SEGREGATION IN NEW ALLOPOLYPLOIDS OF NICOTIANA. II. DISCORDANT RATIOS FROM INDIVIDUAL LOCI IN 6X (N. TABACUM × N. SYLVESTRIS)

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IN the preceding paper of this series (GERSTEL 1960) the relationships between *Nicotiana tabacum* L. (the tobacco of commerce) and two of its close relatives, *N. tomentosiformis* Goodsp. and *N. otophora* Griseb., were explored by means of the amphiploid segregation technique. The latter two species are members of the Tomentosae section of Nicotiana which has contributed the T-genome of amphiploid *N. tabacum*. The present publication is concerned with amphiploids between *N. tabacum* and *N. sylvestris* Speg. et Com. *N. sylvestris* is the only extant relative of the form from which the S-genome is derived (GOODSPEED and CLAUSEN 1928). (The designation of the genomes of tobacco by the symbols S and T is not in general use in the tobacco literature, but is advocated for convenience and in analogy with similar symbolizations employed by wheat and cotton geneticists.)

MATERIALS AND METHODS

As in the previous paper, segregation was studied in duplex (i.e., *ZZzz*) amphiploids which had been synthesized from tobacco and a wild species, in the present case *N. sylvestris*. The synthesis was performed by crossing *N. sylvestris* with a number of genetically marked lines of tobacco, treating F_1 seedlings with colchicine and selecting amphiploids from the treated populations. Since *N. sylvestris* is a diploid and *N. tabacum* a natural tetraploid, these amphiploids were actually hexaploids of the type S′S′SSTT, where S′ denotes a *N. sylvestris* genome while the letters without prime signs stand for the genomes of *N. tabacum*.

Chromosome numbers of the amphiploid parents were usually not confirmed cytologically, but since only highly fertile plants of typical morphology were used the effect of aneuploidy upon genetic segregation was minimized. Furthermore, testcrosses were made on several different amphiploid plants marked for a particular character to exclude the possibility of a ratio being based on the progeny of a single off-type individual. The exceptional ratios which occurred were treated separately.

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Segregation was tested by crossing the duplex amphiploids (*ZZzz*) with recessive marker lines of *N. tabacum*. Several modifications were employed in some instances in order to verify whether certain variables affected the segregation ratios:

a) The effect of cytoplasm was tested by synthesizing genetically marked amphiploids in two ways: (*N. tabacum* × *N. sylvestris*) × 2 with tobacco cytoplasm, and (*N. sylvestris* × *N. tabacum*) × 2 with *N. sylvestris* cytoplasm.

b) The effect of chromosome number of the tester could be analyzed by using testers of different levels of ploidy: *N. sylvestris* (2x), *N. tabacum* (4x), amphiploids *N. tabacum* × *N. sylvestris* (6x) and, in one case, autotetraploid *N. tabacum* (8x). However, crosses between marked amphiploids and *N. sylvestris* proved rather sterile and yielded populations of very small size. Also, for testing with *N. sylvestris* as well as with 6x (*N. tabacum* × *N. sylvestris*) experimentation was limited to dominant mutants of *N. tabacum*.

c) Whether selective elimination or competition of gametes influenced the ratios was studied by testing the amphiploids as both male and female parents. This test was done by using appropriate 6x (*N. tabacum* × *N. sylvestris*) tester plants reciprocally, because 2x female × 6x male crosses were entirely unsuccessful and 4x females × 6x male yielded only 1.5 to 7.4 percent fertile seeds in different trials, whereas 6x female × 6x male gave 79.4 to 82.8 percent germinable seeds. Here again, the 6x female × 6x male test was limited to the study of those amphiploids in which were incorporated dominant marker genes of *N. tabacum* (*Pk* and *Rf*; see below).

Several of the marker stocks of *N. tabacum* came from the collection of the late PROF. R. E. CLAUSEN who had incorporated the genetic markers into the variety "Red Russian" used as a standard in his laboratory. It is not known to what extent the stocks had become isogenic. They differed from each other in many ways, which may have been due to pleiotropy of the markers as well as to substitution of modifiers during transfer. All Red Russian-derived lines carried the *Pk* factor for carmine flower color; separate stocks were homozygous recessive for the following pairs of duplicate genes: sn_1sn_2 (spontaneous necrosis), fs_1fs_2 (fasciated), ws_1ws_2 (white seedling) and yb_1yb_2 (yellow burley). Red Russian lines with the dominant factors *Pt* (petioled) and *Pd* (petioloid) were also employed, but since segregation for the leaf shape characters was far from clear only *Pk* segregation was scored. One stock carried *co* (coral) but the coral factor was not useful, since *N. tabacum* (*co*) × *N. sylvestris* hybrids themselves were coral. The following abbreviations will designate these stocks in tables: RRsn, RRfs, RRws, RRyb, RRPt, RRPd and RRco.

Through the courtesy of PROF. W. D. VALLEAU several mutants were secured: Ruffled (*Rf*), a factor causing great abnormality of leaf and flower shape, was received in the variety Gold Dollar (abbreviation GDRf). In addition, DR. VALLEAU furnished the recessive chlorophyll deficient mutants yellow Crittenden (*yc*), veinbanding (*vb*) and the yellow burley (yb_1yb_2) line Ky 16. Yellow burley (yb_1yb_2) had also been incorporated by DR. T. J. MANN of our Institution into a marker stock of the flue-cured variety 402, and yellowish green (*yg*)

had been introduced by him into another marker line of variety 402.

The still undescribed mutant "Japanese aurea" came to us from DR. H. OKA's laboratory of the Hatano Tobacco Experiment Station in Japan through the courtesy of MR. L. G. BURK. Japanese aurea is phenotypically very much like yellow burley, but proved on analysis to give complementary normal green with the latter. Mendelian analysis of the character has not been completed; probably it differs by two factors from Red Russian (*au* or au_1au_2). In this connection one might mention that the duplicate nature of some of the recessives used was irrelevant to the analysis, since *N. sylvestris* carried in each case a single fully dominant normal factor, the segregation of which was the object of study.

Maryland mammoth (abbreviated MM) carried the mammoth factors (mm_1mm_2) for long-day periodicity.

Three races of *N. sylvestris* were available. The first one, designated here as "Standard," was received some years ago from the U. S. Department of Agriculture. The ultimate origin of this race is not now known. Race "Tucuman" came from the collection of the University of California Botanical Garden, where it had received the accession number UCBG 49-Pagliaga. The third race, "Lumbreras" was received from the same source, as UCBG 37-11. Hybrids and amphiploid hybrids between Red Russian tobacco and Lumbreras were found to exhibit peculiar abnormalities such as leaf edges curled upward, thick and brittle leaves, cork formation on the stem upon approaching maturity, and a moderate dwarfism. The genetic analysis of this syndrome, to be published separately, proved it to be due to a complementary factor symbolized as *Lu.* The Standard race was placed at the disposal of the North Carolina Agricultural Experiment Station through the courtesy of DR. A. KEHR; we owe the other two races of *N. sylvestris* to the kindness of PROF. R. E. CLAUSEN. The races are designated in the tables by Std, Tuc and Lum, respectively.

The relations of the twelve characters under consideration to the chromosomes of *N. sylvestris* are unknown and there exists no evidence as to whether any of the factors are linked in that species. The association with specific tobacco chromosomes is known for ten of the characters, excluding aurea and Lumbreras, and the available information is summarized in Table 1. Occurrence of segregation in a system like the one employed is no evidence of allelism. A dominant factor *Z* from tobacco will segregate in a S′S′SSTT amphiploid if it is located in the S-genome; the ratio will depend on the extent of homology between the S-chromosomes and their S′ homologues (or partial homologues). Segregation will not be affected by the location or even the existence of a recessive *z* anywhere in the S′ genome. If dominant *Z* lies in the T-genome of tobacco, no segregation will occur. The technique may be used, therefore, to find the genome of any particular tobacco dominant. In combinations of amphiploids between tobacco containing a recessive mutant (or a duplicate pair of recessives) with wild-type *N. sylvestris*, the wild-type gene will segregate in accordance with the extent of homology with the *N. tabacum* homologues, regardless of the location of the recessive mutants.

For statistical analyses chi-square contingency tests were applied. No correc-

TABLE 1

Chromosome association of the characters used

Character*	Chromosome designation†	
	T-genome	S-genome
Carmine (*Pk*)	. .	P[1]
Ruffled (*Rf*)	. .	M[4]
yellow burley (yb_1yb_2)	B[1]	O[1]
spontaneous necrosis (sn_1sn_2)	F[4]	N[4]
mammoth (mm_1mm_2)	F[1]	N?[4]
white seedling (ws_1ws_2)	G[2]	T[1]
veinbanding (*vb*)	G[5]	. .
yellow Crittenden (*yc*)	J?[5]	. .
fasciated (fs_1fs_2)	D?[4]	P[3]
yellowish green (*yg*)	. .	S[1]

* Localization of the aurea and Lumbreras factors is unknown.
† Superscripts denote the following references: [1] CLAUSEN and CAMERON 1944; [2] CLAUSEN and CAMERON 1950; [3] CAMERON 1962; [4] CAMERON communication; locations designated with ? need further verification; [5] VALLEAU 1958.

tions for continuity were made in the case of the smaller families, for which reason some of the P-values may have been underestimated.

RESULTS

Carmine: Segregation for the carmine corolla factor (*Pk*) of Red Russian tobacco was studied extensively in amphiploids with the "Standard" race and to some extent with the "Tucuman" race of *N. sylvestris* (Table 2). Four derivative lines of Red Russian were employed in the synthesis of amphiploids: RRPd, RRsn, RRPt, and RRco. Amphiploids between RRPd and RRsn as one parent and *N. sylvestris* as the other were synthesized reciprocally in order to give hybrids with both cytoplasms. Other variables introduced were ploidy of the testers at 2x, 4x and 6x levels; 6x testers were used as male and female parents in test-crosses with amphiploids. Races or combinations which did not have the dominant factor *Pk* served as testers in each case. Chi-square analysis of the data indicated that the resulting ratios were statistically homogeneous. In order to condense Table 2 progeny families of similar origin were combined and the number of individual amphiploid parents tested is given in parentheses following the combined segregation frequencies. Omitted from the analysis were the A × 2x families (amphiploids × *N. sylvestris*) because of their very small size caused by low fertility; their total segregation amounted to 16 carmine:4 pink. The progenies of one amphiploid plant, listed at the bottom, were also omitted from the totals because almost all of the offspring of this plant had pink flowers. Since the parent plant had typical carmine flowers like its sibs one must assume that it was a periclinal chimera in which the core had lost the carmine factors.

Ruffled: The *Rf* factor (Table 3) also gave homogeneous results in various combinations, indicating that none of the variations affected the segregation frequencies. Three different lines of *N. sylvestris* were employed in the synthesis

TABLE 2

Carmine (Pk)*-pink segregation in* N. tabacum × N. sylvestris *and* N. sylvestris × N. tabacum *amphiploids*

Parental strains*		*tabacum* cytoplasm				*sylvestris* cytoplasm				Totals		
tabacum	*sylvestris*	A×2x†	A×4x	A×6x	6x×A	A×2x	A×4x	A×6x	6x×A	Dominant	Recessive	*Ratios*
RRPd	Std	.....	.....	.....	369:92(1)‡	.....	229:64(3)	.....	.....	598	156	*3.8:1*
RRsn	Std	10:3(2)	125:39(2)	107:27(2)	235:56(2)	6:1(2)	84:13(3)	35:14(2)	196:49(2)	798	202	*3.9:1*
RRPt	Tuc	.....	119:19(2)	.....	.....	.....	.....	.....	.....	119	19	*6.3:1*
RRco	Tuc	.....	.....	.....	119:33(1)	.....	.....	.....	.....	119	33	*3.6:1*
Totals		10:3	244:58	107:27	723:181	6:1	313:77	35:14	196:49	1634	410	...
Ratios		...	*4.2:1*	*4.0:1*	*4.0:1*	.....	*4.1:1*	*2.5:1*	*4.0:1*	...	...	*4.0:1*
Homogeneity§: $X^2 = 23.15$; df = 19; P = .2–.3												
Exceptional parent												
RRsn	Std	0:2(1)	0:74(1)	3:95(1)	0:2(1)	.....	.....	.....	.....	3	173	...

* For abbreviations of parental strains see text.
† A stands for Amphiploid; the position indicates whether used as female or male parent.
‡ Figures in parentheses indicate number of amphiploid parents.
§ Homogeneity test for 20 families; the small A×2x families and the progeny from the exceptional parent (bottom row) were not included.

TABLE 3

Segregation for ruffled (Rf) *in reciprocal* N. tabacum × N. sylvestris *amphiploids*

Parental strains*							Totals		
tabacum	*sylvestris*	A×2x†	A×4x	A×6x	6x×A	8x×A	Dominant	Recessive	*Ratios*
tabacum cytoplasm									
GDRf	Tuc	.....	174:27(1)‡	.....	.....	.....	174	27	*6.4:1*
GDRf	Std	57:5(2)	352:35(3)	279:30(3)	94:9(2)	135:14(1)	917	93	*9.9:1*
sylvestris cytoplasm									
GDRf	Lum	52:5(2)	129:13(3)	269:22(3)	189:28(2)	.....	639	68	*9.4:1*
Totals		109:10	655:75	548:52	283:37	135:14	1730	188	...
Ratios		*10.9:0*	*8.7:1*	*10.5:1*	*7.6:1*	*9.6:1*	...	...	*9.2:1*
Homogeneity: $X^2 = 17.91$; df = 21; P = .5–.7									
Exceptional parent (*tabacum* cytoplasm)§									
GDRf	Std	4:4	.....	.....	.....	.....	4	4	...

Footnotes: See Table 2.

of amphiploids, two as females and the third as male parent, providing tests of both cytoplasms. The amphiploids were crossed with 2x, 4x, 6x and 8x recessive testers, 6x being used reciprocally. The mean ratio of 9.2 ruffled:1 normal obtained in these experiments differed very significantly from the 4.0 carmine:1 pink ratio of Table 2 (Chi-square = 81.2; df = 1; P <.001). One small family from a testcross with *N. sylvestris* gave an exceptional segregation of 4:4 (bottom row); the amphiploid parent may have been chromosomally unbalanced but was unfortunately tested only on this small scale.

yellow burley: Segregation ratios (Table 4) were obtained for amphiploids made from three different accessions of *N. sylvestris*; the cytoplasms of both *N. tabacum* and *N. sylvestris* were used and two amphiploids were backcrossed reciprocally. The resulting mean ratio was 12.2:1 and the data proved to be homogenous. The data from the Ky 16 × S amphiploid have been reported previously (GERSTEL and PHILLIPS 1958).

spontaneous necrosis: Testcross data (Table 5) from 3 RRsn × Tuc and 2 RRsn × Std amphiploids were homogeneous, containing 458 normal and 67 necrotic plants, a ratio of 6.8:1. An additional RRsn × Std parent gave 38 normal:41 necrotic offspring, or approximately 1:1; most likely this plant was aneuploid. Three reciprocal Std × RRsn amphiploids produced a highly deviant 95 normal:4 necrotic ratio; the sample was small but the three parents gave very similar results (34:2, 33:1 and 28:1).

For the next group of factors only a single type of test was executed, *viz.* amphiploid × recessive *N. tabacum* tester. The results for eight factors are given in Tables 6 and 7. *mammoth:* Two amphiploids of similar origin gave a combined 3.2 normal:1 mammoth ratio. *white seedling:* Segregation in testcrosses of amphiploids with the three collections of *N. sylvestris* gave a homogeneous total of 4709 green:434 white seedlings which were scored in germination dishes. The *N. tabacum* parent of the amphiploids was in each case Red Russian of the genotype $ws_1ws_1Ws_2ws_2$. Since the character is lethal in the seedling stage, amphiploids were synthesized by crossing $ws_1ws_1Ws_2ws_2$ tobacco with *N. sylvestris* and discarding from the resulting amphiploids those that failed to segregate in a preliminary selfing test. The data entered in Table 6 were obtained in turn by crossing the selected amphiploids with $ws_1ws_1Ws_2ws_2$ testers; the result was corrected for heterozygosity of the testers as follows: (4709 − 434): (434 + 434), which gave a 4.9:1 ratio. Since the data were homogeneous there was little if any influence of the *N. sylvestris* strains used as parents of the amphiploids. *veinbanding:* Three amphiploid sib plants segregating for the veinbanding characteristic gave a total of 87 normal:17 veinbanding offspring, i.e., a 5.1:1 ratio. *Lumbreras syndrome:* Five amphiploids involving two different strains of tobacco with the Lumbreras accession of *N. sylvestris* gave together 183 offspring exhibiting the symptoms of the "*Lumbreras syndrome*" and 32 normal plants, or a 5.7:1 ratio. *yellow Crittenden:* Two amphiploid sibs gave 108 normal:18 recessives, or a 6.0:1 ratio. *fasciated:* Five amphiploids synthesized from the same tobacco stock and two races of *N. sylvestris* gave a 9.2 fasciated:1 normal ratio in a total progeny of 296 plants.

TABLE 4

Segregation for the yellow burley (yb) *character in reciprocal* N. tabacum × N. sylvestris *amphiploids*

Parental strains*				Totals		
tabacum	*sylvestris*	A×4x†	4x×A	Dominant	Recessive	*Ratios*
		tabacum cytoplasm				
KY 16yb	Std	899:76(6)‡	210:20(1)	1109	96	*11.6:1*
402yb	Lum	89:6(3)	.....	89	6	*14.8:1*
		sylvestris cytoplasm				
RRyb	Tuc	139:7(1)	161:14(1)	300	21	*14.3:1*
Totals		1127:89	371:34	1498	123	....
Ratios		*12.7:1*	*10.9:1*	...	...	*12.2:1*
		Homogeneity: $X^2 = 7.59$; df = 9; P = .5–.7				

Footnotes: See Table 2.

yellowish green: Two amphiploid sibs in which were incorporated the yellowish green character from tobacco segregated 558 normal:19 yellowish green offspring or 29.4:1 (data already published, GERSTEL and PHILLIPS 1958). It should be noted here, as was remarked earlier (GERSTEL 1960), that in populations segregating for this character, the yellowish green class is frequently deficient; this is true for parents from both intra- and interspecific crosses.

Japanese aurea: Three amphiploid sibs contrasted for this character gave homogeneous progenies totaling 80 normal and 17 aurea plants, or 4.7:1 (Table 7). One additional sib produced a 21:23 ratio while a fifth had 33 normal and one aurea offspring. The parents of the last two families may have been aneuploids.

TABLE 5

Segregation for spontaneous necrosis (sn) *in reciprocal* N. tabacum × N. sylvestris *amphiploids*

Parental strains*			
tabacum	*sylvestris*	A×4x†	4x×A
		tabacum cytoplasm	
RRsn	Tuc	309:41(3)‡	.....
RRsn	Std	149:26(2)	.....
Total		458:67	.....
Ratio		*6.8:1*	.....
		Homogeneity: $X^2 = 3.13$; df = 4; P = .05–.07	
Exceptional parent			
RRsn	Std	38:41(1)	.....
		sylvestris cytoplasm	
RRsn	Std	.....	95:4(3)
		Homogeneity: $X^2 = 6.84$; df = 1; $P < 0.01$	

Footnotes: See Table 2.

TABLE 6

Segregation for seven loci in 6x (N. tabacum × N. sylvestris) × N. tabacum *testcrosses*

Locus	Parental strains*		Segregation			No. of ampliiploid parents	Homogeneity		
	tabacum	*sylvestris*	Dominant	Recessive	*Ratio*		X^2	df	P
mm	MM	Std	108	34	*3.2:1*	2	1.56	1	.3–.5
ws_2	RRws	Lum	1331	101	...	1	...	.	....
		Std	1689	174	...	3	...	.	....
		Tuc	1689	159	...	2	...	.	....
			4709	434	*4.9:1*	(corrected)†	6.17	5	.2–.3
vb	vb	Tuc	87	7	*5.1:1*	3	0.82	2	.5–.7
Lu	402yb	Lum	86	10	...	3	...	.	....
	yc	Lum	97	22	...	2	...	.	....
			183	32	*5.7:1*	.	4.23	4	.2–.3
yc	yc	Lum	108	18	*6.0:1*	2	0.01	1	.90–.95
fs	RRfs	Std	136	13	...	2	...	.	....
	RRfs	Tuc	131	16	...	3	...	.	....
			267	29	*9.2:1*	.	2.37	4	.5–.7
yg	402yg		558	19	*29.4:1*	2	1.37	1	.2–.3

* See footnote 1, Table 2. † See text.

DISCUSSION

The purpose of the investigation was to ascertain the extent of homology between the chromosomes of *N. tabacum* and *N. sylvestris* as well as to decide to what extent the method employed can provide a reliable criterion of the taxonomic relationship between the two species. On the whole, data obtained for any particular locus proved consistent. Thus, the cytoplasm of the amphiploids, whether from *N. tabacum* or from *N. sylvestris*, did not have a noticeable influence upon segregation (Tables 2, 3 and 4). The case of spontaneous necrosis (Table 5) may be an exception, but the experiment needs to be repeated since it was conducted on such a small scale. Furthermore, the data do not permit a distinction between possible alternatives, even if repeatable. These alternatives are: (1) that segregation for the character is affected by *N. sylvestris* cytoplasm, which is improbable in view of the other results and (2) that the expression of spon-

TABLE 7

Segregation for aurea (au) *in* N. tabacum × N. sylvestris *amphiploids*

Parental strains*		Segregation			No. of amphiploid parents
tabacum	*sylvestris*	Dominant	Recessive	*Ratio*	
Japanese aurea	Std	80	17	*4.7:1*	3
Homogeneity: $X^2 = 1.30$; df = 2; P = .2–.3					
Exceptional parents					
Japanese aurea	Std	21	23	...	1
Japanese aurea	Std	33	1	...	1

* See footnote in Table 2.

taneous necrosis varies with the cytoplasm. If the latter were the case the observations would be irrelevant to the present problem.

Similarity of results from reciprocal crosses (A × 6x *vs.* 6x × A in Tables 2 and 3; A × 4x *vs.* 4x × A in Table 4) excludes large effects of gametic selection since it is against all experience that male and female gametes are equally affected. Also, the observation that the level of ploidy of the tester (e.g., A × 4x *vs.* A × 6x in Table 2; 6x × A *vs.* 8x × A in Table 3) had little influence upon segregation ratios suggested that in each testcross unselected gametes (with respect to the alleles under consideration) were functioning. The wide range of these tests was possible because the Nicotianas are relatively insensitive in their crossing behavior to differences in chromosome numbers (as compared, e.g., with Gossypium).

The uniformity of segregation ratios for any one locus may be contrasted with the variation found among loci. The average ratios for the loci studied are summarized in Table 8, where they are listed according to increasing magnitude. The data are highly nonhomogeneous ($\chi^2 = 187.5$; df = 10; $P < .001$) even though the most deviant ratio for yellowish green (Table 6) has been omitted since it was clearly biased by the much reduced vitality of recessive segregants.

The next highest ratio was produced by segregation for yellow burley which was put in Table 8, perhaps somewhat arbitrarily, since there is evidence also of some bias against yellow burley segregants in intraspecific tobacco populations segregating for this character (HENIKA 1932; STINES and MANN 1960). In the preceding work of the series (GERSTEL 1960) ratios for yellow burley were found to be second largest, after *yg*, in segregating amphiploid hybrids between tobacco and species of the Tomentosae.

In view of these facts it may appear that the varying ratios in Table 8 are

TABLE 8

Summary of segregation frequencies from testcrosses of amphiploids synthesized from N. tabacum *and* N. sylvestris

Character	Segregation		
	Dominant	Recessive	*Ratio*
mammoth	108	34	*3.2:1*
Carmine	1634	410	*4.0:1*
aurea	80	17	*4.7:1*
white seedling	4709	434	*4.9:1*†
veinbanding	87	17	*5.1:1*
Lumbreras	183	32	*5.7:1*
yellow Crittenden	108	18	*6.0:1*
spontaneous necrosis	458	67	*6.8:1*
Ruffled	1730	188	*9.2:1*
fasciated	267	29	*9.2:1*
yellow burley	1498	123	*12.2:1*
Homogeneity: $X^2 = 185.7$; df = 10; $P < .001$			

* Exclusive of exceptional parents and of segregation for yellow green.
† Ratio corrected for heterozygosity of tester; see text.

merely a reflection of different degrees of viability of the recessive phenotypes. But this is true only in part. The evidence indicates that fasciated plants, which also figure with a low frequency in Table 8, were probably not the subject of discrimination by selection. In the *N. tabacum* × Tomentosae amphiploids studied in the paper just mentioned *Fs:fs* segregations were among the smallest ratios, and in an intraspecific F_2 family segregating for one of the two pairs of *fs* alleles PINILLA (1960) found close agreement with an expected 3:1 ratio provided the plants were grown under uncrowded conditions, as was also the case in the present study. One must add, however, that in experiments by PINILLA in which seedlings were crowded in the seedling cultures, fasciated offspring were deficient in number.

The most convincing contrast in the array of Table 8 is provided by a comparison of the ratios for the carmine and ruffled characters which are controlled by dominant tobacco factors. Segregation for ruffled gave more than nine times as many ruffled as normal plants; a selective advantage of zygotes carrying *Rf* is highly improbable since homozygous *Rf Rf* tobaccos are very weak as a rule. Therefore, if the observed ratio were biased the true ratio would be even larger. One must conclude that the M-chromosomes of tobacco marked by *Rf* (Table 1) conjugate preferentially in the amphiploid. Carmine segregants, on the other hand, were only four times as frequent as pinks. This can by no means be attributed to a reduced viability of plants carrying the carmine factor *Pk*, since in the literature on flower color inheritance in tobacco no evidence was found to point in this direction (ALLARD 1919; MACRAE 1941; VAN DER VEEN 1957, and others; PINILLA (1960) obtained a small excess of carmine flowered plants in F_2). Since the observed ratio of 4.0:1 was within the limits of autotetrasomic inheritance one may assume that the P-chromosome of *N. tabacum* on which *Pk* is located is still nearly homologous with a chromosome of *N. sylvestris*. Without further discussing in detail each of the characters listed in Table 8 one may conclude that the extent of differentiation is not the same for all chromosomes of the two species. Apparently, some of the *N. tabacum* and *N. sylvestris* chromosomes are still completely homologous whereas others have become differentiated to some extent. This differentiation has not proceded very far, since preferential pairing becomes rapidly complete in an amphiploid as the relationship between the two parent species diminishes, if one may generalize from observations made in Gossypium (GERSTEL and PHILLIPS 1958). This is emphasized further by the recent observation by SHAVER (1962) that the introduction of a single inversion into the chromosomes of one parent of an amphiploid may greatly increase the differential affinity of chromosomes bearing that inversion. Therefore, amphiploid segregation studies of distantly related species yield little critical evidence. A method for estimating differences in homologies among the chromosome pairs formed in hybrids from more distantly related species has recently been developed by SFICAS (1962).

The precision of the amphiploid segregation method is diminished by the varying viabilities of the mutants used as markers of chromosomes. This effect could be measured if the offspring from the three classes of gametes *ZZ*, *Zz* and

zz were distinguishable (GERSTEL 1956), but because of complete dominance no such discrimination was possible in the cases reported here.

In the evaluation of the method one must also recognize the genetic effect of nondisjunction of chromosomes in the amphiploid parents which results in aneuploid progeny. YANG (1962) found that 20 out of 47 offspring of a cross 6x (*N. tabacum* × *N. sylvestris*) × *N. tabacum* had one or two chromosomes less than the euploid number, but since the distribution of the losses over the three subgenomes of the S'ST gametes was unknown, only rough estimates of the effect on genetic segregation could be made. Under extreme assumptions a 5:1 segregation would be transformed into a 4.1:1 phenotypic ratio (YANG 1962). In any case, the effect would be some reduction of the estimate of chromosome differentiation.

The inaccuracy introduced by aneuploidy was more pronounced in Nicotiana than that found in a similar study in Gossypium. BERNARDO (1962) observed in the latter genus only 17 numerically deficient plants out of a total of 174 in three 6x × 4x crosses comparable to those studied by DR. YANG. The difference between the genera may be one of relative frequency of nondisjunction rather than of degree of tolerance of chromosomal imbalance since an amphiploid with a factor causing asynapsis had 73.7 percent numerically deficient offspring (BERNARDO 1962). This is also borne out by the observation that aneuploid microspores are rarer in Gossypium than in Nicotiana amphiploids (YANG 1962). In this connection one might mention that among many Gossypium amphiploids studied cytologically by PHILLIPS (communication) over a number of years, numerically unbalanced plants were very rarely encountered, whereas in the present study alone four or five of the amphiploids gave abnormal ratios which were ascribed to aneuploidy for a specific chromosome (Tables 2, 3, 5 and 7). Occurrence of aneuploidy among Nicotiana amphiploids was cytologically demonstrated by YANG (1962) and SMITH (1943) has shown previously that colchicine treatment can cause aneuploidy in the Nicotianas.

(The various abnormal segregations which were observed may be accounted for in the following ways. *Table 2:* Segregation of 3 carmine to 173 pink offspring from a carmine parent could be due to an exclusion of both tobacco P-chromosomes bearing *Pk* from most of the tissue which produced the sampled gametes. The possibility that the three carmine plants were contaminants cannot be gainsaid. *Tables 3, 5 and 7, first case:* A 1:1 ratio could result from loss of a chromosome with a dominant factor in the parent amphiploid; i.e., a change from *ZZzz* to *Zzz*. Simplex trisomic ratios will approach 1:1. *Table 7, second case:* Loss of the chromosomes carrying the recessives will result in only dominant progeny; nondisjunction of the remaining homologues may give an occasional recessive. These explanations were verified neither cytologically nor by progeny tests.)

SUMMARY

Homology of the chromosomes of diploid *Nicotiana sylvestris* with those of allotetraploid *N. tabacum* was evaluated by the amphiploid segregation tech-

nique previously employed in our laboratory. For this purpose genetically marked amphiploids *N. tabacum* × *N. sylvestris* and *N. sylvestris* × *N. tabacum* were crossed with diploid, tetraploid, hexaploid and octoploid testers used as male and female parents. Only a few of the possible combinations were applied to the testing of any particular locus, but for those which were used the results were remarkably consistent (with the possible exception of a cytoplasmic effect in the case of *sn*). A few abnormal segregation ratios encountered were attributed to aneuploidy of the parents.

Differences between loci were pronounced. Only in part were these differences due to differential viability of the segregating phenotypes; differences in preferential affinity of the chromosomes of the parent species probably played an effective role in causing differences in segregation ratio. It was concluded that some chromosomes of the two species have remained completely homologous while others have become differentiated to some degree during evolution.

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