>)	3	6	29	50	26	9	2	125	30.4	29.6	60.0
Total	12	48	135	229	101	23	2	550	34.5	22.9	58.4
	$\chi^2 = 8.15$ P = 0.10-0.25 degrees of freedom = 6										

* Abbreviated names for species: *tbc* for *N. tabacum*, *sy1* for *N. sylvestris*, *oto* for *N. glauca* and *tfr* for *N. glauca*.

TABLE 2
*Frequency distributions of pentaploid progenies**

Pentaploids	Somatic chromosome number								Aneuploids (percent)			
	56	57	58	59	60	61	62	64	Total	Hypo-	Hyper-	Total
5x(<i>tbc-tbc-syl</i>)†	.	.	4(3)	16(9)	24(7)	3(1)	.	.	47(20)	42.6	6.4	48.9
5x(<i>tbc-tbc-oto</i>)	.	.	1	10(6)	22(11)	1(1)	.	.	34(18)	32.4	2.9	35.3
5x(<i>tbc-tbc-tfr</i>)	1	2(1)	9(2)	30(3)	33(10)	8(4)	2	1	86(20)	48.8	12.8	61.6
Total	1	2(1)	14(5)	56(18)	79(28)	12(6)	2	1	167(58)	43.7	9.0	52.7
5x(<i>tbc-tbc-syl</i>)‡	.	3	5	14	1	.	.	.	23	95.7	0.0	95.7
$\chi^2 = 2.72$ P = 0.25-0.50 degrees of freedom = 2												

* To facilitate further statistical treatment, one plant of 5x(*tbc-tbc-oto*) with only 34 chromosomes was excluded from the data in this table (see YANG, for more information on this plant). Numbers in parentheses are the part of the data obtained from hexaploids as maternal parents.
† For abbreviations of specific names see footnote in Table 1.
‡ The parents of the two pentaploid progenies summarized in this row had not been checked cytologically—see text.

plants 6x (*N. tabacum* × *N. sylvestris*) which had not been confirmed as 72-chromosome individuals (Table 2, last row). A mode of 59 instead of 60 chromosomes distinguished these progenies from the other families; this suggested that the parental amphiploids were unbalanced. The data were excluded from further analyses.

Selective elimination of microspores: The recurrent parent in the backcrosses which produced the pentaploids was the self-pollinated natural species *N. tabacum*. Chromosomal irregularity in this species was observed only infrequently in root tips (YANG in preparation). This may also occur rarely in meiosis and produce abnormal spores. However, a large proportion of the progenies examined were derived from crosses using *N. tabacum* as the male parent, and all crosses were made with an abundance of pollen grains; presumably rare pollen grains with aberrant chromosome numbers did not succeed in fertilization. Therefore, chromosomal abnormalities in the progenies were considered to have come from the hexaploids and the scores at the gametophytic stage of the hexaploids were compared with frequencies among the pentaploids (Table 3). A highly significant chi-square, calculated from the actual numbers indicated an unequal rate of fertilization by microspores with different chromosome numbers. There was generally an increase of the euploid and hypoploid numbers and a decrease of hyperploids in the pentaploid generation. A large proportion of the chi-square value came from the hyperploids, the column headed by $\geq (N+1)$ in the table.

DISCUSSION

It is not unexpected for artificial allopolyploids to produce some unbalanced gametes and offspring; the high frequencies observed here are nevertheless remarkable. One may ask when in the life cycle these cytological imbalances arose.

Since most of the hexaploids were found to have 72 chromosomes and were of normal morphology and fertility, it is unlikely that the process of colchicine doubling had induced aneuploidy or pseudo-aneuploidy as was found in *Avena* (GRIFFITHS, ROWLANDS and PEREGRINE 1959), *Datura* (BERGNER, AVERY and BLAKESLEE 1940) and *Nicotiana* (SMITH, STEVENSON and KEHR 1958). Irregular somatic divisions during later development could also have occurred. An example can be found in the interspecific hybrids of *Nicotiana* described by MOAV and

TABLE 3
*Comparison of the frequency distributions of spores and progenies
(combined from Tables 1 and 2)*

Source	Chromosome numbers*				Total	Sample size
	$\leq (N-2)$	$(N-1)$	N	$\geq (N+1)$		
Microspores	10.91%	24.55%	41.64%	22.90%	100.00%	550
Progenies	10.18%	33.53%	47.31%	8.98%	100.00%	167
$\chi^2 = 17.52$ $P < 0.005$ degrees of freedom = 3						

* N represents the expected chromosome number, 36 in microspores and 60 in progenies.

CAMERON (1961) which showed somatic as well as meiotic elimination of the chromosomes of one of the parents. Thus, the hexaploids may have been mosaics. However, should somatic abnormalities occur so frequently and/or so early during ontogeny as to produce a considerable proportion of aneuploid gametes and offspring, the modes of the frequency distributions would probably have been shifted. Since the modes were as expected, i.e., 36 chromosomes in spores and 60 in progenies, abnormal somatic divisions before meiosis can be excluded as a major cause leading to the frequent appearance of deviants.

The nature of the two plants which had the normal appearance of hexaploids but gave rise to progenies composed predominantly of hypoploids is uncertain. Unfortunately, these plants were not analysed cytologically and they may have been either colchicine induced hypoploids or mosaics of cells with differing chromosome numbers (YANG, in preparation).

Behavior at meiosis may be considered next. Chromosomes of artificial polyploids do not always pair and disjoin regularly at meiosis. Since meiotic chromosomes in *Nicotiana* species are much contracted and difficult to study, the associations at metaphase could not be analyzed with certainty. However, irregularities were seen immediately prior to and after meiosis which could result in unbalanced products of meiosis. Cytomixis was often found in PMC's at the early stages of sporogenesis; furthermore, micronuclei were frequent in sporads. It seems clear that numerical unbalance found in spores and progenies must have arisen in the main as a result of disturbances during sporogenesis.

As can be seen in Tables 1 and 2, hyperploid spores and progeny plants were relatively few. This may have been due to chromosome elimination during sporogenesis in the amphiploids or to lower viability at various later stages. From the present data one cannot decide whether the biased distribution among microspores (Table 1) was due to chromosome elimination or to failure of hyperploid products of meiosis to reach the stage of first microspore division, or to both factors. The further reduction of hyperploidy among the offspring (Table 2) must have been due, however, to selective elimination at later stages.

Some time ago, CLAUSEN (1928) analysed chromosome elimination in spore quartets of aneuploid derivatives of synthetic polyploid *Nicotiana digluta*; he concluded that the biased chromosome distribution found among the progenies of aneuploids was mainly due to chromosome elimination during the two meiotic divisions and not to inviability. The aneuploids studied by CLAUSEN and the amphiploids investigated here may not be strictly comparable.

In view of the frequent occurrence of unbalanced offspring, the genetic consequences need to be evaluated (Tables 4 to 6). Genetic segregation in duplex amphiploids, $AAaa$, can be affected in two opposite ways by chromosome unbalance. One is by an increase of segregants carrying a dominant allele A by receiving an extra chromosome on which the dominant allele is located, i.e., gametes of Aaa type would result from type aa by gaining a chromosome carrying A . The other is by an increase of recessives through loss of the chromosome carrying A , i.e. a gamete of type a resulting from Aa . Table 2 shows that the abnormal progenies were primarily hypoploids with a relatively small proportion

of hyperploids. Thus, to simplify the matter, only chromosome elimination will be considered in the following and the aspect of chromosomal gain will be neglected.

The variables to be considered here are (i) the frequency of elimination of specific chromosomes and (ii) the fraction of gametes affected by chromosomal loss—affected in the sense that the resulting offspring would be of a different phenotype owing to the loss of specific chromosomes. The former can be estimated from the empirical mean loss of chromosomes per plant. The latter depends on the type of meiotic pairing and the amount of double reduction.

To compute mean chromosomal loss, a correction was made for compensated unbalance in the case of the progenies from $6x(N. tabacum \times N. tomentosiformis)$ where plants with 59 and 61 chromosomes were relatively frequent. The fraction of simultaneous loss of one and gain of another chromosome, $30/86 \times 8/86$ or 3 percent (Table 2), was added to the observed frequency of the 59-chromosome class and subtracted from that of the 60-chromosome class. The chance for any particular chromosome to be lost is estimated from the empirical mean under two assumptions. First, the chromosomes of one, two or three genomes may be lost, i.e. the numbers may be 12, 24 or 36—the reasoning will be explained below. Second, all chromosomes within these categories may have an equal chance to be lost. No evidence against this assumption was obtained from fitting the frequency distributions obtained from each of the three pentaploid types to Poisson distributions; the resulting chi-squares were nonsignificant with probabilities between 0.50 and 0.75 in two cases and approaching 0.50 in the third.

The question whether chromosomes of one, two or three genomes can be lost may now be considered. In amphiploids and other hybrids derived from crosses between *N. tabacum* and *N. plumbaginifolia*, MOAV and CAMERON (1961) found that certain chromosomes were frequently lost, both in meiosis and in mitosis. These turned out to be chromosomes only from *N. plumbaginifolia*, in the opinion of those authors. Therefore, it is conceivable that elimination in the hexaploids studied here may also be limited to the chromosomes of the genomes derived from the diploid species *N. sylvestris*, *N. otophora* and *N. tomentosiformis*. But the possibility that chromosomes of *N. tabacum* may also be lost was shown in derivatives of another amphiploid. PATEL and GERSTEL (1961) studied 37 progeny plants from a hexaploid hybrid between *N. tabacum* and *N. glutinosa*. Twelve of these lacked a *N. glutinosa* chromosome, and two or perhaps three, lacked a *N. tabacum* chromosome. Thus, even in an amphiploid which combines distantly related species and forms at meiosis predominantly bivalents, there is elimination of chromosomes from either parent; conceivably, this may occur also in the present material.

There are the two following possibilities if chromosomes of *N. tabacum* origin as well as those from the diploid species can be lost. Since *N. tabacum* itself is of amphiploid origin and the diploid species *N. sylvestris*, *N. otophora* and *N. tomentosiformis* carry chromosomes which are more or less homologous with one or the other genome of *N. tabacum* (GOODSPEED 1954) the synthetic hexaploids

are to a degree autopolyploids for four genomes and meiotic associations typical for autopolyploids are formed, as quadrivalents, trivalents and univalents. Chromosomes of all four genomes associated in this way may separate irregularly—the fraction affected in a gamete may be 24 out of 36. Finally, loss may also occur to those chromosomes from *N. tabacum* which are present in only two doses in the hexaploid and are unrelated to those introduced from the diploid parents. All three situations are considered in Tables 4 to 6, i.e. loss of members of one, two or three genomes.

TABLE 4

Effect of chromosome elimination on segregation in 5x(N. tabacum-tabacum-sylvestris)

Observed chromosome loss/plant <i>a</i>	Chromosomes affected <i>b</i>	Fraction of gametes affected <i>c</i>	Fraction of recessives			Segregation ratio*	
			From loss $d = a \times c/b$	From segregation <i>e</i>	Total $f = d + e$	No loss <i>e'</i>	With loss <i>f'</i>
0.5454	12	(i)† 1	0.0454	0.0000	0.0454	no seg.	21.0:1
		(ii) 2/3	0.0303	0.1667	0.1970	5.0:1	4.1:1
		(iii) 4/7	0.0260	0.2143	0.2286	3.7:1	3.4:1
0.5454	24	(i) 1	0.0227	0.0000	0.0227	no seg.	43.0:1
		(ii) 2/3	0.0152	0.1667	0.1818	5.0:1	4.5:1
		(iii) 4/7	0.0130	0.2143	0.2273	3.7:1	3.4:1
0.5454	36	(i) 1	0.0152	0.0000	0.0152	no seg.	64.8:1
		(ii) 2/3	0.0101	0.1667	0.1768	5.0:1	4.7:1
		(iii) 4/7	0.0086	0.2143	0.2229	3.7:1	3.5:1

* Observed ratios for different loci varied from 3.2:1 to 9.2:1 (ignoring the larger ratios obtained for certain loci with subvital recessives) according to GERSTEL, 1963.

† (i) In case of complete preferential pairing.

(ii) Random pairing, 0 percent chromatid segregation.

(iii) Random pairing, 100 percent chromatid segregation.

TABLE 5

Effect of chromosome elimination on segregation in 5x(N. tabacum-tabacum-otophora)

Observed chromosome loss/plant <i>a</i>	Chromosomes affected <i>b</i>	Fraction of gametes affected <i>c</i>	Fraction of recessives			Segregation ratio*	
			From loss $d = a \times c/b$	From segregation <i>e</i>	Total $f = d + e$	No loss <i>e'</i>	With loss <i>f'</i>
0.3636	12	(i)† 1	0.0303	0.0000	0.0303	no seg.	32.0:1
		(ii) 2/3	0.0202	0.1667	0.1869	5.0:1	4.4:1
		(iii) 4/7	0.0174	0.2143	0.2317	3.7:1	3.3:1
0.3636	24	(i) 1	0.0151	0.0000	0.0151	no seg.	65.2:1
		(ii) 2/3	0.0101	0.1667	0.1768	5.0:1	4.7:1
		(iii) 4/7	0.0086	0.2143	0.2229	3.7:1	3.5:1
0.3636	36	(i) 1	0.0101	0.0000	0.0101	no seg.	98.0:1
		(ii) 2/3	0.0067	0.1667	0.1734	5.0:1	4.8:1
		(iii) 4/7	0.0058	0.2143	0.2220	3.7:1	3.5:1

* Observed ratios for different loci varied from 3.5:1 to 10.7:1 (ignoring the larger ratios obtained for certain loci with subvital recessives; GERSTEL 1960).

† (i) In case of complete preferential pairing.

(ii) Random pairing, 0 percent chromatid segregation.

(iii) Random pairing, 100 percent chromatid segregation.

TABLE 6

Effect of chromosome elimination on segregation in 5x(N. tabacum-tabacum-tomentosiformis)

Observed chromosome loss/plant <i>a</i>	Chromosomes affected <i>b</i>	Fraction of gametes affected <i>c</i>	Fraction of recessives			Segregation ratio*	
			From loss $d = a \times c/b$	From segregation <i>e</i>	Total $f = d + e$	No loss <i>e'</i>	With loss <i>f'</i>
0.8571	12	(i) $\frac{1}{2}$	0.0476	0.1667	0.2143	5.0:1	3.7:1
		(ii) $\frac{4}{7}$	0.0408	0.2143	0.2551	3.7:1	2.9:1
0.8571	24	(i) $\frac{2}{3}$	0.0238	0.1667	0.1905	5.0:1	4.2:1
		(ii) $\frac{4}{7}$	0.0204	0.2143	0.2347	3.7:1	3.3:1
0.8571	36	(i) $\frac{2}{3}$	0.0159	0.1667	0.1826	5.0:1	4.5:1
		(ii) $\frac{4}{7}$	0.0136	0.2143	0.2279	3.7:1	3.4:1

* Observed ratios for different loci varied from 2.3:1 to 3.5:1 (ignoring the larger ratios obtained for certain loci with subvital recessives; GERSTEL 1960).

† (i) Random pairing, 0 percent chromatid segregation.

(ii) Random pairing, 100 percent chromatid segregation.

The amphiploids examined gave little indication of pronounced differential affinity when analysed by genetical techniques (GERSTEL 1960, 1963), though differences in segregation ratios for different loci occurred. However, the theoretical effect of chromosome elimination on segregation in cases where chromosomes pair preferentially is listed in sections (a) of Tables 4 and 5 for convenient comparison; the figures may serve as estimates of the proportion of recessive segregants expected on the basis of chromosome elimination in "good" allopolyploids. Preferential pairing was not considered in Table 6 since it certainly did not occur at all in the amphiploids involving *N. tomentosiformis*, as indicated by the small segregation ratios found there by GERSTEL (1960).

As to the proportion of gametes of type *Aa* affected (point (ii) above), the highest fraction possible would be unity following completely preferential pairing in the case of a "good" allopolyploid. With completely random pairing the proportion of *Aa* gametes would be $\frac{4}{6} = \frac{2}{3}$ and $\frac{16}{28} = \frac{4}{7}$ for zero and 100 percent of chromatid segregation, respectively. However, the latter extreme is only a very unlikely limiting condition since double reduction could have occurred only rarely.

Taking into account all the above mentioned factors, the resulting fractions of recessive segregants have been calculated and presented in Tables 4 to 6. Effects of chromosome loss, of normal segregation and of the two together are listed. Finally, the computed segregation ratios are given under "no loss" and "with loss." For convenient comparison, the ranges of genetic ratios observed by GERSTEL (1960, 1963) are indicated in footnotes below each table.

For example, one may see from Tables 4 and 5 how chromosome elimination could yield 4.5 and 3.0 percent, respectively, of recessive segregants following completely preferential pairing, in which case no segregation at all should be expected. The percentages of recessives increased from 16.7 percent to 19.7 percent and to 18.7 percent following random pairing without chromatid segregation.

It is worth noting that among the three types of amphiploids, $6x(N. tabacum \times$

N. tomentosiformis) gave the smallest segregation ratios (GERSTEL 1960; see footnote in Table 6) and the highest frequency of unbalanced offspring. The small genetic ratios, however, such as 2.3:1 which had been obtained by GERSTEL were smaller than is explainable by any of the conditions set out in Table 6, where the minimum is 2.9:1. It is likely that other unknown factors were operating. GERSTEL and PHILLIPS (1958) discussed the possible roles played by genetic instability and by differential survival of gametes and zygotes. Excess of recessives could come from frequent mutation of dominant alleles to recessives in somatic as well as in meiotic divisions and from selective advantages of gametes or zygotes carrying recessive alleles. It is also possible that the assumption of random loss of chromosomes in a set of 12 was not warranted, even though statistical tests had revealed no evidence against it. Finally, somatic crossing over in heterozygous individuals during pre- or postmeiotic mitoses could produce recessive segregants. However, the fact that all loci located on several chromosomes gave similar genetic ratios makes none of these explanations very likely.

Comparable data for the genus *Gossypium* were obtained by BERNARDO (1962) who studied the chromosomal variations in the three artificial hexaploids 6x-(*Gossypium barbadense* × *G. arboreum*), 6x(*G. hirsutum* × *G. raimondii*) and 6x(*G. barbadense* × *G. gossypioides*). The frequency distribution of spores with various chromosome numbers differed significantly among the three amphiploids ($P < 0.005$). Excluding the amphiploid listed last, because of the presence of genetically determined asynapsis, reduces the heterogeneity chi-square drastically ($P = 0.50-0.75$). For a comparison the combined results from all three *Nicotiana* amphiploids (Tables 1 and 2) were compared with the pollen data from the first two *Gossypium* amphiploids, with a total of 396 spores and 175 pentaploid back-cross progeny. Table 7 gives the actual scores and Figure 1 the frequency distributions in percentages. As can be seen, *Gossypium* has the larger proportion of normal microspores indicating either less tolerance for chromosome unbalance or a lower frequency of the events that lead to aneuploidy. The lower frequency of micronuclei or microcytes (Table 8) in *Gossypium* spore quartets points to the latter. It is possible that differences in multivalent formation in the amphiploids

TABLE 7

*Comparison of frequency distributions from Nicotiana and Gossypium**

Material	Chromosome numbers [†]									Total
	N—4	N—3	N—2	N—1	N	N+1	N+2	N+3	N+4	
Microspores										
Nicotiana	.	12	48	135	229	101	23	2	.	550
Gossypium	1	5	16	53	240	56	18	6	1	396
Progenies										
Nicotiana	1	2	14	56	79	12	2	0	1	167
Gossypium	.	.	6	8	143	13	4	.	.	174

* Data for *Gossypium* are taken from BERNARDO (1962).

[†] N represents the normal number of chromosomes, being 36 and 39 in spores, and 60 and 65 in progenies of *Nicotiana* and *Gossypium* respectively.

FIGURE 1.—Frequency distributions of microspores and pentaploid progenies of *Nicotiana* and *Gossypium*.

chosen from the two genera may be the explanation. However, BERNARDO's (1962) data show no association between multivalents at meiosis and the frequency of microspores with aberrant chromosome numbers. Multivalent frequencies in the *Nicotiana* amphiploids used are not known. The curves also show for *Gossypium* a more drastic narrowing of the spread and a lowering of the peak from spore to progeny distributions, suggesting a more severe discrimination against abnormal spores, zygotes or young seedlings. Furthermore, selection against off-types in *Nicotiana* favors chromosome deficiency over hyperploidy, whereas the distribution in *Gossypium* is more symmetric.

TABLE 8

*Frequencies of micronuclei or microcytes in amphiploids of Nicotiana and Gossypium**

Amphiploids	Mean per quartet	Quartets counted
<i>Nicotiana</i>		
6x(<i>N. tabacum</i> × <i>N. glutinosa</i>)	0.87	198
6x(<i>N. tabacum</i> × <i>N. sylvestris</i>)	0.31	400
6x(<i>N. tabacum</i> × <i>N. otophora</i>)	1.15	600
6x(<i>N. tabacum</i> × <i>N. tomentosiformis</i>)	0.61	600
<i>Gossypium</i>		
6x(<i>G. hirsutum</i> × <i>G. armourianum</i>)	0.26	8397
6x(<i>G. hirsutum</i> × <i>G. harknessii</i>)	0.21	999
6x(<i>G. barbadense</i> × <i>G. arboreum</i>)	0.22	1776
6x(<i>G. barbadense</i> × <i>gossypioides</i>)	0.46	2492

* Data from GERSTEL and PHILLIPS (unpublished), except those for 6x(*N. tabacum* × *N. glutinosa*) from a publication by CLAUSEN (1928), who counted microcytes.

In considering the effect of chromosome imbalance upon genetic segregation, BERNADO (1960) concluded that the proportion of recessive segregants attributable to chromosomal unbalance can only be very small in the two amphiploids used in the above comparison. This differs from the situation in the *Nicotiana* hexaploids where the relatively frequent occurrence of chromosomally deficient spores and progeny plants must have an appreciable effect upon segregation frequencies.

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SUMMARY

Variation of chromosome numbers was studied among microspores and progeny plants of the artificial amphiploids $6x(N. tabacum \times N. sylvestris)$, $6x(N. tabacum \times N. otophora)$ and $6x(N. tabacum \times N. tomentosiformis)$. Considerable chromosome unbalance occurred which appeared to have arisen mainly from irregular meiosis in the hexaploids. Selection among abnormals favored chromosomal deficiency over hyperploidy in progenies. It was estimated that the amount of chromosome elimination increased the fraction of recessive offspring to an appreciable extent over what could be obtained from regular meiotic segregation alone. However, even under extreme assumptions the high frequencies of recessives obtained in the progenies of $6x(N. tabacum \times N. tomentosiformis)$ could not be explained. Comparable data from *Gossypium* cited from the work of BERNARDO (1962) demonstrate a lower frequency of chromosomal aberrancy in that genus.

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