INHERITANCE IN *NICOTIANA TABACUM*

XXVI. STERILITY GENES FROM TOMENTOSAE SPECIES

By ABBAS H. AR-RUSHDI

*Department of Genetics, University of California, Berkeley**

(With Plate 1 and Three Text-figures)

(*Received* 4 *January* 1955)

Nicotiana tabacum ($2n = 48$) was one of the first species to be analysed in terms of Winge's (1917) hypothesis as to the origin of polyploid species by doubling of chromosome number following interspecific hybridization. The extensive body of evidence from studies of distribution, morphology, taxonomy, karyology, genome analysis, and from experimental cytogenetical studies, reviewed by Goodspeed (1945), has established, it would seem beyond doubt, its origin from hybridization of *N. sylvestris* ($2n = 24$) with some one of four species of the section Tomentosae, viz. *N. otophora* ($2n = 24$), *N. setchellii* ($2n = 24$), *N. tomentosa* ($2n = 24$), or *N. tomentosiformis* ($2n = 24$). Of special interest was Greenleaf's (1941, 1942) evidence obtained by actual production of amphidiploid types roughly equivalent to *N. tabacum* from the above-mentioned species.

Although the evidence regarding the ancestry of *N. tabacum* appeared to be conclusive, actual establishment of the amphidiploid *N. tabacum* equivalents revealed not only the existence of morphological features in these products at some variance with those of *N. tabacum*, but also presence of genetical conditions interfering with fertility of the amphidiploids. The sterility of these amphidiploids, despite their regular meiotic behaviour, was a novel and unexpected observation, which became the central object of Greenleaf's investigations. He was able to show that in the amphidiploid *sylvestris-tomentosiformis* and *sylvestris-tomentosa* hybrids the difficulty was due to inability to mature an embryo sac following meiosis. However, other collections of *N. tomentosa*, namely, 'Acomayo' and 'Machu Picchu', were observed to give amphidiploids of very limited fertility, but Greenleaf failed to determine the mechanism of sterility in these amphidiploids, though he did note a difference in this respect between amphidiploid *sylvestris-setchellii* and the two mentioned above to which most attention was devoted. Furthermore, later experience with these amphidiploids and with the related sesquidiploid hybrids, as well as hybrids with *N. tabacum*, revealed phenomena pointing unmistakably to essential differences among them, as may be seen from the following tabulation of some critical comparative observations:

Amphidiploid hybrids crossed with tabacum, *approximately regular synaptic association*

	As female		As male	
	Fertile	Sterile	Fertile	Sterile
4x (*N. sylvestris* × *N. otophora*) × 2n *N. tabacum*	+	·	+	·
4x (*N. sylvestris* × *N. setchellii*) × 2n *N. tabacum*	·	+	+	·
4x (*N. sylvestris* × *N. tomentosa*) × 2n *N. tabacum*	+	·	+	·
4x (*N. sylvestris* × *N. tom.* 'Acomayo') × 2n *N. tabacum*	·	+	+	·
4x (*N. sylvestris* × *N. tomentosiformis*) × 2n *N. tabacum*	+	·	+	·

* Present address: Zoology Department, Arts and Science College, Baghdad, Iraq.

Sesquidiploid hybrids, mostly approximately 12 *bivalents and* 12 *trivalents*

	As female		As male	
	Fertile	Sterile	Fertile	Sterile
4*n N. tabacum* × 2*n N. sylvestris*	+	.	+	.
4*n N. tabacum* × 2*n N. otophora*	+	.	+	.
4*n N. tabacum* × 2*n N. setchellii*	.	+	+	.
4*n N. tabacum* × 2*n N. tomentosa*	+	.	+	.
4*n N. tabacum* × 2*n N. tmn.* 'Acomayo'	.	+	+	.
4*n N. tabacum* × 2*n N. tomentosiformis*	+	.	+	.

The important difference here recorded is that the F_1 hybrids of *N. tabacum*, with amphidiploids *sylvestris-setchellii* and *sylvestris-tomentosa* 'Acomayo', are sterile, whereas those of *sylvestris-tomentosa* and *sylvestris-tomentosiformis*, the main subjects of Greenleaf's studies, are fertile. Furthermore, the results of crossing 4*n tabacum* with these species demonstrate that the difference depends upon differences in the elements introduced by *N. setchellii* and *N. tomentosa* 'Acomayo' as contrasted with those contributed by *N. tomentosa* and *N. tomentosiformis*.

The present investigations were directed towards a clarification of this difference in behaviour, particularly towards an elucidation of the mechanism of sterility in the hybrids of *N. setchellii* and *N. tomentosa* 'Acomayo' and to a determination of its genetical basis, for these phenomena are of interest not only to the specific problem of the origin of *N. tabacum*, but also to the general problem of the limitations effective in the production of new species by amphidiploidy.

Material and methods

Of the *N. tabacum* varieties available (about 200) at the Genetics Department, University of California, Berkeley, *N. tabacum* 'Purpurea' (U.C.B.G. 06–25) was used. It is better known to growers as Red Russian, which name will be used throughout this presentation. This variety was highly inbred, having been maintained at this University over the past 40 years by continuous selfing. Of section Tomentosae, *N. setchellii* and the 'Acomayo' form of *N. tomentosa* were used. *N. setchellii* was derived from the original and apparently only collection of the species, and *N. tomentosa* 'Acomayo' was one of a numerous set of forms of *N. tomentosa* collected by Goodspeed. It is particularly notable for its self-sterility which does not conform to the oppositional system established by East and Mangelsdorf for the *N. alata* assemblage.

The original *tabacum-tabacum-setchellii* sesquidiploid was obtained by Prof. R. E. Clausen through the pollination of a tetraploid *N. tabacum* by *N. setchellii* pollen. From the above cross, fifty seeds were placed in the germinator, but only two plants (48087 p1 and 48087 p2) were raised to maturity. Both were completely sterile in terms of failure to set seeds, although their pollen was good and was used for subsequent backcrosses.

The technical methods employed in these investigations were as follows:

I. *Embryo-sac development*

Ovaries were divided longitudinally into four parts, fixed in Randolph's CRAF solution (1935) containing about 0·05 % alkanol. They were embedded in paraffin blocks according to Randolph's procedure. Transverse sections were cut at thicknesses of 14–40 μ, depending

on the age of the ovary and the purpose of the study. The triple stain of Flemming as modified by Stockwell (1934), with the omission of Orange G, was used.

Ovular development was observed at meiosis and later stages in flowers fixed on successive days up to 17 days after pollination.

II. *Pollen-tube growth*

(1) *The dissection technique.* The dissection technique followed is essentially that of Buchholz & Blakeslee (1927). Magenta red in an aqueous solution of 0·01 % proved to be a satisfactory stain for the *setchellii* hybrids only, while aceto-carmine was more satisfactory for the 'Acomayo' hybrids.

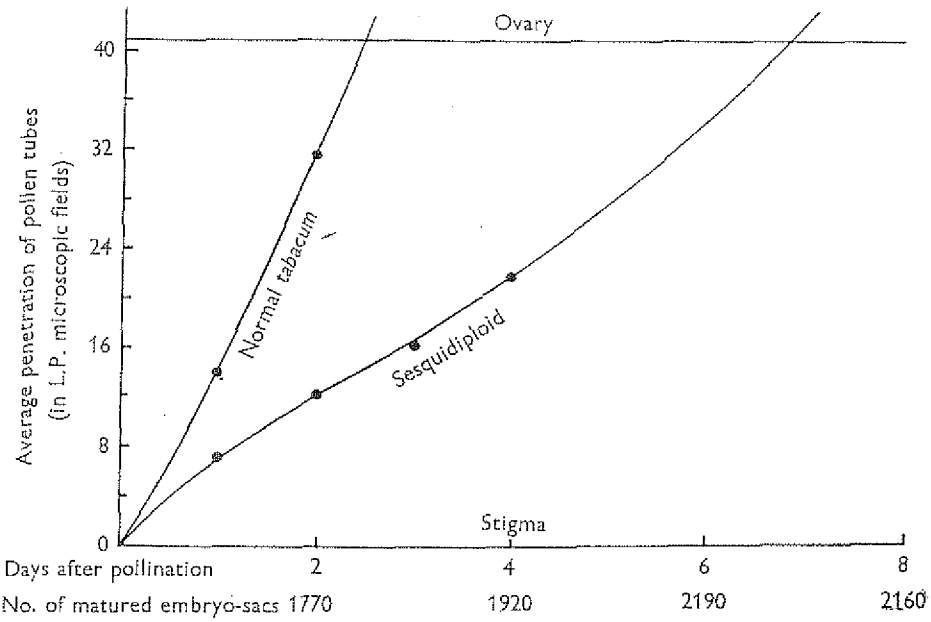
(2) *The radioactive tracer method.* The dissection technique, although quite satisfactory and most informative for pollen-tube studies, besides being rather time-consuming, is difficult, if not impossible, to apply to styles collected more than 6 days after pollination. Consequently, pollen grains labelled with radioactive phosphorus, ^{32}P, were used for pollination, and radio-autographs were made for a study of pollen-tube penetration (Ar-Rushdi, 1955).

EXPERIMENTAL RESULTS

Both sesquidiploid plants obtained from the cross of 4*n* *N. tabacum* by 2*n* *N. setchellii* failed completely to set seed from self-pollination, but they also produced no seeds with pollen from diploid and tetraploid *N. tabacum*, nor with pollen of any other *Nicotiana* species available at that time. Such a failure of seed setting was inexplicable on the basis of meiotic behaviour of the chromosomes, particularly in view of the fact that comparable sesquidiploids, produced from other members of the Tomentosae, are fertile. Moreover, the sterile plants had about 32 % good pollen, about 70 % of their embryo sacs reached maturity, and no difficulty was encountered in securing viable seed from application of their pollen to *N. tabacum*.

Studies of pollen-tube growth revealed a characteristic pattern of retardation. Pollen-tube growth was roughly estimated in a number of styles in intervals of 24, 48, 72 and 96 hr. after pollination. Text-fig. 1 shows the average tube penetration as measured in arbitrary microscopic field units as compared with numbers of available matured embryo-sacs. The abscissa represents the number of matured sacs at 48 hr. intervals computed on the assumption that an ovary contains an average of 3000 ovules, a very conservative estimate. The ordinate represents the average length of the style in the same units used for measuring pollen-tube growth. It is observable by extrapolation, that at the rate of penetration observed in the first 4 days, the tubes should reach the ovary about 7 days after pollination when many receptive embryo-sacs are present. However, no evidence of fertilization was seen in the embryo-sacs studied. This suggests the possibility of a stylar block which results in the failure of tube growth some time after the period of 4 days. It would have been desirable to make more detailed examinations of the styles for longer periods following pollination. However, this was not done on these particular plants as the necessary techniques had not been developed at the time.

Preliminary examination of pollen-tube growth in the sesquidiploid *tabacum-tabacum-tomentosa* 'Acomayo' showed a similar, but stronger, retardation in pollen-tube growth. No examination was made of embryo-sac development.



Text-fig. 1. Comparison of pollen-tube growth in normal *N. tabacum* and in the sesquidiploid hybrid. Graphs are based on average penetrations of five styles for each time interval.

Table 1. *Segregation for fertility in the first backcross generation*

Population no.	Total plants	Sterile plants	Fertile plants	Doubtful plants
49,325	44	32	12	—
49,375	46	39	6	1
49,492	45	30	13	2
Total	135	101	31	3

The first backcross generation

Three populations of the first backcross, *2n N. tabacum* × *3n* (*tabacum-tabacum-setchellii*), were grown in the field in the summer of 1949. Table 1 shows the segregation for sterility in these populations. The frequency of sterile plants was much higher than expected on the basis of segregation of a single dominant gene in a trivalent association with the corresponding recessives. Cytological examination of twelve sterile plants showed that none was reduced to the 24-pair condition. Furthermore, three plants were recognized as being intermediate with regard to sterility. In each, very few ovules had developed in the ovaries of abscissed flowers. The number of enlarged ovules per ovary was between five and nine, which is far below the minimum number (about fifty) required to prevent abscission.

Pollen fertility in one of the intermediate plants proved to be about 10 %. That of five sterile and two fertile plants ranged from 20 to 70 %, the two fertile plants having 42·8 ± 1·12 and 60·3 ± 0·99 % fertile pollen respectively.

The observed frequencies of fertile and sterile plants and the variability in pollen fertility and seed setting are, no doubt, a consequence of segregation in the 'tomentosa' subgenome of the sesquidiploid. A comprehensive understanding of the various nuclear mechanisms involved in bringing about failure of seed setting required a detailed analysis

of individual plants over several generations. However, only three plants were selected in which retardation of pollen-tube growth was evident. These were used as male parents in a second backcross to Red Russian tobacco.

The second and subsequent backcross generations

(1) *Segregation ratio for sterility*

Seven second backcross populations were grown in the summer of 1950. Of these three sterile plants, each with 24 pairs of chromosomes, were selected for subsequent backcrossing; for studies of pollen-tube penetration and embryo-sac development as well as for determining the *tabacum* chromosome associated with the genic material responsible for sterility.

Table 2. *Segregation for fertility in the second, third and fourth backcross generations*

Generation	Total plants	Sterile plants	Fertile plants	Doubtful plants
Second backcross	323	150	168	5
Third backcross	147	67	78	—
Fourth backcross*	492	241	251	—

* Backcrosses made to monosomics.

Table 2 records the pooled segregation frequencies as regards seed setting in each of the backcross generations. All generations were in full agreement with a 1 : 1 ratio for a single genic differential substituted or introgressed into *tabacum*. However, five intermediate plants were observed in the second backcross generation, three of which were examined cytologically and found to have extra chromosomes, suggesting that their intermediacy was due to chromosomal imbalance.

(2) *Embryo-sac development*

The development of embryo-sacs in the 24-paired sterile plants follows the normal pattern described by Fansler (1941) for diploid Red Russian tobacco. Meiosis observed in a few E.M.C.'s appeared normal, and with no laggards. The enlargement of the chalazal megaspore to form the single nucleate embryo-sac coincides with the degeneration of the three micropylar cells occurring progressively from the uppermost downwards. This is accompanied by the degeneration of the nucellar tissue which continues until about the four-nucleate stage. The sac elongates and the first nuclear division takes place. This is followed by further enlargement of the embryo-sac in all dimensions, and the occurrence of the second nuclear division at a varying angle to the long axis of the embryo-sac. The third division takes place after still further enlargement of the embryo-sac and the resulting eight nuclei become the egg apparatus, the polar nuclei and the antipodals.

No evidence of fertilization, embryo or endosperm development was seen even as late as 14 days after pollination. Nor were any pollen-tube contents observed in or about the embryo-sacs.

Ovule abortion was very rarely observed in this material as compared to 2% abortion reported by Fansler (1941) in normal *tabacum*. Furthermore, due to lack of fertilization, it was possible to study changes in ovaries which were unfertilized for a period as long as 14 days. Lack of fertilization and ageing do not change the ovules except for a frequent degeneration of the antipodals or their less frequent enlargement by vacuolation. Occasional

fusion of the polar nuclei was also observed. Similar effects were seen in normal *tabacum* which was emasculated, protected from pollination and fixed after a comparable time.

Embryo-sac development was followed in the first backcross of $2n$ *N. tabacum* $\times 3n$ (*tabacum-tabacum-tomentosa* 'Acomayo'). As in the above *setchellii* hybrid development was completely normal.

(3) *Pollen-tube growth*

Text-fig. 2 represents free-hand sketches depicting penetration of pollen tubes as observed by the dissection method at different time intervals. Frequencies are recorded in terms of tube ends per unit microscope field (magnification 150 ×). Counts are recorded from the stigmatic end, to the left of the figures, down to the ovular end, to the right of the figures. Total numbers of tube ends are plotted above the datum line, the proportion with swollen or burst ends (Pl. 1) below the line. No attempt was made to record ungerminated pollen. Accurate counts at the region immediately below the stigma were impossible to obtain.

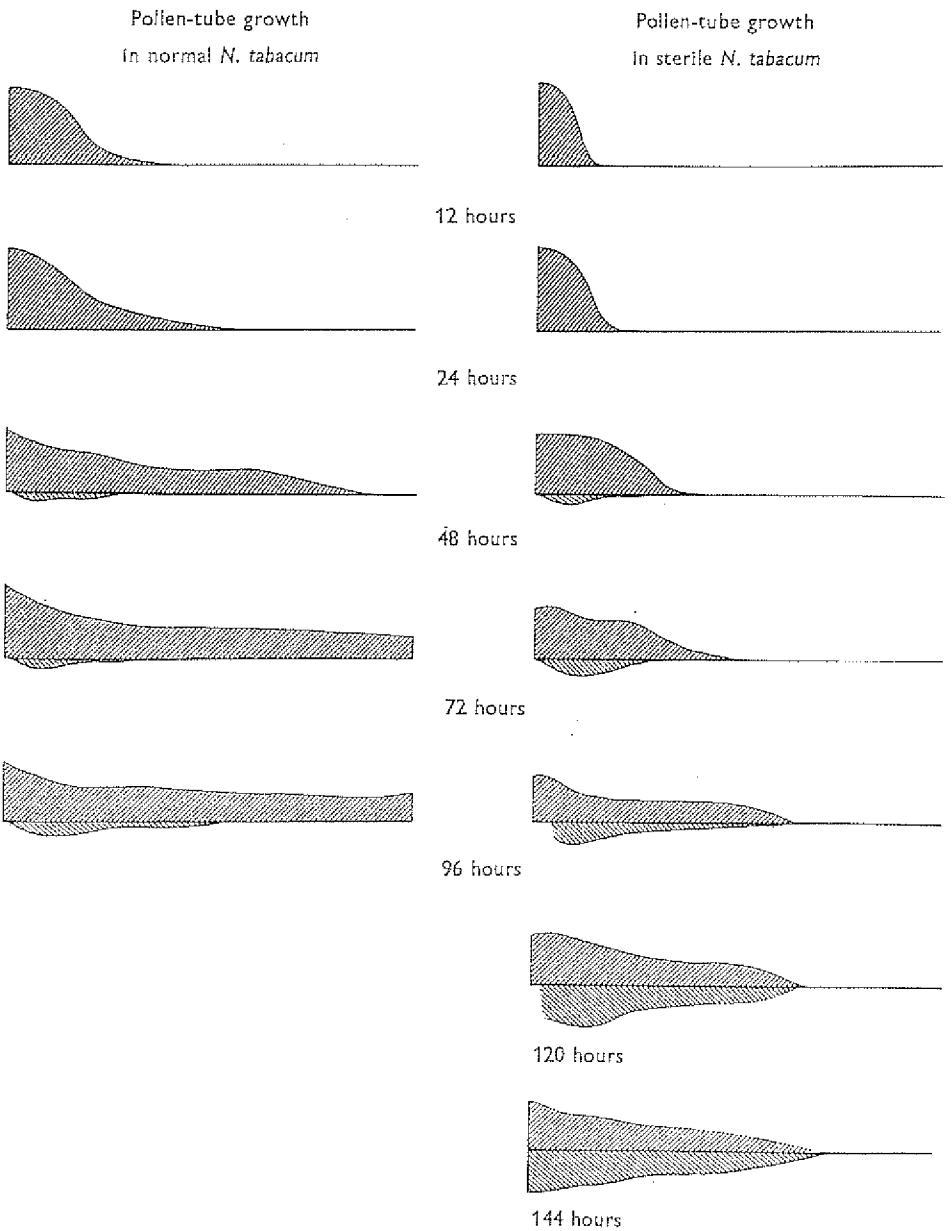
It is readily evident from the figures that on the average, pollen-tube ends in fertile diploid plants reach the ovary in slightly over 48 hr. with a mode near the advancing front. Tube ends much behind the mode may represent pollen grains that were slower in germination, possibly due to the lack of good contact with the stigma, or may represent retarded growth due to crowding. The distribution of abnormal tube ends and their restriction to the stigmatic half of the style together with the increase in their frequency with time in these fertile plants suggest that bursting may well be due to stagnation, presumably due to crowding in this case. That such bursting is due to differences in the nuclear or the cytoplasmic constitution of the pollen (Buchholz & Blakeslee, 1929) is unlikely in a highly inbred line such as Red Russian. The low frequency of such tubes renders such an explanation less tenable.

A comparison of the pollen-tube growth in the sterile plants with that in the fertile ones reveals at once a pronounced retardation in the rate of growth. This is associated with the bursting of the tubes and the ultimate failure of transmission of sperm nuclei to the ovules.

The phenomena of pollen-tube swelling and bursting have been observed in *Datura* by Buchholz & Blakeslee (1936). Their extensive studies revealed two major conditions giving rise to such abnormalities. One is that associated with the presence of certain genes and has been demonstrated for the gene tricarpel (Buchholz & Blakeslee, 1927). This recessive gene causes a bursting of part of the pollen tubes. The other condition is the growth of otherwise normal gametophytes in styles presumably unsuitable for normal pollen-tube growth, due to quantitative chromosomal relationships as illustrated by the crosses $2n \times 4n$, $3n \times 4n$, $n \times 4n$, and $2n \times 2n+1$, or in certain interspecific hybrids where pollen-tube growth rate was observed to be very slow. The observations made in the present study indicate that bursting is a consequence of slow rate of growth. This may be true for some of the above results of Buchholz & Blakeslee. Yet, it does not account for all their observations, especially in those cases where a mutation was responsible for the abnormality. This point is illustrated by their work on five radium-induced mutants where two (sb-1 and s-2) were characterized by slow growth as well as bursting, two (s-1 and s-2) with very slow growth of tubes without bursting and a fifth (1-p) in which pollen grains failed to germinate but became lobed at the germ pores.

In contrast to the known cases of incompatibility where specific responses of the game-

tophyte or sporophytes involved in a cross is demonstrated, the crossability barrier reported here involves no specificity of reaction of any kind, and the effect is quite general in the sense that pollen of any genetic constitution fails to effect fertilization. This is true even of pollen from species normally crossable with *tabacum* and with species of the Tomentosae. This behaviour indicates a physiological condition in these variants in which a fundamental process is impaired; a condition also suggested by observations on grafting.



Text-fig. 2. Graphic representation of pollen-tube penetration (dissection method). Graphs are based on average counts of tube ends in 6–15 styles for each time interval.

(4) *Grafting*

Grafting floral branches of sterile plants on stocks of fertile plants resulted in the setting of viable seeds consistently. The reciprocal graft had no adverse effect on the fertility of the scion. Furthermore, grafts of the mutual type rendered the sterile partner fairly fertile. Control grafts of sterile scions on sterile stocks did not change sterility. These observations together with those on crossability, point to a lack in sterile plants of the production of a substance essential for the normal growth of pollen tubes in general. The chemical identification of such a substance should be, in comparison to incompatibility, a simple procedure.

(5) *Bud pollination*

Extensive bud pollination on sterile plants over a period of two flowering cycles gave one capsule of seeds from over 200 pollinations. The capsule was the result of selfing. Its occurrence is felt to be a rare event under specific environmental conditions.

Other trials, such as stylar grafting, decapitation of styles, and pollination with the use of stigmatic squashes, failed to give any positive results.

(6) *Pollen dimorphism*

Microscopic examination of pollen from plants of the sterile type revealed a percentage of abortive pollen comparable to that found in normal *N. tabacum* (Table 3). However, among the stainable grains there were two distinct size classes, normal-sized grains with a mean diameter of about 35 μ and smaller ones with a mean diameter of 22·5 μ (Text-fig. 3). No such dimorphism was observed in the stainable pollen of fertile sibs. The phenomenon is apparently associated with the introduction of alien chromosomal material. It has not been established whether the dimorphism results from a pleiotropic effect of the sterility gene or whether it is the result of some other cause. It is not known whether the small grains are able to germinate and effect fertilization. At any rate the extensive backcross data indicate that there is no direct relation between frequency of occurrence of small grains and transmission ratios of sterile *v.* normal plants.

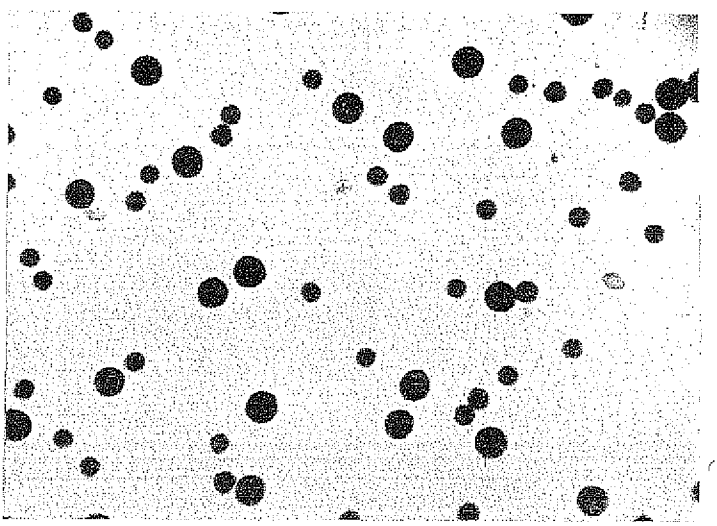
Genetic nature of sterility

Sesquidiploids obtained from tetraploid Red Russian tobacco pollinated by species of the Tomentosae other than *N. setchellii* and *N. tomentosa* 'Acomayo' are fairly fertile. The parental species in all the above-mentioned crosses, excepting 'Acomayo', are self-fertile. Hybrids of *4n N. sylvestris* × *2n N. setchellii*, are also completely sterile. The sterility in all cases mentioned above was brought about by pollen-tube retardation. It was accordingly concluded that a dominant complementary action of the nuclear constituents of the species involved was responsible for producing sterility. The consistent backcross ratios obtained over three generations from obligate heterozygotes indicates the segregation of a single genic differential for pollen-tube retardation, a tube retarder (Tr^1), in the *tomentosa* subgenome. The gene was introduced into *tabacum* from *setchellii* by backcrossing and selection for two generations. The *sylvestris* subgenome is regarded as being the contributor of the other complementary genic material and as being homozygous for it. As a consequence of this homozygosis, the determination of the exact nature of this genic material was not possible. A survey of the other *tabacum* varieties for a 'non-tube-retarding'

hybrid with *N. setchellii* and a cross of such a variety with Red Russian might furnish the heterozygosity necessary for determining the number of genes in the *sylvestris* subgenome complementary in action to the gene found in *N. setchellii*. A scheme has been suggested (Ar-Rushdi, 1953) which examines the hypothesis of a single dominant complementary gene residing in the *sylvestris* subgenome.

Table 3. *Pollen dimorphism in female-sterile plants*

Plant no.	No. of flowers examined	Pollen grains counted	Percentage normal	Percentage small	Percentage abortive
52398p1	3	1639	55·82	37·27	6·89
399p10	2	1272	59·19	33·25	7·54
399p18	2	1777	58·58	31·90	9·51
612p1	3	2489	54·27	39·33	6·38
612p4	3	2275	57·40	35·56	7·03
612p9	3	1947	54·39	42·93	2·67
613p2	3	2013	57·12	35·56	7·30
613p5	3	2556	57·23	38·92	3·83
613p6	3	2812	56·57	40·32	3·09



Text-fig. 3. Photomicrograph of pollen from one of the sterile plants showing the marked dimorphism of stainable grains.

Thus, if Red Russian tobacco is designated as $tr^1/tr^1\ Tr^2/Tr^2$, *N. setchellii* as Tr^1/Tr^1 and the sterile sesquidiploid as $Tr^1/tr^1/tr^1\ Tr^2/Tr^2$, then the selected sterile diploid plants must be of the constitution $Tr^1/tr^1\ Tr^2/Tr^2$, giving, when used as pollen parents on Red Russian tobacco, the observed ratio of 1 sterile to 1 fertile.

Though the effects of nuclear differentials for sterility were observed, the role of the cytoplasm in conjunction with the nuclear genes has not been studied, as all the crosses planned involving *N. setchellii* cytoplasm were unsuccessful. A number of male-sterile cases have been found to depend in part on cytoplasmic interaction as reported in *Linum* (Bateson & Gairdner, 1921; Chittenden, 1927), *Nicotiana* (East, 1932; Clayton, 1951; Clausen, unpublished), *Epilobium* (Lehmann, 1939) and *Allium* (Jones & Clarke, 1943).

The association of Tr^1 *with the 'L' chromosome*

The location of *Tr*^{1} in a specific chromosome was accomplished by application of monosomic analysis (Clausen & Cameron, 1944). The procedure was to cross sterile plants as males to the twelve *tomentosa* monosomics whereupon the expected results would be:

P_1 Haplo-X fertile × diplo-X sterile
F_1 Haplo-X fertile
Haplo-X sterile
Diplo-X fertile
Diplo-X sterile

The ratio of the monosomics to the disomics depends on the ovular transmission of the monosomic type involved. The ratio of sterile plants within each chromosomal group should be equal to the fertile plants as evidenced by the backcross ratios.

The selection of a haplo-X sterile plant and its subsequent crossing as a male to a diplo-X normal should give progeny consisting of fertile and sterile diploid plants, in equal proportions, whenever the monosome is not associated with the *Tr*^{1} gene:

Diplo-X fertile × Haplo-X sterile
Diplo-X fertile
Diplo-X sterile

The progeny of the same cross using the monosome associated with *Tr*^{1} locus would all be sterile:

Diplo-X fertile × Haplo-X sterile
Diplo-X sterile

In both cases no monosomic offspring are expected as there is practically no pollen transmission of *n*-1 gametes.

Table 4 gives the results obtained from such crosses with haplo-L. Thus *Tr*^{1} is shown to be located in chromosome L.

Table 4. *Data on the association of* Tr^1 *with the 'L' chromosome*

Haplo-L normal × Diplo-L sterile

Population no.	Haplo-L		Diplo-L	
	Sterile	Fertile	Sterile	Fertile
51,412	3	3	12	21
52,400	4	2	23	17
Totals	7	5	35	38

Diplo-L normal × Haplo-L sterile

Population no.	Total plants	Diplo-L fertile	Diplo-L sterile
52,398	38	0	38
399	38	0	38
612	12	0	12
613	11	0	11
Totals	99	0	99

DISCUSSION

The separation of hybrid sterility phenomena into genic and chromosomal types was first suggested by Dobzhansky (1933, 1936). His studies of sterility in the hybrid *Drosophila pseudoobscura* × *D. persimilis* (1936) represented the first fully analysed example of genic sterility in animals, gonad development being affected by sterility genes present throughout the genetic system of both species. In plants, the first clear example was studied by Greenleaf (1941, 1942). This investigator showed that complementary sterility genes are present in *Nicotiana sylvestris* on the one hand and *N. tomentosiformis* and *N. tomentosa* on the other. The genes in question resulted in the arrest of embryo-sac development in the two- or four-nucleate stages. Other examples of genic sterility are reported in *Aegilops umbellata* × *Haynaldia villosa* allopolyploids (Sears, 1941), where a high degree of asynapsis or desynapsis with sterility in respect both to pollen and to seed was observed. Love & Suneson (1945) found a similar asynaptic mechanism underlying the sterility in *Triticum-Agropyron* allopolyploids. A few other such cases, not so fully analysed, are known in plants and have been cited by Stebbins (1950). It appears then that the types of genic sterility hitherto examined are connected with gene complexes acting specifically during the development of the gonads, at the time of meiosis, or during the later developmental stages of the gametophytes or gametes. In the type reported here a still later stage in the life cycle is affected. Sterility is manifested in physiological inability of functional male and female gametes to unite during an otherwise normal course of events, as a consequence of which it prevents the maintenance of the amphidiploid condition. Yet, the association of such an effective barrier, here as well as in the other instances of genic sterility observed, with a pronounced and effective chromosomal barrier raises a question as to the significance of genic sterility as an independent isolating mechanism. The fact that the cases of genic sterility observed are relatively few, that they are invariably associated with differentiations and alterations of chromosomes, and that chromosomal barriers are comparatively universal as internal barriers (at least in plants where hybridity is extensively analysed) suggests that genic isolation may not be an independent event but rather a consequence of chromosomal divergence—somewhat in line with Muller's (1940, 1942) views of 'position-effect'.

In a discussion of the mode of origin of *N. tabacum* in the light of its genetic relations to the species of the Tomentosae, it is necessary to consider two relevant problems. One is the assumption that the observable differences between the raw amphidiploid and present-day tobacco have not occurred in the progenitor species subsequent to the establishment of the amphidiploid condition. Such an assumption, though extremely difficult to investigate experimentally, is nevertheless broadly acceptable in view of the relatively recent origin of cultivated tobacco. The second is a consideration of the trend and nature of genetic alterations which have occurred in the cultivated tobacco since its origin as an amphidiploid. This is significant in relation to the feasibility of drawing conclusions regarding the *sylvestris* subgenome of *N. tabacum*. Difficult as this task is, experimental evidence regarding such transformational changes have been presented (Clausen, 1941), and it was concluded that modification of the raw amphidiploid has been most likely through gene mutation and mainly from the dominant to the recessive. It is interesting to observe in this connexion that the sterility system found in the amphidiploid *N. sylvestris-N. tomentosiformis* is recessive to the fertility of *tabacum*. It could then be said that,

as far as the analogy between *N. tabacum* subgenomes and their ancestral genomes are concerned, no pronounced changes, aside from the trend shown above, have occurred. Consequently, conclusions drawn from the genetic behaviour of *N. tabacum* can be extended to the raw amphidiploid.

Though simplification through mutation seems to account for the change in some of the duplicate gene systems in *N. tabacum*, it does not account for all such conditions apparently. In some cases it would be appropriate to consider variation in the progenitor species and hybridization of their recombination products as an alternative mechanism. This is especially true for the dominant complementary sterility genes reported here. Its adoption would aid in resolving the mode of origin of *N. tabacum* and the complexities confronted in selecting any one species of the Tomentosae as an ancestor. It would comply with the restrictions imposed on the possibilities of origin of *N. tabacum* by the different genic sterility systems hitherto examined.

N. otophora is readily excluded on morphological grounds as a possible progenitor despite the fertility of its amphidiploid with *N. sylvestris* and the overlapping of their geographical distributions. With the existence of two independent genic sterility systems, one recessive (*N. tomentosiformis* and some forms of *N. tomentosa*) and one dominant (*N. setchellii* and other forms of *N. tomentosa*), it would seem plausible to conclude that *N. tabacum* originated by the doubling of the chromosomes of an F_1 hybrid involving *N. sylvestris* and a recombination product which was free from both sterility genes, from a cross between two sterility types in the Tomentosae. A limited attempt was made to verify this hypothesis experimentally. Tetraploid *N. sylvestris* was crossed to an F_1 (*N. setchellii* × *N. tomentosiformis*) with the hope that some fertile segregants would result. However, only small populations were obtained and seed germination was not good, so that no results can be reported until further trials are made.

The author is fully aware that some of the assumptions made in developing the background for the above hypothesis are founded on indirect evidence. However, it is hoped that sufficient evidence has been adduced, pending further observations on additional collections of species and forms of Tomentosae species and of *N. sylvestris*, to show that the genic restrictions discussed are important for the proper understanding of the dynamics of the origin of *N. tabacum*.

Summary

1. The sesquidiploids *N. tabacum-tabacum-setchellii* and *N. tabacum-tabacum-tomentosa* 'Acomayo', despite having normal embryo-sacs and good pollen, were completely sterile in terms of seed setting.

2. Sterility was found to be due, primarily, to a retarded rate of pollen-tube penetration which resulted in bursting of tube-ends and their ultimate failure to reach the ovary.

3. A single dominant complementary gene (Tr^1) was found in *N. setchellii* which was responsible for the sterility. The other complementary locus is present in the *sylvestris* subgenome of *N. tabacum* as well as in *N. sylvestris* in the homozygous dominant condition.

4. 'Tr^1' was introduced into *N. tabacum* by a series of backcrosses and was located in the 'L' chromosome. Its introduction was found to be associated with pollen dimorphism.

5. The significance of the two systems of genic sterility hitherto found in the progenitors of *N. tabacum* and the restrictions imposed by them on the origin and establishment of *N. tabacum* were discussed. It was concluded that *N. tabacum* arose as an

amphidiploid involving *N. sylvestris* and a recombination product which was free from both genic sterility systems, obtained from a cross between two sterility types in the Tomentosae.

These investigations were made in the laboratories of the Department of Genetics at the University of California. They were facilitated by a scholarship from the Government of Iraq.

The writer wishes to express his indebtedness to Prof. R. E. Clausen, who suggested the problem and provided the material for its execution. His continuous advice and criticisms were essential in the conduct of the study.

Thanks and gratitude are due to Dr D. R. Cameron for his personal supervision throughout the investigation and during the preparation of the manuscript.

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EXPLANATION OF PLATE I

Photomicrographs of pollen-tubes studied by the dissection method

(*a*) Normal pollen tubes. ×120.

(*b*) Three stages leading toward the bursting of pollen-tube ends. ×400.

(*c*) A burst pollen-tube end. ×400.

(*d*) Normal pollen-tube ends at the ovular end of a style in normal *N. tabacum*. One pollen tube entering an ovule.

(*e*) Burst pollen-tube ends in a style of a sterile plant 144 hr. after pollination. ×120.