GENIC STERILITY IN *TABACUM*-LIKE AMPHIDIPLOIDS OF *NICOTIANA*[1]

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(With Plates 3–6 and Twenty-one Text-figures)

Introduction

The hypothesis that *Nicotiana Tabacum* ($2n=48$) is of amphidiploid origin, involving *N. sylvestris* ($2n=24$) and *N. tomentosa* ($2n=24$) was advanced by Goodspeed & Clausen (1928). This hypothesis is based mainly on the pairing relations ('Drosera scheme') of the chromosomes at meiosis in the F_1 hybrids *N. Tabacum* × *N. sylvestris* and *N. Tabacum* × *N. tomentosa*, on the absence or slight amount of pairing in *N. Tabacum* haploid (Chipman & Goodspeed, 1927; Clausen & Mann, 1924; Lammerts, 1934), and on the slight amount of pairing in the F_1 *N. sylvestris* × *N. tomentosa* (Goodspeed, 1934), as well as on the morphological features of this last hybrid (Pl. 3 and Pl. 6, fig. 2) which resemble those of *N. Tabacum*. For the more complete evidence in support of this hypothesis reference is made to an earlier paper (Greenleaf, 1941).

It was soon recognized that *N. tomentosiformis*, a species closely related to *N. tomentosa*, could be substituted for the latter species in the *N. sylvestris*—*N. tomentosa*—*N. Tabacum* triangle (Clausen, 1932; Kostoff, 1938). A fertile amphidiploid, *N. sylvestris-tomentosiformis*, was synthesized by Kostoff (1938). The method of synthesis and the probable origin of this amphidiploid will be discussed below. The same amphidiploid made by somatic doubling of the F_1 hybrid, using the same strains of the parental species as were used by Kostoff (Greenleaf, 1938), is completely female sterile but highly male fertile (95% of good pollen).

Since the summer of 1937 the writer has made several amphidiploids which are concerned with the problem of the origin of *N. Tabacum*. All involve *N. sylvestris* and some species or race of the *N. tomentosa* group which are known to give the 'Drosera scheme' of pairing or probably show this behaviour with *N. Tabacum*. *N. sylvestris-tomentosa* ($4n$), *N. sylvestris-tomentosiformis* ($4n$) *Gr*,[2] *N. tomentosiformis-sylvestris* ($4n$),

[1] Part of a dissertation submitted in partial satisfaction for the degree of Doctor of Philosophy in Genetics at the University of California, Berkeley, California, December 1940.

[2] The female sterile, male fertile amphidiploid of Greenleaf as contrasted with the female fertile, male fertile one of Kostoff.

and *N. sylvestris-Setchellii* (4*n*) (Pl. 3 and Pl. 6, figs. 1, 3, 4) have been obtained from callus of the corresponding F_1 hybrids. Several amphidiploids have also been made by crossing callus-induced autotetraploids of the species. They are: *N. sylvestris* (4*n*) × *N. tomentosa* race Acomayo (4*n*), *N. sylvestris* (4*n*) × *N. tomentosa* race Machu Picchu (4*n*), *N. sylvestris* (4*n*) × *N. tomentosa* race Pincos (4*n*), *N. sylvestris* (4*n*) × *N. tomentosa* race Ticempaya (4*n*), and *N. sylvestris* (4*n*) × *N. tomentosiformis* (4*n*) (Pl. 4 and Pl. 5, fig. 1).

It has been found that the callus-induced amphidiploids *N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis Gr*, and *N. tomentosiformis-sylvestris* which employ the long inbred *N. tomentosa* (U.C.B.G. 08–193[1]) and *N. tomentosiformis* (U.C.B.G. 25–12) are completely female sterile but highly male fertile. Most plants of the amphidiploid populations involving the races Acomayo and Machu Picchu were partially fertile, and set one or more capsules when left to open pollinate in the field. There was, however, considerable variation in fertility between plants, for a few sterile or almost sterile plants, including some obvious chromosomal aberrants (as judged by the morphological features), appeared. The amphidiploid line involving race Pincos failed to set any seed when left to open pollinate in the field, but two plants pollinated with pollen from the amphidiploid Acomayo line set some seed, showing that at least some of these plants were potentially slightly fertile. Only two amphidiploid plants of the Ticempaya line flowered. One was tested and found to be sterile, the other also appeared to be sterile. An entire population of fifty-four plants of *N. sylvestris* (4*n*) × *N. tomentosiformis* (4*n*) was completely female sterile and male fertile, just like the corresponding callus-induced amphidiploid. All of the above amphidiploids except *N. sylvestris-Setchellii* have been found to have very good pollen (Table 1).

A callus-induced amphidiploid *N. glutinosa-tomentosa* (Pl. 6, fig. 5) has also been made. It, too, is completely female sterile and male fertile. It has only 64% of good pollen.

A detailed cytological study of ovaries of the female sterile amphidiploids *N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis Gr*, *N. tomentosiformis-sylvestris*, *N. glutinosa-tomentosa*, and *N. sylvestris-Setchellii* has shown that ovule abortion is not due to irregular chromosome behaviour at meiosis, but to arrested development of embryo sacs while still in the two- or four-nucleate stage (Greenleaf, 1941). This is followed by disintegration. Only *N. sylvestris-Setchellii* (4*n*) has shown a few eight-

[1] University of California Botanical Garden accession number. The first number refers to the year when received.

Table 1. *Pollen fertility counts*

Name of plant	No. of grains counted	Total no. of grains counted	% good pollen	Mean % good pollen
N. tomentosa race Acomayo (2*n*)	434		98·85	—
N. tomentosa race Pincos (2*n*)	717		90·10	
	509	1226	87·62	89·07
N. tomentosa race Ticempaya (2*n*)	387		96·38	
	488		97·95	
	508	1383	91·75	95·23
N. tomentosiformis (2*n*)	895		95·98	—
N. Setchellii (2*n*)	430		75·58	
	533		69·61	
	515	1478	79·03	74·63
N. sylvestris (2*n*)	402		98·26	
	408	810	97·79	98·02
N. tomentosa race Pincos (4*n*)	764		78·66	
	604		88·74	
	416		87·50	
	442	2226	76·02	82·52
N. tomentosa race Ticempaya (4*n*)	494		93·72	
	498		90·36	
	391	1383	93·10	92·34
N. tomentosa race Machu Picchu (4*n*)	905		90·94	—
N. tomentosiformis (4*n*)	231		77·06	
	414		82·12	
	207		92·75	
	305		86·89	
	573		92·50	
	743		97·04	
	768		85·81	
	748	3989	86·77	88·59
N. sylvestris (4*n*)	712		91·57	
	480		93·33	
	133		93·23	
	374		91·71	
	187	1886	85·03	91·52
F_1 *N. sylvestris* × *N. tomentosa*	432		0·23	
	350	782	0·57	0·38
F_1 *N. sylvestris* × *N. tomentosiformis*	209		1·44	
	309	518	1·62	1·54
F_1 *N. sylvestris* × *N. Setchellii*	423		0·24	
	394	817	0·51	0·37
F_1 *N. tomentosiformis* × *N. Setchellii*	631		42·31	
	349		34·10	
	329		46·81	
	380		39·47	
	544	2233	31·62	38·60
F_1 *N. tomentosiformis* (4*n*) × *N. Setchellii*	183		68·85	
	250		61·20	
	157		70·70	
	104		57·69	
	1238	1932	63·09	63·72

Table 1 (*cont.*)

Name of plant		No. of grains counted	Total no. of grains counted	% good pollen	Mean % good pollen
F_1 *N. glutinosa* × *N. tomentosa*		Whole slide		0	
		310		0·65	—
F_1 *N. tomentosiformis* × *N. tomentosa*		626		98·08	
		273	899	97·80	98·00
N. Tabacum		323		92·57	
		473		93·45	
		402	1198	98·26	94·82
N. sylvestris-tomentosa (4*n*)	P1	506		90·51	
		373		91·69	
		915		93·33	
		771		95·33	
		284		95·42	
	P2	590		94·92	
		321	3760	83·49	92·77
N. sylvestris (4*n*) × *N. tomentosa* race Acomayo (4*n*)	P6	112		90·18	
		471		95·12	
		173	756	93·06	93·92
	P18	285		96·14	
		415	700	92·29	93·86
N. sylvestris (4*n*) × *N. tomentosa* race Machu Picchu (4*n*)	P10	457		92·56	
		329	786	90·58	91·73
	P11	210		97·14	
		125	335	98·40	97·61
	P20	253		96·44	
		360	613	93·06	94·45
	P23	350		94	
		258	608	96·90	95·27
	P39	197		97·46	
		229	426	93·01	95·07
N. sylvestris (4*n*) × *N. tomentosa* race Pincos (4*n*)	P9	288		86·11	
		247	535	70·04	78·69
	P19	209		93·78	—
	P22	166		68·07	
		140	306	88·57	77·45
	P29	134		95·52	
		229	363	96·07	95·87
N. sylvestris-tomentosiformis (4*n*) *Gr*		149		95·97	
		153		96·08	
		32		100	
		88		97·73	
		128		88·28	
		102		97·06	
		93		93·55	
		172		96·51	
		187	1104	94·12	95·02
N. tomentosiformis-sylvestris (4*n*)		306		91·83	
		512		90·23	
		345		91·30	
		516	1679	94·96	92·20

Table 1 (*cont.*)

Name of plant		No. of grains counted	Total no. of grains counted	% good pollen	Mean % good pollen
N. sylvestris-tomentosiformis (4*n*) Kostoff	P2	239		75·73	
		1076		77·23	
		378		90·74	
		264		81·82	
	P3	229		92·14	
	P5	261		93·87	
		210		83·81	
		201	2858	87·06	83·21
N. sylvestris-Setchellii (4*n*)	P3	613		81·24	
		351		81·48	
		537		72·44	
		632		81·17	
	P10	657		68·34	
		534		75·66	
		1414		89·11	
		829		85·77	
		724		56·77	
		1053		57·64	
		794		80·23	
		378		74·87	
		214		80·84	
		403	9133	76·18	75·86
N. glutinosa-tomentosa (4*n*)		1737		47·67	
		852		61·50	
		209		72·25	
		189		88·36	
		277		83·39	
		139		95·68	
		359		88·30	
		158	3920	94·94	63·80
F_1 *N. Tabacum* × *N. sylvestris-tomentosa* (4*n*)		99		89·90	
		341	440	91·79	91·36
F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr*		528		81·44	
		456	984	80·70	81·10
F_1 *N. Tabacum* × *N. sylvestris-Setchellii* (4*n*)	P1	416		40·87	
		351		60·11	
		498		63·65	
		220		53·64	
	P2	359		45·68	
		177		52·54	
		975		63·69	
		1292		62·93	
		285		47·72	
		591		51·44	
		245	5409	51·84	56·83
F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) Kostoff	P1	336		87·80	
		348		87·93	
	P2	835		86·23	
		1008		86·41	
		526		93·73	
	P3	295		87·80	
		173	3521	93·06	88·18
F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × *N. Tabacum*		180		82·78	
		364	544	79·40	80·51

Table 1 (*cont.*)

Name of plant		No. of grains counted	Total no. of grains counted	% good pollen	Mean % good pollen
F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × *N. sylvestris-tomentosiformis* (4*n*) *Gr*		574		91·99	
		181	755	97·24	93·24
F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × *N. sylvestris-tomentosa* (4*n*)		110		96·36	
		350		96	
		253	713	94·86	95·65
F_1 *N. Tabacum* × *N. tomentosa*		318		2·52	—
		Whole slide		0	
		Whole slide		0	
F_1 *N. Tabacum* × *N. tomentosiformis*		264		3·41	
		176	440	2·27	2·95
F_1 *N. Tabacum* haplo Σ × *N. sylvestris*—normal plants	P1	387		15·76	
		475		10·63	
	P4	816		16·67	
		1068		10·95	
		537		13·59	
		151	3434	18·54	14·39
F_1 *N. sylvestris* × *N. sylvestris-tomentosa* (4*n*)		726		14·88	
		1280		12·11	
		653		20·37	
		1078		13·45	
		558		17·74	
		660	4955	21·06	15·72
F_1 *N. sylvestris* × *N. sylvestris-tomentosiformis* (4*n*) *Gr*	P1	498		35·14	
		606		46·04	
		298		41·95	
		270		45·19	
	P2	368		51·09	
		242		61·57	
	P7	291		70·79	
		107		54·21	
		303		66·67	
		486		47·53	
		267	3736	58·80	50·64
F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × *N. sylvestris*	P1	186		36·56	
		245		40·41	
		209		40·19	
	P7	145		40	
		156		32·69	
		434		26·04	
	P9	268		31·72	
		248	1891	27·82	33·16
F_1 *N. sylvestris* (4*n*) × *N. sylvestris-tomentosiformis* (4*n*) *Gr*	P2	553		34·90	
		597		27·97	
		346		20·52	
		498	1994	33·53	29·99
F_2 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr* (Female sterile plants)	P11	385		85·19	
		291	676	76·63	81·50
	P95	558		71·15	—

nucleate embryo sacs. Complete or almost complete sterility in this last amphidiploid is due to failure of pollen tubes to reach the ovary when flowers are selfed or crossed with *N. Tabacum*.

A cytological study of embryo-sac development has also been made of the partially fertile F_1 hybrids of *N. Tabacum* with *N. sylvestris-tomentosiformis* (4*n*) *Gr* and *N. sylvestris-Setchellii* (4*n*) and of *N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*. In all of these the majority of the embryo sacs reach only the two- or four-nucleate stage when they disintegrate, i.e. abortion is like that in the amphidiploids.

Early experiments designed to study segregation for sterility in F_2 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr* and in the backcross [F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr*] × ditto *Gr* showed almost no sterile plants. The results from the F_2 and the backcross were about the same, viz. three female sterile plants in seventy-three and two in fifty-three, respectively. The pollen of the sterile plants was good (Table 1). It was observed, however, that the plants from these populations varied greatly in fertility, ranging from completely sterile through plants of very low fertility (about 20–30 seeds per pod) to quite fertile ones. The failure of sterile plants to segregate was understood after a cytological examination of ovaries. These studies showed that abortion is gametic, and that many of the embryo sacs disintegrated while still in the two- or four-nucleate stage just as in the female sterile amphidiploids. It became clear that all megaspores which receive a certain combination of genes abort. Similar cytological results were observed in F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*. An F_2 and backcrosses of this F_1 to the Kostoff and to the sterile *Gr* amphidiploids gave only partially fertile but no sterile plants (Table 3).

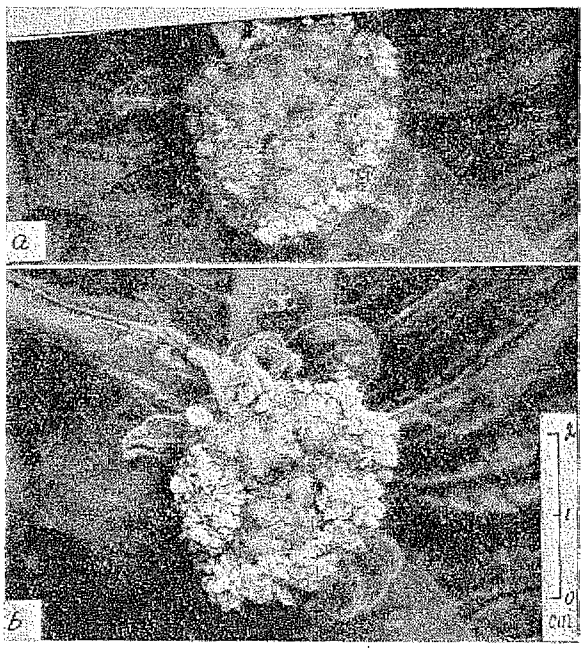
Abortion of the embryo sacs is thought to be due to the action of complementary genes. Each of the parental species has genes controlling embryo-sac development which probably do so by different reactions or reaction rates. When brought together in the amphidiploid, they would oppose each other, thus arresting embryo-sac development. The following working hypothesis of the cause of the sterility in the amphidiploids has been evolved: Only those megaspores which do not carry the complementary sterility genes can give rise to functional embryo sacs. These genes are invariably present in the megaspores of the sterile amphidiploids. They do not, however, affect the viability of the pollen (Greenleaf, 1941). Completely sterile plants like those rarely found in F_2 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr* could be explained by the infrequent survival of an embryo sac's carrying sterility genes being

fertilized by pollen carrying such genes. A similar explanation can be given for the variation in fertility between plants. Data from seed counts supporting this hypothesis will be presented later.

In this paper evidence will be presented from morphology, microsporogenesis, pollen fertility counts, pollen measurements, and seed fertility counts to show that some of the above amphidploids could well represent the ancestral type of *N. Tabacum*, that these amphidiploids exhibit genic sterility affecting the female gametophyte only, and that the sterility genes are borne on not more than three chromosomes in either of the parental genomes.

Methods

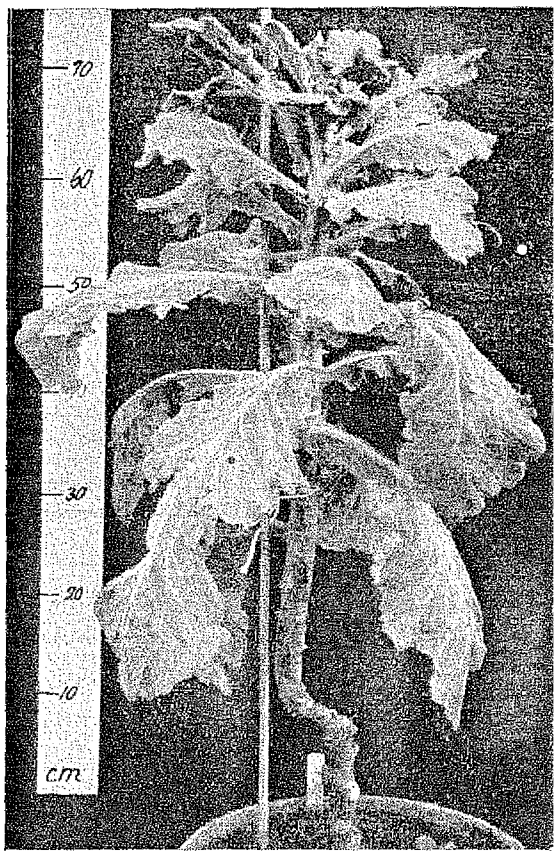
As already mentioned, the amphidiploids herein reported were made in either of two ways: by callus doubling of the F_1 species hybrids (Greenleaf, 1938) or by crossing of similarly induced autotetraploids of the species. This modified callus method is highly successful with some species and species hybrids of *Nicotiana* (Text-figs. 1, 2) but fails with others.



Text-fig. 1*a*. Callus shoots on F_1 *N. sylvestris* × *N. Setchellii* 20 days after decapitation and treatment.
Text-fig. 1*b*. Same as Text-fig. 1*a* 2 days later.

Pollen mother cells were stained with iron aceto-carmine (Belling, 1921).

Pollen fertility counts were made in iron aceto-carmine, care being taken to use just enough liquid to spread to the edges of the cover-slip so that no pollen grains would reach the edge. This was done to overcome



Text-fig. 2. Octoploid callus shoot from F_1 *N. sylvestris* × *N. tomentosa* Ruiz & Pavon.

a tendency for the smaller bad grains to escape to the periphery, while the larger good ones were held by surface tension between the cover and slide. To insure getting a representative sample, three counts were made of each slide, one through the middle, and one on either side. All grains which were well-stained, round, thin-walled, and above a certain minimum size (usual for non-staining aborted grains) were counted as good.

Pollen measurements were made of grains mounted in diaphane. In this medium they retain their original size and form.

Seed fertility counts on ovaries were made of uniformly large areas by means of a ruled square inserted into the eyepiece of a dissecting microscope. The counts were best made about 18 days after pollination when the still immature, white seeds (mature seeds are dark brown) were carefully removed to a moistened black paper where they were counted. The very small, aborted ovules remained on the placenta where they could also be counted.

Materials

Several distinct species are now known which are related to *N. tomentosa*. Of these the writer has employed *N. tomentosiformis* and *N. Setchellii*.[1] The former is much more closely related to *N. tomentosa* than is the latter, but a brief examination of the pollen mother cells has shown that *N. Setchellii* also exhibits the 'Drosera scheme' of pairing with *N. Tabacum*. A number of races of *N. tomentosa* itself are known. Of these five very distinct ones have been used. They differ in numerous vegetative features, e.g. amount of tomentum on the leaves, amount of anthocyanin in leaves and stems, and the shape and size of leaves, as well as in features of the flowers. All are biennial to perennial. The following plants of the *tomentosa* group have been used:

N. tomentosa Ruiz & Pavon. U.C.B.G. 08–193. Brazil and Peru. Long inbred line. This is the type originally described and pictured by Setchell (1912). Pl. 3.

N. tomentosa race Acomayo. U.C.B.G. 36–40. Acomayo, Dpto. Huanuco, Peru. Pl. 4, fig. 2.

N. tomentosa race Machu Picchu. U.C.B.G. 36–165. Machu Picchu, Dpto. Cuzco, Peru. Pl. 4, fig. 3.

N. tomentosa race Pincos. U.C.B.G. 36–32. Pincos, Dpto. Apurimac, Peru. Pl. 4, fig. 4.

N. tomentosa race Ticempaya. U.C.B.G. 36–59. Ticempaya, Dpto. La Paz, Bolivia. Pl. 4, fig. 1.

N. tomentosiformis Goodsp. U.C.B.G. 25–12. Amazon basin. Described by Goodspeed (1933). This species has been used under the name *N. Rusbyi* by some workers. Annual. Pl. 4, fig. 5.

N. Setchellii Goodsp. U.C.B.G. 37–30. Annual to perennial. Pl. 3.

[1] A new species of *Nicotiana* collected by B. Beauchamp near Chachapoyas, Dpto. Amazonas, Peru, in 1937, named and to be described by T. H. Goodspeed in *University of California Publications in Botany*.

Of *N. sylvestris* two races have been used by the writer:

N. sylvestris Speg. & Comes. U.C.B.G. 07–69. 'Spegazzini sent the seeds from Argentina to Comes in 1897' (Setchell, 1912). Also described by Setchell (1912). Annual. Pl. 5, fig. 2.

N. sylvestris. U.C.B.G. 37–11. Sierra La Lumbrera, Dpto. Anta, northern Argentina. Annual.

Except where otherwise stated the former has been employed throughout these experiments.

Callus-induced autotetraploids (Pl. 4, figs. 1–5) have been made of *N. tomentosiformis, N. sylvestris* Speg. & Comes, and the recently introduced races of *N. tomentosa*.

Allooctoploids (Text-fig. 2 and Pl. 5, fig. 2) have been obtained from the callus of the F_1 hybrids *N. sylvestris* × *N. tomentosa* Ruiz & Pavon and *N. sylvestris* × *N. tomentosiformis* (Pl. 3 and Pl. 5, fig. 2).

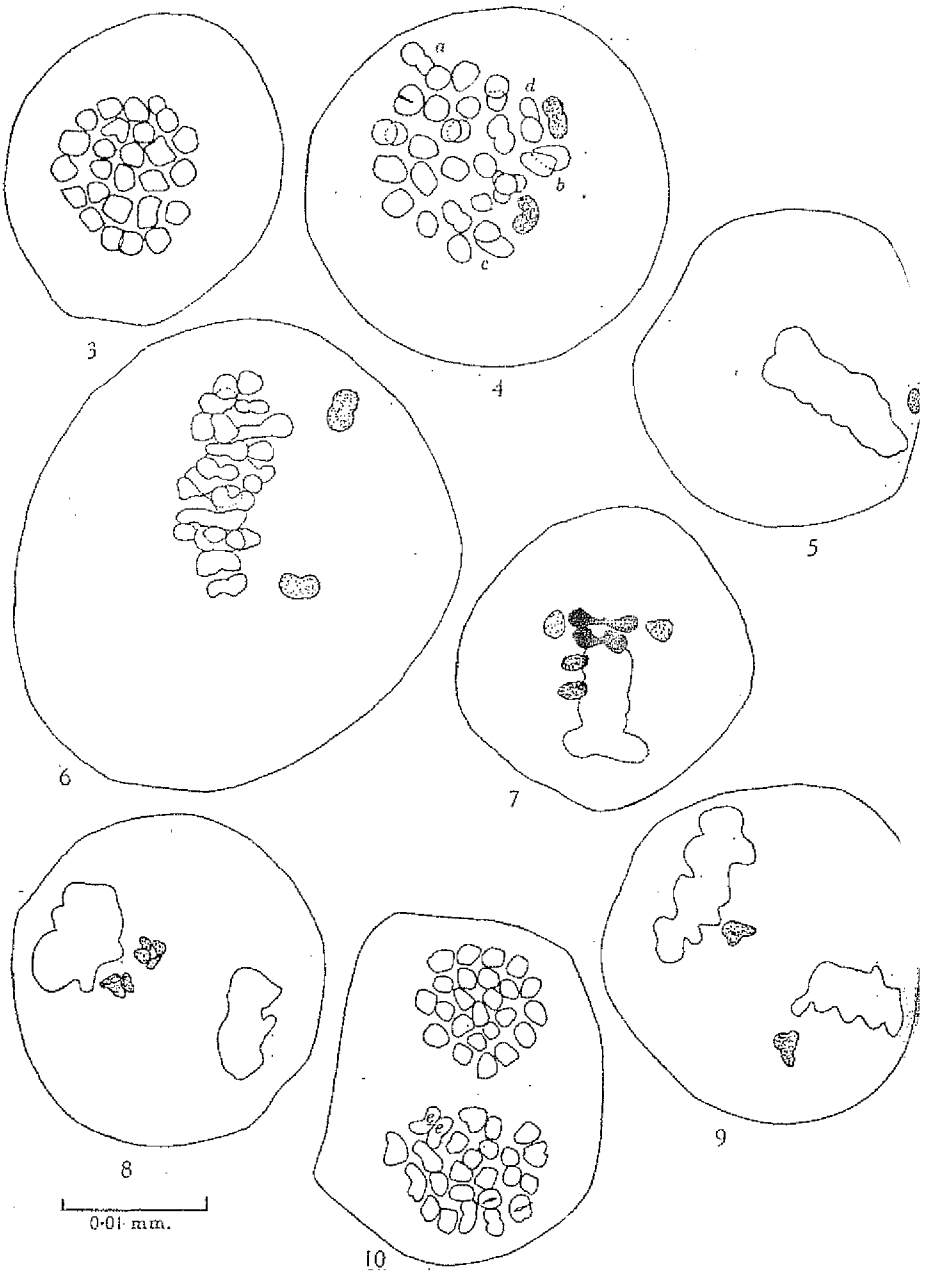
In all crosses involving *N. Tabacum* in this paper *N. Tabacum purpurea* (Pl. 5, fig. 1) has been used.

EXPERIMENTAL RESULTS

Microsporogenesis

Microsporogenesis in sterile amphidiploids. Pollen mother cells have been studied in the female sterile amphidiploids *N. sylvestris-tomentosa*, *N. sylvestris-tomentosiformis Gr*, *N. sylvestris-Setchellii*, and *N. glutinosa-tomentosa*.

N. sylvestris-tomentosa (4*n*) proved to be better cytological material than *N. sylvestris-tomentosiformis* (4*n*) *Gr* or *N. tomentosiformis-sylvestris* (4*n*), but since these three amphidiploids are all very similar in their chromosome behaviour, only the first one will be described in detail. The chromosome configurations found, in order of frequency of occurrence, are: 23 II + 2 I (24 cells), 24 II (16 cells), 22 II + 4 I (3 cells), 22 II + 1 III + 1 I (2 cells), and 21 II + 1 III + 3 I (1 cell) (Text-figs. 3–10). Occasional formation of a trivalent indicates that some chromatin material of *N. tomentosa* is also present in the genome of *N. sylvestris*. This is supported by studies of the F_1 *N. sylvestris* × *N. tomentosa* which has a mean of 2–3 bivalents per cell (Goodspeed, 1934). Some pairing is to be expected in any hybrid between two species which are closely enough related to cross. Side views of I M plates confirm the observations of polar views (Text-figs. 5–7). One or two univalent chromosomes can sometimes be seen lying off the II M plates (Text-figs. 8, 9). Text-fig. 10 clearly shows 24 I in each of the II M plates. The frequent failure of two chromosomes



Text-figs. 3–10. *N. sylvestris-tomentosa* (4*n*). Single chromosomes stippled. Dotted lines indicate lower focal level.

Text-fig. 3. I M, 24 II.

Text-fig. 4. I M, 23 II + 2 I; note single terminalized chiasma in several dumbbell-shaped (loose) pairs.

Text-fig. 5. I M with I off the plate.

Text-fig. 6. I M with two I's off the plate (not all bivalents are shown).

Text-fig. 7. I M with four I's, two from early separation of a pair.

Text-fig. 8. II M with two divided I's between the plates.

Text-fig. 9. II M with two I's between the plates, or perhaps a I has divided at I M.

Text-fig. 10. II M with 24 I in each plate; note early separation of dyad *e*, *e*.

to pair, though difficult to explain, is not uncommon in pure species. The writer found this behaviour in *N. Setchellii* in which an occasional plate with as many as four univalents was seen. Similar behaviour may also explain the infrequent occurrence of monosomics in *N. Tabacum*. The value of the amphidiploid as breeding material is only slightly impaired by these irregularities, as certation will tend to eliminate the deficient gametes, particularly on the male side.

Fewer pollen mother cells have been studied of *N. sylvestris-tomentosiformis* (4*n*) *Gr*, for its behaviour is very similar to that of *N. sylvestris-tomentosa* (4*n*). Of fourteen cells studied at I M seven showed 24 II and seven 23 II + 2 I. There usually appear to be two or three bivalents each with one non-terminal chiasma (Text-figs. 12, 13). They can be recognized readily at diakinesis (Text-fig. 13). Most of the bivalents are, however, rings, having two terminalized chiasmata.

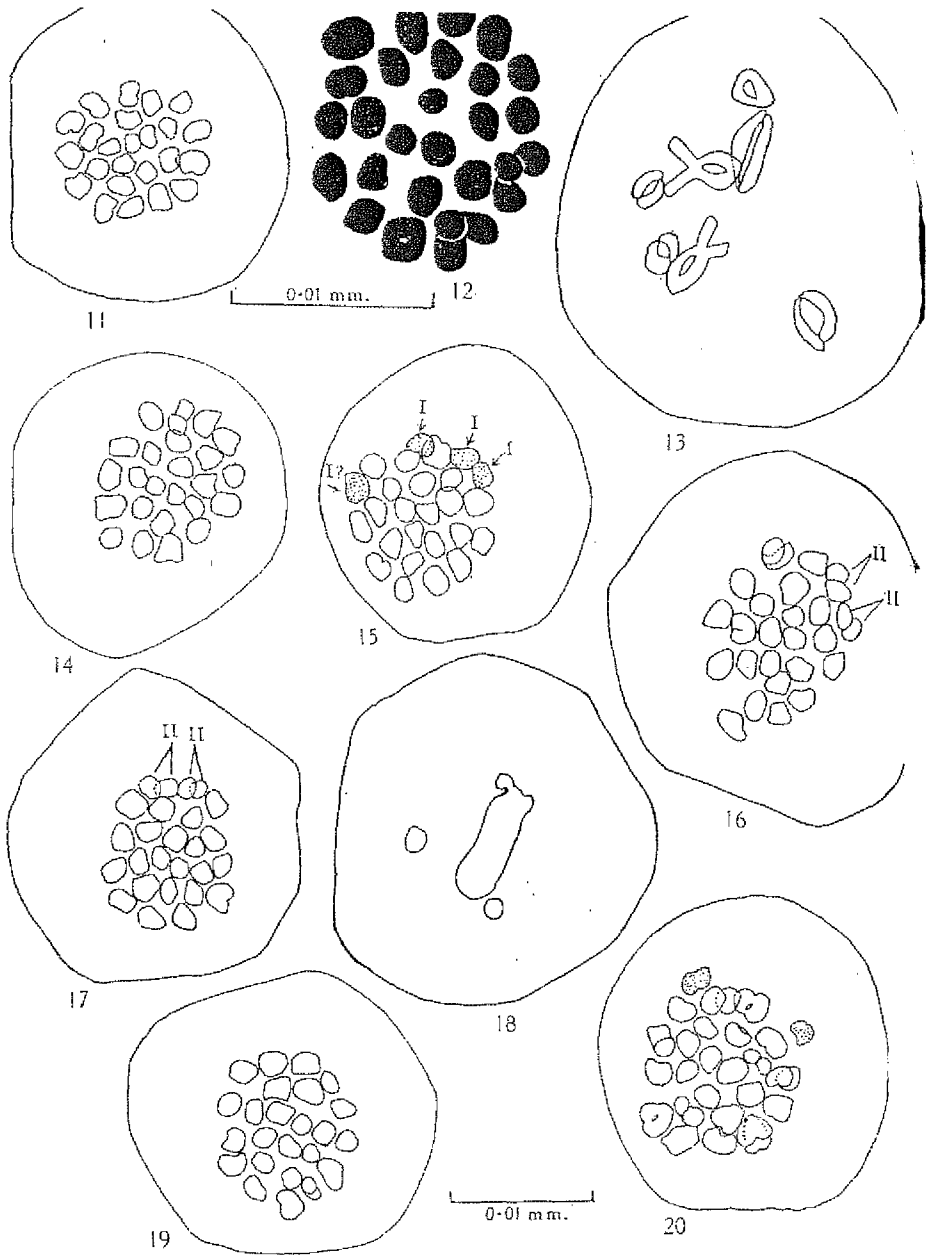
Of the amphidiploid *N. sylvestris-Setchellii*, which has much less regular meiosis than the two described above, pollen mother cells at I M showed 24 II (9 cells), 23 II + 2 I (10 cells), 22 II + 4 I (2 cells), 22 II + 1 III + 1 I (4 cells), 21 II + 1 II + 3 I (2 cells) (Text-figs. 14, 15). Some of the cells studied had more than four univalents, and could not be analysed. Hence the pollen mother cells studied are not a random sample like those of *N. sylvestris-tomentosa* (4*n*) already discussed.

The amphidiploid *N. glutinosa-tomentosa* showed very regular I M plates. Fifteen I M plates each showed 24 II (Text-figs. 16, 17). Fourteen II M plates each had 24 I, and in one cell 24 I could be counted in each plate. Side-views of I M have confirmed the highly regular pairing seen in polar views (Text-fig. 18). Data from pollen fertility counts to be presented later show that pairing behaviour in this amphidiploid is apparently very subject to seasonal fluctuation.

Microsporogenesis in partially fertile amphidiploids. Two plants of the amphidiploid *N. sylvestris* (4*n*) × *N. tomentosa* race Acomayo (4*n*) have been briefly examined. Five pollen mother cells from one of them showed 24 II (Text-fig. 11) and two showed 23 II + 2 I. Pollen mother cells of the other plant showed 24 II (4 cells) and 23 II + 2 I (4 cells).

Pollen mother cells of the F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr* have revealed the following configurations at I M: 22 II + 1 IV (16 cells), 24 II (5 cells), 23 II + 2 I (5 cells), 21 II + 1 IV + 2 I (4 cells), 22 II + 1 III + 1 I (1 cell), 21 II + 1 III + 3 I (1 cell), 20 II + 1 IV + 1 III + 1 I (1 cell). Attention is drawn to the frequent occurrence of a quadrivalent.

Microsporogenesis in crosses of N. Tabacum *with some of the ampli-*



Text-figs. 11–20.

Text-fig. 11. *N. sylvestris* (4*n*) × *N. tomentosa* race Acomayo (4*n*); I M, 24 II.

Text-figs. 12, 13. *N. sylvestris-tomentosiformis* (4*n*) *Gr*; Text-fig. 12, I M, 24 II; Text-fig. 13, diakinesis (not all chromosomes shown); note the two II's in Text-figs. 12 and 13, each with one terminal and one interstitial chiasma.

Text-figs. 14, 15. *N. sylvestris-Setchellii* (4*n*); Text-fig. 14, I M, 24 II; Text-fig. 15, I M, 22 II + 4 I.

Text-figs. 16–18. *N. glutinosa-tomentosa* (4*n*); Text-figs. 16 and 17, I M, 24 II; Text-fig. 18, side view of I M with two I's.

Text-fig. 19. F_1 *N. Tabacum* × *N. sylvestris-tomentosa* (4*n*); I M, 24 II.

Text-fig. 20. F_1 *N. Tabacum* × *N. sylvestris-Setchellii* (4*n*); I M, 23 II + 2 I.

diploids. In F_1 *N. Tabacum* × *N. sylvestris-tomentosa* (4*n*) and F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × *N. Tabacum* pairing of the chromosomes is good, there usually being 24 II or 23 II + 2 I at I M (Text-fig. 19). Pollen mother cells of the former showed 24 II (6 cells) and 23 II + 2 I (2 cells) at I M and of the latter, 24 II (10 cells) and 23 II + 2 I (1 cell). Kostoff (1933) reported 24 II as being quite regularly formed in *N. triplex*. *N. triplex* corresponds closely to the F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr.* F_1 *N. Tabacum* × *N. sylvestris-Setchellii* (4*n*) is much less regular in its chromosome behaviour (Text-fig. 20). Two pollen mother cells showed 24 II and 23 II + 2 I. Pollen fertility counts reported below support the less regular chromosome pairing observed at I M.

Microsporogenesis in N. Setchellii *and in the* F_1 N. tomentosiformis × N. Setchellii. Pollen mother cells of the one plant of *N. Setchellii* examined have frequently shown two unpaired chromosomes. The following configurations have been seen at I M: 12 II (9 cells), 11 II + 2 I (13 cells), and 10 II + 4 I (6 cells). However, probably more than half of the cells had 12 II.

The F_1 *N. tomentosiformis* × *N. Setchellii* apparently exhibits mostly 12 II at I M. Pollen mother cells at I M showed 12 II (6 cells) and at M II, 12 I (4 cells). At first anaphase, however, irregularities are occasionally encountered. Fragmentation of chromatin bridges has been observed, but it is not likely that their frequency would account for the high percentage of pollen abortion (61 %) found. It is more likely that most of the recombination products are inviable. Evidently these two species are rather closely related, but are much more distant than *N. tomentosiformis* and *N. tomentosa.*

Pollen fertility counts

Pollen fertility counts of many species and species hybrids which are related to the problem of the origin of *N. Tabacum* are given in Table 1. In examining the table the parallelism between the results of microsporogenesis and the percentage of good pollen should be kept in mind.

Pollen measurements

If *N. Tabacum* has originated as an amphidiploid from *N. sylvestris* and *N. tomentosiformis* or *N. tomentosa*, one might expect it to be intermediate in many respects between the two parental species. This is found to be the case. Thus, it is interesting to compare pollen size of the amphidiploids with that of the parental species and that of *N. Tabacum*

(Table 2). Twenty-five grains were measured in each case. The comparisons should be made between the callus-induced autotetraploid species and their corresponding amphidiploids. *N. sylvestris-tomentosiformis* (4*n*) *Gr* and *N. Tabacum* with means of 43 and 42, respectively, are near the size 42μ of the *N. tomentosiformis* (4*n*) parent. It would seem that *N. tomentosiformis* has the dominant size factors. *N. sylvestris-tomentosa* (4*n*) has slightly larger pollen and *N. sylvestris-Setchellii* (4*n*) has the largest of any of the amphidiploids measured. The mean difference,

Table 2. *Measurements of long diameters of pollen grains in microns*

	Mean and standard error of mean
N. sylvestris-tomentosa (4*n*)	43·9 ± 0·5
N. sylvestris-tomentosiformis (4*n*) *Gr*	43·3 ± 0·7
N. sylvestris-tomentosiformis (4*n*) Kostoff	39·8 ± 0·5
N. sylvestris-Setchellii (4*n*)	45·1 ± 0·7
N. Tabacum (2*n*)	42·2 ± 0·4
N. Tabacum (4*n*)	48·0 ± 0·5
N. sylvestris (2*n*)	43·8 ± 0·4
N. sylvestris (4*n*)	52·9 ± 0·4
N. tomentosiformis (2*n*)	36·3 ± 0·7
N. tomentosiformis (4*n*)	41·7 ± 0·5

3·5 ± 0·9, between *N. sylvestris-tomentosiformis* (4*n*) Kostoff and ditto *Gr* is statistically highly significant, as is also the mean difference, 2·4 ± 0·6, between *N. Tabacum* and the Kostoff amphidiploid. However, the mean difference, 1·1 ± 0·8, between *N. sylvestris-tomentosiformis* (4*n*) *Gr* and *N. Tabacum* is not significant. The difference, 1·7 ± 0·6, between *N. sylvestris-tomentosa* (4*n*) and *N. Tabacum* is also significant. There probably is a correlation between size of chromosomes and of pollen grains, for those of *N. sylvestris* are on the whole larger than those of *N. tomentosiformis*. The proportional increase in size of pollen, following doubling of the chromosome number, is about the same in all the species compared.

The sterility problem

Seed fertility counts. Three categories of ovules have been distinguished in the seed counts. They are: very small, aborted ovules due to gametic abortion; well-developed ovules with well-developed embryo and endosperm; and seemingly good ovules, but actually empty shells or only partially filled. This last class is thought to represent zygotic abortions; only a few of these occur. They usually comprise 2–3% of the total number of seeds. In Table 3 they are classified with the bad ovules. Seeds have been counted at the lower part of the ovary where the set is

best. The reader is reminded that species of *Nicotiana* have a large number of seeds per capsule. *N. Tabacum* itself has about 4000 seeds per capsule (Olmo, 1934).

The following seed counts were made in an effort to discover how many chromosomes of the female sterile amphidiploids carried the sterility genes. Kostoff (1933) reports that four *N. triplex* plants made by crossing *N. Tabacum* with the extremely sterile F_1 *N. sylvestris* × *N. tomentosiformis* are 'fully fertile', and that all but one of 127 F_2 progeny from these four F_1 *N. triplex* plants 'were fully fertile after selfing'. The writer cannot confirm these results. Although superficially quite fertile, seed

Table 3. *Seed fertility counts*

	Total seeds counted	No. of samples	Mean % good seeds	Standard error of mean
N. Tabacum	630	9	89·6	±1·9
N. sylvestris-tomentosiformis (4*n*) Kostoff	1025	11	65·3	±1·8
F_1 *N. Tabacum* × *N. sylvestris-tomentosa* (4*n*)	1625	10	17·4	±1·4
F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr*	883	6	17·0	±1·5
F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) Kostoff	1155	10	30·2	±2·3
F_1 *N. Tabacum* × *N. sylvestris-Setchellii* (4*n*)	1877	12	14·0	±1·2
F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*	1464	8	13·2	±1·1
[*N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*] × ditto *Gr*	1554	7	14·4	±1·9
[*N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*] × ditto Kostoff	1462	14	70·6	±3·3
N. sylvestris-tomentosiformis (4*n*) Kostoff × [ditto Kostoff × ditto *Gr*]	1510	9	25·2	±3·3

counts of the F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) *Gr*, which is very similar to *N. triplex*, show that this hybrid is only 17% fertile (Table 3). Also, the F_2 plants from this hybrid show great variation in fertility. The same is true of the first backcross to the sterile amphidiploid. Variation in fertility in these populations is expected if the pollen transmits various numbers of sterility genes. In these populations involving *N. Tabacum* two causes for ovule abortion are probably superimposed one upon the other, thus complicating the analysis. One of these is thought to be due to a mixture of *N. Tabacum* and of amphidiploid chromosomes of which certain combinations are inviable as female gametophytes (cf. the sterile F_2 segregates), and the other is that different numbers of sterility genes of the amphidiploid are transmitted to the F_2 through the pollen. It may be that elimination on the female side

is not absolute, and that occasionally some sterility genes are transmitted.

The F_1 *N. Tabacum* × *N. sylvestris-tomentosa* (4*n*) likewise shows only 17% of good seeds, again confirming the general genetic equivalence of *N. tomentosa* and *N. tomentosiformis*. The F_1 *N. Tabacum* × *N. sylvestris-Setchellii* (4*n*), however, has only 14% of good seeds and the F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*, 13% (Table 3). The percentage of surviving gametes in these hybrids indicate that only two or three chromosomes carry the sterility genes.

Because of the possible complicating effect of the dissimilar *N. Tabacum* chromosomes, another series of hybrids has been thought to be better material for the sterility analysis. These are the backcross [F_1 *N. sylvestris-tomentosiformis* (4*n*) Kostoff × ditto *Gr*] × ditto Kostoff, the reciprocal of this cross, and the backcross of the F_1 to ditto *Gr*. According to the gametic lethal hypothesis, the first of these crosses should give more highly fertile plants than the reciprocal cross, and the last cross mentioned should give the least fertile plants. This, as can be seen from Table 3, is actually found to be the case. Unfortunately, Kostoff's amphidiploid itself has a rather low fertility (Table 3). It has been noticed that its seed pods often have no placental tissue in some portions of the upper part of the ovary.

Evidence for the complicating effect of *N. Tabacum* chromosomes in analysing the sterility is furnished by the fertility of F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* (4*n*) Kostoff (Table 3) which is less than that of either parent. It would appear, therefore, that the lowered fertility of this hybrid is due to the complementary action of sterility genes of the two genetic systems. This conclusion is strengthened by the regular pairing observed and the high percentage of good pollen (Table 1).

The possible origin of Kostoff's amphidiploid

The fertile amphidiploid *N. sylvestris-tomentosiformis* was reported in 1938 by Kostoff, who over a period of several years persistently crossed the extremely sterile F_1 hybrid *N. sylvestris* × *N. tomentosiformis* with *N. sylvestris* ♂, finally obtaining a few seeds. Among the resulting seedlings there were two with 36 chromosomes. He then crossed these much more fertile, but still relatively sterile, plants with *N. tomentosiformis* and obtained a large number of seeds. Among the plants from this last cross there were two with 48 chromosomes. These two plants are described by him as having been 'almost fully fertile and very much *N. Tabacum* like. Morphologically they were not quite alike...'. It

must be added that their progeny also was not uniform, although a stable type was established after a few generations. Naturally this manner of obtaining the amphidiploid leads to chromosome changes, and it is not surprising that it differs from the *Gr* amphidiploid in morphology and fertility. The callus method of making polyploids precludes chromosome alterations. The chromosome complement of the resulting amphidiploid is an exact duplication of that of the decapitated plant. It is thought that the Kostoff amphidiploid has undergone a quantitative alteration of chromosome material with respect to the strictly duplicate condition of the sterile amphidiploid. The method by which this change may have occurred during the synthesis employed by Kostoff and the various steps leading to a fertile amphidiploid will now be outlined.

Designate the chromosomes of *N. sylvestris* 1 – – – 2, 3 – – – 4, 5 – – – 6, ... , 23 – – – 24, and those of *N. tomentosiformis* *a* – – – *b*, *c* – – – *d*, *e* – – – *f*, ... , *x* – – – *y*. Assume some genes of the 3 – – – 4 chromosome of the former duplicated in the *c* – – – *d* chromosome of the latter, and that this leads to exchange between these two chromosomes at meiosis in the F_1 hybrid. Assume one case of such similarity to be gene **S** in chromosome 3 – – – 4 and gene **T** in chromosome *c* – – – *d* (Text-fig. 21), and that these particular genes are concerned with the same phase of embryo-sac development. Both genes are considered to have had a common ancestor but have since diverged in such a way that they now affect embryo-sac development by different reactions or reaction rates. When present in the same embryo-sac mother cell, their complementary action results in arrested development of the embryo sac in the two- or four-nucleate stage. Translocation results in dyads $\begin{matrix}3\text{–}\mathbf{T}\text{–}d\\3\text{–}\mathbf{S}\text{–}4\end{matrix}$ and $\begin{matrix}c\text{–}\mathbf{T}\text{–}d\\c\text{–}\mathbf{S}\text{–}4\end{matrix}$. Assume that functional gametes are unreduced. Segregation, following formation of a restitution nucleus, will give the four gametic combinations shown in Text-fig. 21. Assume in accordance with the hypothesis of complementary sterility genes that only 3–**T**–*d*, *c*–**T**–*d* and 3–**S**–4, *c*–**S**–4 gametes function on the female side. Assume 3–**S**–4, *c*–**S**–4 to be the functional female gamete in the backcross to *N. sylvestris*. This gamete is deficient for the *d* end and duplicated for the 4 end of the chromosomes *c*–**T**–*d* and 3–**S**–4, respectively. The resulting 36-chromosome plant will then have the constitution:

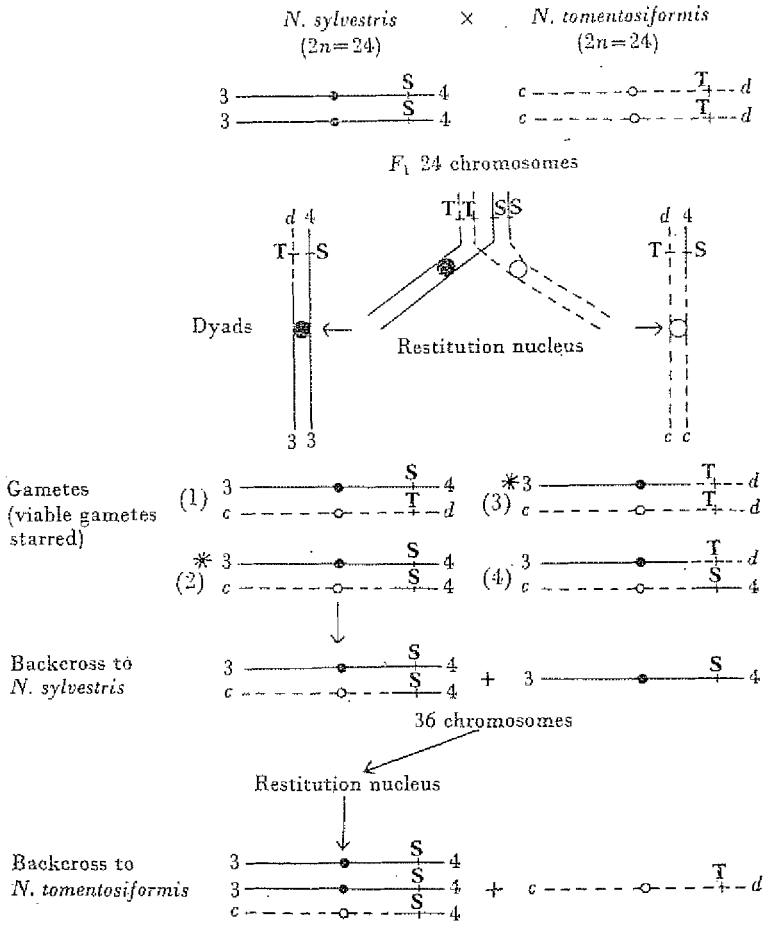
$$a\text{–}\text{–}\text{–}b,\ c\text{–}\mathbf{S}\text{–}4,\ e\text{–}\text{–}\text{–}f,\ \ldots\ldots,\ x\text{–}\text{–}\text{–}y \quad \begin{matrix}1\text{–}\text{–}\text{–}2,\ 3\text{–}\mathbf{S}\text{–}4,\ \ldots\ldots,\ 23\text{–}\text{–}\text{–}24\\1\text{–}\text{–}\text{–}2,\ 3\text{–}\mathbf{S}\text{–}4,\ \ldots\ldots,\ 23\text{–}\text{–}\text{–}24\end{matrix}$$

Backcrossed to *N. tomentosiformis*, once more assuming restitution

nucleus formation, an amphidiploid is obtained with the constitution:

a---*b*, *c*–**S**–4, *e*---*f*, ..., *x*---*y* 1---2, 3–**S**–4, ..., 23---24.
a---*b*, *c*–**T**–*d*, *e*---*f*, ..., *x*---*y* 1---2, 3–**S**–4, ..., 23---24

The functional eggs of this amphidiploid will be of the *c*–**S**–4, 3–**S**–4 constitution.



Text-fig. 21. Scheme for the origin of a fertile amphidiploid from diploid species having complementary sterility genes.

In the above scheme it is assumed that only the simultaneous presence of **S** and **T** results in abortion of embryo sacs. The combinations **St**, **sT**, and **st** are all considered to be viable as female gametophytes.

Such an amphidiploid as the above is not expected to breed true, and will be only partially fertile. This is exactly what Kostoff (1938) has reported. Kostoff also found that on selfing his original amphidiploid some more fertile and other less fertile types appeared, which is in accord with the complementary gene (duplication-deficiency) hypothesis outlined above. According to it, one pair of complementary genes (one duplication-deficiency) results in 50% of aborted gametes, two independent pairs of complementary genes (two duplications-deficiencies), in 75% of aborted gametes, etc. The theoretical amphidiploid with one translocation, hypothecated above, would on selfing be expected to give fully fertile individuals homozygous for the *c*–**S**–4 chromosome, and others heterozygous like the parent plant. If two or more pairs of independent complementary sterility genes were involved, the progeny of this theoretical amphidiploid would be still more variable in morphological features and in fertility. The simplest possible case is presented in the above scheme.

The Kostoff amphidiploid, in accordance with the hypothesis outlined, would soon become homozygous for its duplications. When crossed with the sterile *Gr* amphidiploid, the F_1 would sometimes be expected to show a quadrivalent chain of the type *d*–**T**–*c*.*c*–**S**–4.4–**S**–3.3–**S**–4 in some cells and only bivalents in others. This agrees with the pollen mother cell data of this hybrid (cf. Microsporogenesis). The quadrivalent could not, however, be identified for certain as a chain, but some observations indicate that it may possibly be one. The data also show that five cells have 23 II + 2 I. The two singles could possibly be the *c*–**S**–4 and *c*–**T**–*d* chromosomes which would occasionally be expected to remain unpaired.

One duplication-deficiency would best fit the pollen mother cell data, but it cannot explain the percentage of seed (gametic) abortion found in the F_1 hybrid (Table 3). A chain of the kind shown would regularly give only *c*–**T**–*d*, 3–S–4 and *c*–**S**–4, 3–**S**–4 gametes of which only the latter would be functional. A second duplication-deficiency, however, if small, would not contradict the pairing evidence, for differential affinity between chromosomes would only rarely permit quadrivalent formation, and cannot, therefore, be detected in a study of the pollen mother cell. The mean percentage of good seeds in the Kostoff amphidiploid is 65·3. The calculated value on the basis of two independent duplications-deficiencies is 52·8% (Table 3). These agree fairly well, considering the experimental error in data such as these.

There still remain difficulties. One is that the Kostoff plant is only

partially fertile, even though it must soon have become homozygous for its duplications-deficiencies. The other is that duplications, unless small, cannot lead to a stable type. To overcome this last difficulty, the writer assumes that the duplications are small, and, therefore, interfere but slightly or not at all in meiosis. For the first it would seem that some complementary sterility genes are still present in the genetic system of this amphidiploid, but that they are of minor importance and become lethal only under certain environmental conditions. Removal of a few additional sterility genes and their replacement by corresponding genes from the other parental species would buffer the system against environmental influence. Hence, the writer is of the opinion that the Kostoff amphidiploid can be made more fertile, and will eventually, through mutation and natural selection, become completely fertile. There will, of course, always remain some residual sterility due to the crowding of ovules, ensuing competition for foodstuffs, and occasional irregularities at meiosis. These are thought to be the causes for ovule abortion in *N. Tabacum* (Table 3). It is of interest that ovule abortion in the latter is higher than pollen abortion (Table 1).

Discussion

To the writer's knowledge these are the first cases of genic sterility in amphidiploids to be reported in the literature. That complementary sterility genes are actually involved is made very probable by the partial female fertility of some amphidiploids made with certain wild races of *N. tomentosa*, the complete female sterility of others involving different, long inbred races or species of the *tomentosa* group (cf. *N. sylvestris* (4*n*) × *N. tomentosiformis* (4*n*)), and the regular meiosis and very high percentage of good pollen found. The hypothesis of complementary gene action could explain the partial fertility of the amphidiploid lines, as well as the occasional appearance of a completely sterile plant, if the wild *tomentosa* races were heterozygous for complementary sterility genes. It could similarly explain the partial fertility of F_1 hybrids of *N. Tabacum* with the completely female sterile amphidiploids. These considerations emphasize the method of synthesis of the partially fertile, apparently heterozygous, amphidiploids, for callus-induced ones are of necessity homozygous and are expected, therefore, to be either sterile or fertile, but not partially fertile.

It is clear from these experiments that genic sterility in F_1 hybrids may be superimposed on chromosomal sterility, as in the F_1 hybrids *N. sylvestris* × *N. tomentosa*, *N. sylvestris* × *N. tomentosiformis*, *N. syl-*

vestris × *N. Setchellii*, and *N. glutinosa* × *N. tomentosa*. It follows, then, that fertility of amphidiploids, contrary to Darlington (1937), cannot be predicted from the amount of chromosome pairing in F_1 hybrids but must be experimentally tested.

It is significant that either the long inbred *N. tomentosa* U.C.B.G. 08–193 or *N. tomentosiformis* is involved in all of the sterile amphidiploids, and that only the female gametophyte aborts while the pollen is unaffected. The phenomenon of unilateral fertility is not so strange as one might suppose if one recalls that different genes may be concerned with the development of the male and female gametophytes, e.g. the male sterile genes and the lethal ovule gene in maize (Beadle, 1932; Singleton & Mangelsdorf, 1940). It is possible that other races of *N. sylvestris* would give fertile amphidiploids with the same race of *N. tomentosa* as that which gave the sterile one and with *N. tomentosiformis*. It has, however, been found that a recently introduced race of *N. sylvestris* U.C.B.G. 37–11 gives very abnormal, spindly hybrids with *N. tomentosiformis* and *N. Setchellii*. These hybrids continually lose the lower leaves on the stem, retaining only an apical tuft. They have not flowered. This race does not appear to be a likely ancestor. Other races need to be tested.

A possible case of genic sterility in an amphidiploid has been reported by Beasley (1940) for one between a 13-chromosome Asiatic and an American species of *Gossypium*. This amphidiploid has quite regular chromosome pairing, is female fertile, but only slightly male fertile. Other cases which resemble the female sterile *Nicotiana* amphidiploids are the numerous male steriles in maize (Beadle, 1932), especially those in which disintegration occurs between the end of meiosis and the first division in the pollen grain. A gene in maize which causes abortion of the ovules receiving it has been described by Singleton & Mangelsdorf (1940), but no cytological study has been reported of it. Female gametic lethals are rare as compared with the number of male steriles recorded in the literature.

The two best studied cases of genic sterility in animals are the male sterile interracial hybrids of *Drosophila pseudoobscura* (Dobzhansky, 1936) and the intersexes from interracial crosses in *Lymantria dispar* (Goldschmidt, 1931). Dobzhansky explains his results by complementary genes. He found sterility genes in each of the four largest chromosomes tested.

The origin of *N. Tabacum* from races which possess complementary sterility genes is not excluded if it occurred by the Kostoff method or by the union of unreduced gametes in the F_1 hybrid, for, as has been

indicated, probably not more than two or three chromosomes of the parental genomes carry these genes, and translocations (Text-fig. 21) could eliminate the genes in one of the sets. Origin by somatic doubling, however, would require the absence of these genes. It is perhaps more likely that *N. Tabacum* originated from races of the parental species which were not yet differentiated in the genes controlling embryo-sac development. Some of the amphidiploid plants of *N. sylvestris* (4*n*) × *N. tomentosa* race Acomayo (4*n*) are quite fertile. Over 500 seeds were counted in one capsule. In general, however, 50–200 seeds per capsule is more representative. A further test of the complementary gene hypothesis will be the fertility of future generations of the partially fertile amphidiploid lines. Selection for greater fertility should be automatic.

N. sylvestris-tomentosiformis (4*n*) *Gr* probably resembles most varieties of *N. Tabacum* more closely than do the other amphidiploids. Like *N. Tabacum* it does not have a gene for anthocyanin in the stem. The purple factor is present in *N. tomentosa*. This amphidiploid like *N. Tabacum* matures earlier than those involving the biennial *N. tomentosa* which flower about three weeks later. Also its pollen size is near that of *N. Tabacum*. It is not possible on such evidence to state definitely that this amphidiploid rather than one of the others is the predecessor of *N. Tabacum* of to-day, for the latter itself has changed by mutation and selection under cultivation since its origin. All of these amphidiploids, as expected, differ physiologically as well as morphologically from *N. Tabacum*, but the relationship is also obvious. The physiological-chemical differences are well illustrated in a comparison between the allooctoploids *N. sylvestris-tomentosiformis* (Pl. 5, fig. 2) and *N. sylvestris-tomentosa* (Text-fig. 3) and autotetraploid *N. Tabacum*. All have 96 chromosomes, but the first two are monstrosities with enormously enlarged cells while the last has the features typical of an autotetraploid. Evidently *N. Tabacum* has undergone physical-chemical changes since its origin. These changes are probably associated with a readjustment in the ratio of cell volume to nuclear volume. The evidence from pollen measurements indicates that *N. Tabacum* pollen has been reduced in size since *N. Tabacum* originated. The writer has found that autooctoploid *N. tomentosa* is also a monstrosity. Octoploid sectors on plants which are the result of colchicine treatment show the same phenomenon. A readjustment in the cellular physiology must, therefore, take place before another doubling can occur without deleterious results, and this probably requires a very long time. These considerations are important when contemplating the time factor in evolution, and they also furnish addi-

tional evidence of the more important role of alliopolyploidy in evolution, for it is probable that if the amphidiploid were crossed with a third species, followed by doubling, and this process were repeated, that the higher polyploid numbers could be obtained with less harmful effects. Some evidence supporting this view has been obtained by Wettstein (1927) in mosses where $16n$ was found to be the upper limit of viability of intergeneric polyploids of *Physcomitrella* × *Funaria*, and $4n$ that of autopolyploids of the species themselves.

From the results to date the writer concludes that *N. Tabacum* has originated as an amphidiploid from *N. sylvestris* and *N. tomentosiformis* or from *N. sylvestris* and a race of *N. tomentosa* similar to those which to-day give partially fertile amphidiploids, and that this event occurred somewhere in the general region of South America (Peru, Bolivia, and northern Argentina) where these species and races occur to-day.

SUMMARY

A study of microsporogenesis of the two callus-induced amphidiploids *N. sylvestris-tomentosa* and *N. sylvestris-tomentosiformis Gr* has shown that meiosis is regular. Pollen fertility counts have shown that their pollen is 93% or more good. They are, nevertheless, completely female sterile.

Four other amphidiploids have been made using wild races of *N. tomentosa*. Races Machu Picchu and Acomayo have given partially fertile ones. The pollen is good in all four.

The complete or partial female sterility in the several amphidiploids made is, therefore, genic and not chromosomal. It is interpreted to be due to the interaction of complementary genes of the two parental species. These genes are thought to control embryo-sac development by different reactions or reaction rates. Their interaction in the amphidiploids results in arrested development of embryo sacs while still in the two- or four-nucleate stage.

A fertile amphidiploid *N. sylvestris-tomentosiformis* involving the same strains of the parental species as were used by the writer in obtaining his corresponding female sterile amphidiploid was synthesized by Kostoff (1938). The manner in which the Kostoff amphidiploid may have arisen is depicted by means of a scheme which incorporates the complementary sterility gene hypothesis.

Seed fertility counts of F_1 *N. sylvestris-tomentosiformis* ($4n$) Kostoff × ditto *Gr*, F_1 *N. Tabacum* × *N. sylvestris-tomentosa* ($4n$), and F_1 *N. Tabacum* × *N. sylvestris-tomentosiformis* ($4n$) *Gr* indicate that the

sterility genes are probably carried on two, or at the most three, chromosomes of each of the parental genomes.

Pollen fertility counts have been made of many different plants concerned with or related to the problem of the origin of *N. Tabacum*. These counts support the thesis of the amphidiploid origin of *N. Tabacum*, as well as the hypothesis of complementary sterility genes.

A comparison of pollen size of the synthetic amphidiploids with that of *N. Tabacum* has shown that the difference in size of pollen between the amphidiploid *N. sylvestris-tomentosiformis Gr* and *N. Tabacum* is not significant, while that of some of the other amphidiploids is significant.

The physiological-chemical differences between any of the synthetic *Tabacum*-like amphidiploids and *N. Tabacum* are illustrated by means of a comparison of morphological features of the allo-octoploids *N. sylvestris-tomentosa* and *N. sylvestris-tomentosiformis* with autotetraploid *N. Tabacum*. The first two are monstrosities, while the last has the features typical of any experimental autotetraploid.

It is concluded that *N. Tabacum* has originated as an amphidiploid from *N. sylvestris* and *N. tomentosiformis* or a race of *N. tomentosa* similar to one of those which to-day give partially fertile amphidiploids, and that this event occurred in the general region of South America (Peru, Bolivia, and northern Argentina) where these species and races occur to-day.

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EXPLANATION OF PLATES 3–6

PLATE 3

Topmost: *N. sylvestris.*
Top row, left to right: *N. tomentosiformis, N. Setchellii, N. tomentosa.*
Middle row, left to right: the corresponding F_1 hybrids with *N. sylvestris.*
Bottom row, left to right: the corresponding amphidiploids.

PLATE 4

Middle row:

Fig. 1. *N. tomentosa* race Ticempaya.
Fig. 2. *N. tomentosa* race Acomayo.
Fig. 3. *N. tomentosa* race Machu Picchu.
Fig. 4. *N. tomentosa* race Pincos.
Fig. 5. *N. tomentosiformis.*

To the right, diploids; to the left, tetraploids of the identical diploid plants.

Bottom row: corresponding seed pods.

Top row: the corresponding amphidiploids made by crossing *N. sylvestris* (4*n*) with the races of *N. tomentosa* (4*n*) and with *N. tomentosiformis* (4*n*).

Fig. 3 shows two tetraploid flowers of *N. tomentosa* race Machu Picchu, one white and the other mauve.

The background is ruled in square cm.

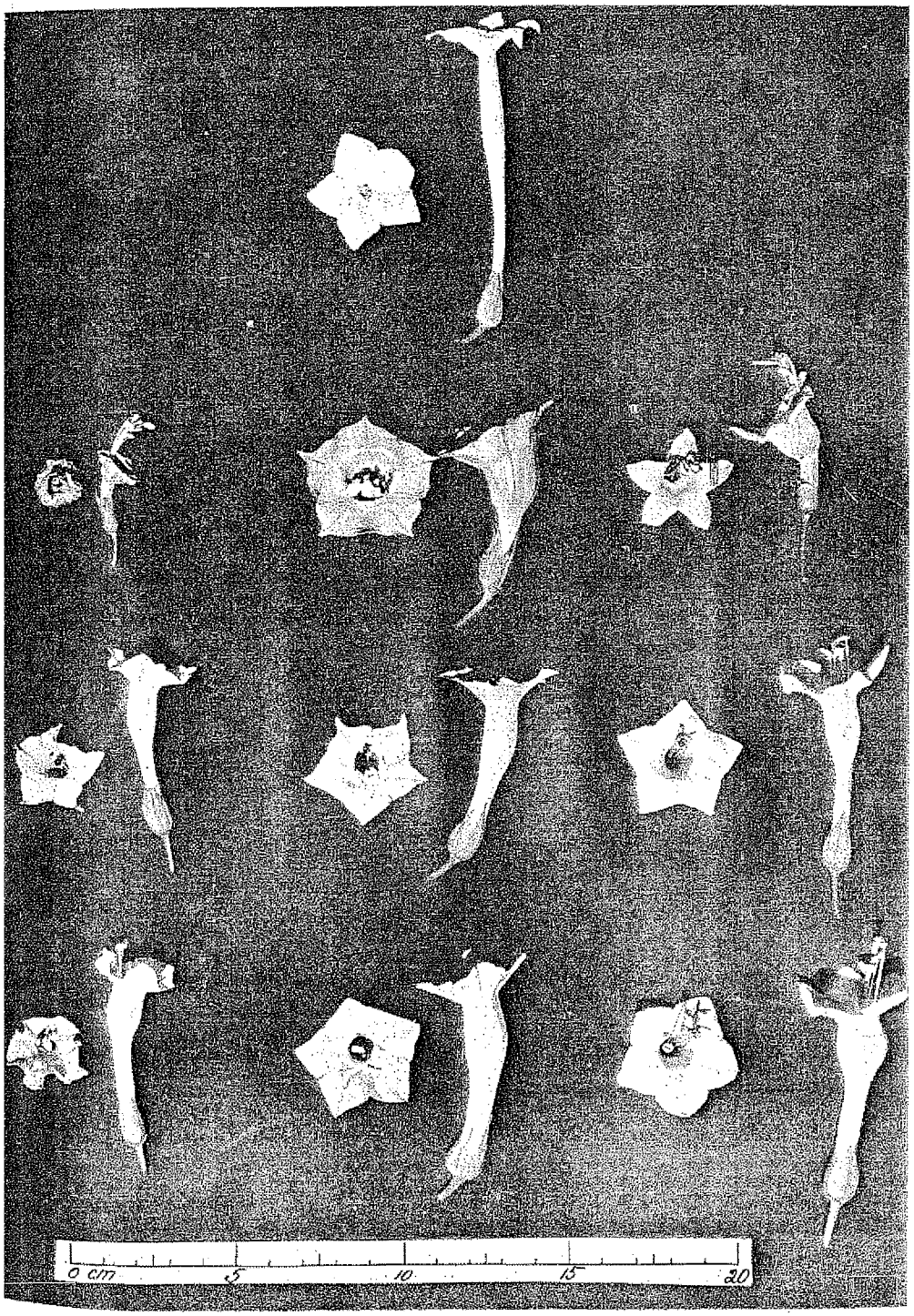
PLATE 5

Fig. 1. Top row, left to right: *N. Tabacum*, *N. sylvestris-tomentosa* (4*n*), F_1 *N. sylvestris* (4*n*) × *N. tomentosa* race Acomayo (4*n*). Bottom row, left to right: F_1 *N. sylvestris* (4*n*) × *N. tomentosa* race Pincos (4*n*) P4, P1, P30, and P28, F_1 *N. sylvestris* (4*n*) × *N. tomentosa* race Machu Picchu (4*n*) P11, P23, P10, and P31.

Fig. 2. Topmost: left, *N. sylvestris*; right, *N. tomentosiformis.* Below: centre, the F_1 hybrid; left, the callus-induced amphidiploid; right, the callus-induced allooctoploid. Photographed on mm. paper.

PLATE 6

Fig. 1. *N. sylvestris-tomentosa* (4*n*).
Fig. 2. F_1 *N. sylvestris* × *N. tomentosa.*
Fig. 3. *N. sylvestris-tomentosiformis* (4*n*) *Gr.*
Fig. 4. *N. sylvestris-Setchellii* (4*n*).
Fig. 5. *N. glutinosa-tomentosa* (4*n*).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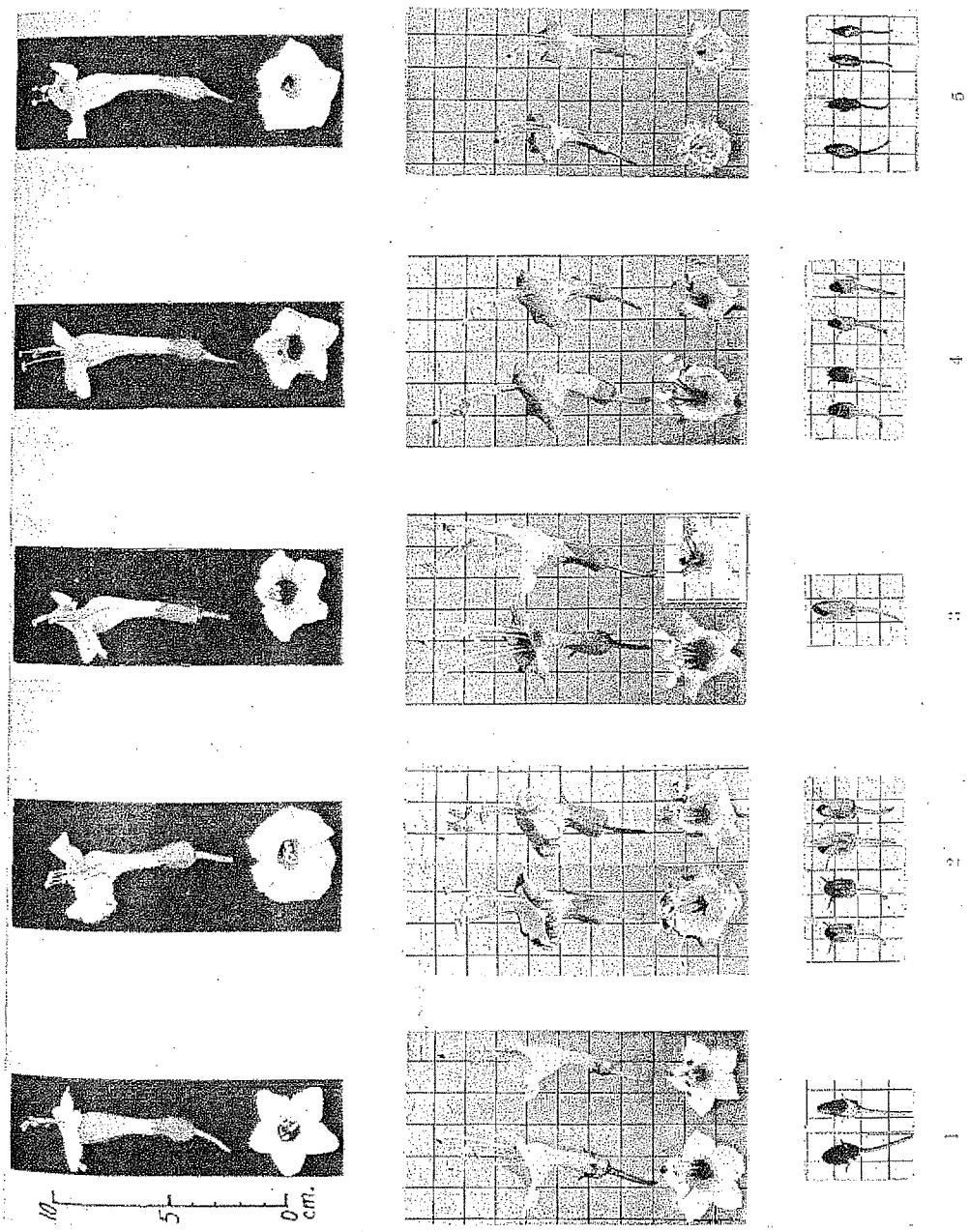
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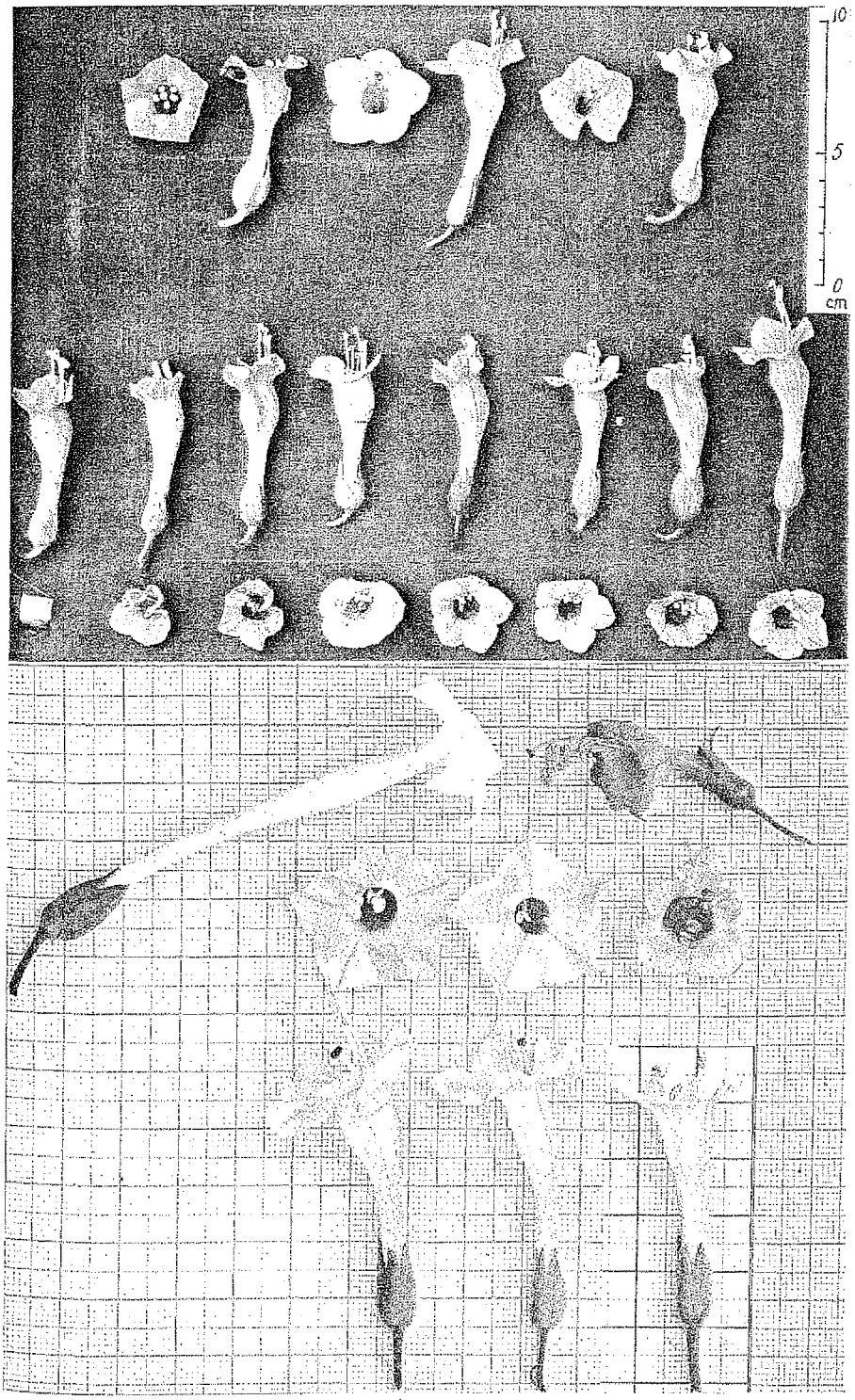
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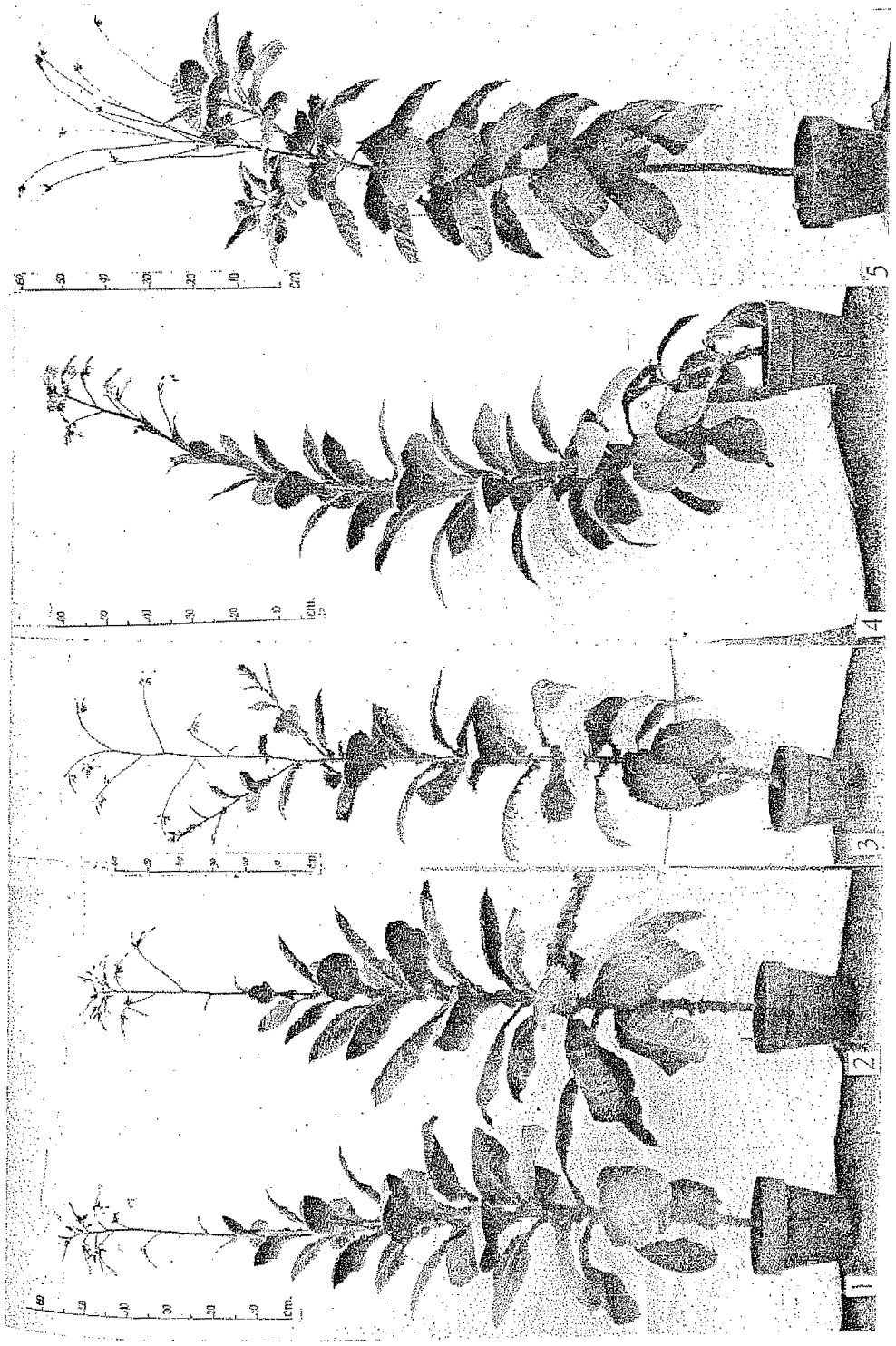
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