CYTOLOGICAL STUDIES IN THE GENUS NICOTIANA

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INTRODUCTION

The genus Nicotiana has been a favorite subject for study since the time of KÖLREUTER, his investigation of crosses between *N. paniculata* and *N. rustica* being the first genetical research of which we have a record. Unfortunately, the work of the hybridizers of the eighteenth and nineteenth centuries produced few generalizations of value to genetics, because of the numerous variables under consideration. The foundations of genetics have been laid by experiments where a limited number of genetic differences have been followed and where the fertility of the hybrids was virtually perfect. More recently, however, there has been a trend back to the study of species hybrids, using both the cytological and the pedigree culture methods of technique. The work of SAX on the genus Triticum is an excellent example. Using the general philosophy of genetics built up by intra-specific studies as a basis of his inductions, SAX has been able to throw considerable light , not only upon the genetic behavior of the species composing this genus, but also upon their origin. It was

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thought, therefore, that perhaps a similar series of investigations upon the genus Nicotiana might yield results of some value. There was the further hope that the genus might again be brought to the favorable attention of geneticists. It deserves a high place in their regard, for the plants are easy to grow, crosses are easy to make, large quantities of seed are produced, and the seed retains its viability for several years.

Since the genus is fairly large (about 60 species having been described) it has been impossible for me to make even a relatively complete survey. I have been able to obtain 19 species. In each of these forms, the chromosome number has been determined. In addition, 15 species were selected for hybridization work, and an effort made to cross each of these species with all of the others. In the case of nine hybrids, the cytological behavior of the F_1 plants was investigated.

The species used correspond with the descriptions found in COMES' Monographie du genre Nicotiana (1899), with one or two exceptions. These exceptions are species described since the appearance of this work. Their descriptions can be found in the original publications cited in the bibliography.

METHOD AND TECHNIQUE

The haploid chromosome number in each species was determined both from aceto-carmine preparation made according to Bellings' method and also from permanent slides. A very small part of the bud was cut away at the tip so that a part of each anther could be examined by the aceto-carmine method. The rest was immediately fixed with modified Bouin's solution as developed by ALLEN. Sections were cut 7 to 10 microns thick, and were stained with Haidenhain's iron-hæmatoxylin.

The diploid chromosome numbers of the pure species, and in a few cases of the dwarf species hybrids, were determined by a study of the root tips at a time when the plant, grown in sterile soil, had produced its 6th or 7th leaf. The fixative in this case was strong Flemming's solution, the stain being again Haidenhain's iron-hæmatoxylin.

In the hybridization work, 3 or 4 plants of both species were always used. The parent plants were kept together, but isolated as far as possible from the plants of the other species. The hybridization was made in the greenhouse, and in order to prevent contamination, all buds which could not be used for hybridization were cut off at a very early stage. The flowers were not bagged. Flying insects were excluded from the house by cheesecloth covers; and experiments have shown that with plants having heavy pollen, as is the case with all Nicotiana species, this is a sufficient

precaution. Crosses and reciprocals were made in each case using five buds of every combination. The seeds were always grown in sterilized soil.

THE CHROMOSOMES OF THE VARIOUS NICOTIANA SPECIES

Tabacum section

1. *N. Tabacum* L. Within this species I have made cytological studies of the following varieties: Cuba, *macrophylla*, and a white flowering tobacco. According to COMES, Cuba belongs to the var. *havanensis*. It has lanceolate leaves and pink flowers. *Macrophylla* is a very strong plant with large ovate leaves gradually tapering to a broad clasping base, with rounded but not prominent basal lobes. The flowers are deep red with a stout tube and an abruptly swollen, broad infundibulum; the limb is almost pentagonal, and at the base of every lobe is a whitish, triangular spot. The white tobacco is a small variety bearing a large number of small lanceolate and broadly decurrent leaves tapering towards the base on a relatively short stem. The corolla is relatively small, short, and cream colored.

In each of these three varieties, I found 24 chromosomes in the pollen mother cells during the different stages of the reduction division. The number was very easy to count at the second metaphase or at the first anaphase because the chromosomes at these stages, though small, are clearly separated. Sometimes the first metaphase (figure 19) is exceptionally clear and favorable for the study of chromosome size. At this stage the chromosomes are twice as large as in the second metaphase and lie close together. Figure 19 shows clearly the differences in the chromosome size. The small chromosomes are arranged in the center, the larger chromosomes at the periphery.

There is great difference in the chromosome shape at meiosis and at mitosis. In the cells of the root tips the chromosomes are long and slender, usually curled, and difficult to count. In figure 1 is represented one of the root tip cells of *N. Tabacum* var. *macrophylla* in which it is relatively easy to distinguish 48 chromosomes.

Rustica section

2. *N. rustica* L. In this species I have studied the varieties *humilis*, *brasilia*, and one which is apparently *texana*. The 24 haploid chromosomes of the pollen mother cells, in contrast to those of *Tabacum*, are uniform in size and shape. Their shape is markedly different from that of the *Ta-*

bacum chromosomes, being bean-shaped instead of spherical (figure 18). During the second metaphase they are to be seen clearly because of their separation, but are so small that their shape is vague, and they appear to be spherical.

The somatic chromosomes, though somewhat larger than those of *Tabacum*, are entwined and difficult to distinguish. It was impossible to make counts on preparations of root tips from the small *humilis* variety because the cells are very small and the chromosmes much entwined. Only in *brasilia* was I able to find clear plates (figure 2) where the chromosomes are manifestly 48.

3. *N. paniculata* L. The material used had all of the characteristics of true *N. paniculata*—a long, slender corolla tube with short, symmetrical, greenish corolla lobes. The upper leaves were lanceolate, the basal leaves were ovate, slightly cordate, and with long petioles.

The 12 chromosomes counted during different stages of the reduction division in the pollen mother cells (figure 25) seem to be identical in shape with those of *N. rustica*. In the cells of the root tips the chromosmes are very slender, long, and twisted; but 24 are easily distinguished (figure 8).

4. *N. glutinosa* L. The material used in this study was a small branched plant with small, cordate, long petioled, extremely glandular leaves. The flowers were short and cylindrical below, but suddenly swollen above into an irregular, obliquely one-sided funnel. The limb was bilabiate, the stigma and anthers being connivent just under the middle lobe of the upper lip. The color was light yellow tinged with red.

Twelve chromosomes were counted in the different stages of the pollen mother cells. The chromosomes are uniform in size and shape, the spherical shape giving them a greater resemblance to those of *N. Tabacum* than to the bean-shaped chromosomes of *paniculata* or *rustica*. They are only one-half as large as those of *paniculata*. During the first metaphase they lie very close together (figure 26), but are very clear and distinct in the first anaphase. The differences between the chromosomes of *glutinosa* and *paniculata* are visible also in the somatic cells, those of the former (figure 6) being shorter and slenderer than the latter.

5. *N. glauca* Grah. This species is a tree tobacco with long, petioled, glaucous leaves. The flowers are pale yellow with tubes similar to those of *paniculata*. The stem and leaves are bluish green. The chromosome number was found to be 12 in the pollen mother cells (figure 23) and 24 in the root tips (figure 9). The somatic chromosomes are extremely large, long, and entwined, but because of the large cells and nuclei they are well scattered and therefore are easily distinguished.

6. *N. tomentosa* R. et. P. The two year old plants are not yet in flower, but their general character shows them to be true *N. tomentosa* (*Lehmannia tomentosa* Spr.). The chromosome number was counted in the cells of the root tips and found to be 24 (figure 10).

Petunioides section

7. *N. Bigelovii* Wats. According to SETCHELL (1912) different varieties of *N. Bigelovii* exist. The plants used were small, their first leaves being slightly decurrent, nearly petioled, in fact, the later leaves becoming characteristically truncate, auriculate, and partly clasping at the base. The first leaves were more or less elongate deltoid, the later leaves more lanceolate. The corolla tube was narrow, and the corolla limb deeply divided into long pointed lobes. The corolla was pure white. Chromosome counts were made most easily during the first and second metaphases. During the first metaphase (figure 16) the chromosomes appear as bean-shaped objects about equal in size but not quite so large as those of *N. rustica* and *N. Tabacum*. In the root tips 48 chromosomes were counted (figure 3).

8. *N. viscosa* Lehm. (?). This species was raised from seeds obtained from a Gray Herbarium sheet labeled *N. viscosa*. At the same time I obtained seeds from another Gray Herbarium sheet labeled *N. attenuata*. Both groups of plants had the same morphological characters. Both lots may be *N. attenuata*, but I have labeled them *N. viscosa* (?) because I raised other specimens received from Dr. T. H. GOODSPEED as *N. attenuata* which differed somewhat from the first two lots. The latter group of plants was rather similar to *N. acuminata*, but very much smaller. These specimens, in fact, seemed to be much reduced acuminatas, and therefore correspond more closely to the taxonomic description of *N. atenuata*. I am not certain of the identification, however, for these species are quite similar to each other, and perhaps should be grouped together.

The pollen mother cells are nearly as large as those in *macrophylla*. The chromosomes also are quite large, lying close together during the first metaphase (figure 20) and more distant during the second. During the first and second metaphases as during the first anaphase I counted 24 chromosomes.

9. *N. nudicaulis* Wats. *N. nudicaulis* is a very small plant with dark green ovate leaves narrowly decurrent. The corolla is shorter and broader than in *N. trigonophylla*. The tube is somewhat brownish, the limb cream. The corolla lobes overlap.

The chromosomes of the pollen mother cells are not easy to count at any stage of the reduction division, being small and usually rather dumb-

bell shaped. In the very early first metaphase the chromosomes are somewhat more spherical, however, and 24 could be distinguished (figure 21). In the root tips, it is relatively easy to count 48 chromosomes, the chromosomes being slender and well separated (figure 4).

10. *N. suaveolens* Lehm. The plants used were branched, with the lower leaves ovate, sessile, clasping at the base and pointed at the tip. The upper leaves were sharply lanceolate and clasping. The corollas were ivory white, salver shaped and with long slender, non-infundibular tubes. The corolla limb is large with broad, shallow rounded lobes.

During the first metaphase of the pollen mother cells, the 16 chromosomes always lie close together (figure 22). They are most easily counted in the first anaphase or in the second metaphase. Cells were found with both plates of a first anaphase lying in the same section in such a manner that by focussing 16 chromosomes can be counted in each. In the first anaphase, the chromosomes are seen as curved rods or as spheres depending on their position. In both the first and the second metaphases they are more or less spherical. During the second metaphase they are more separated and are easy to count. The chromosomes in this species are relatively very small though the cells in comparison with those of the other species are very large. In the cells of the root tips 32 chromosomes could be counted. The chromosomes here are rather slender, comparatively short, and often U-shaped (figure 5).

11. *N. sylvestris* Speg. *et* Comes. In general habit as in leaf shape this species of the Petunioides section is very similar to certain *Tabacum* varieties. Its corolla is white, and very long and slender, the tube being inflated in the middle. The corolla limb consists of 5 broadly triangular lobes. The flowers ordinarily droop.

In the pollen mother cells during the heterotypic metaphase 12 spherical chromosomes, closely arranged at the plate, were counted (figure 24). The first anaphase and the second metaphase are the best for making counts, the 12 chromosomes then lying fairly distant from each other. In the cells of the root tip preparations the chromosomes are long, U-shaped, quite large, and spread out, so that it is easy to distinguish 24 chromosomes (figure 7).

12. *N. acuminata* Grah. This is a branched plant with petioled leaves, cordate below and lanceolate above. The coarse, spine-like glandular hairs on the surface and on the margin are characteristic. The corolla is white with a cylindrical tube and shallow, rounded lobes on the limb.

Twelve chromosomes were counted during the different stages of the reduction division of the pollen mother cells (figure 27). It is easier to

count them at the second metaphase or the first anaphase. The chromosomes after the second division are long and U-shaped, somewhat similar to those of the somatic cells, though during the other stages they are almost spherical. In some other plants this is a common phenomenon; but in the rest of the Nicotiana species it apparently does not occur, the chromosomes after the second division ordinarily resembling those of the earlier stages.

13. *N. trigonophylla* Dun. This is a very small plant with broadly lanceolate leaves, narrow at the base and then expanded into broad, partly clasping auricles. The corolla is very small with a short, cylindrical tube. The corolla limb consists of five broad, obtuse, and shallow lobes. The flowers are a deep cream tinged with green.

In all stages of the reduction division in the pollen mother cells 12 chromosomes were found (figure 28). The chromosomes were all of about the same size, spherical, and distinctly spread, especially during the second metaphase. In the somatic cells of the root tip they are easily distinguished as 24 relatively straight and slender chromosomes (figure 11).

Seeds from a specimen at the Gray Herbarium were obtained under the name of *N. Palmeri* A. Gray. Plants raised from them exhibited no particular differences from *N. trigonophylla*, except that the corolla was lighter and the leaves more lanceolate than those of my *trigonophylla* plants. Twelve chromosomes were counted in the different stages of the pollen mother cells (figure 29). Permanent slides of the young root tips show 24 nicely spaced chromosomes (figure 12).

14. *N. longiflora* Cav. This is quite a tall plant with broad, lanceolate or oblanceolate leaves, coarsely bullate and rugose above, the epidermis smooth except for coarse, spine-like, glandular hairs on the surface and margins. It early develops a characteristically compact rosette of large, coarse leaves which lie flat on the ground, the rosette persisting for a considerable time before the flowering stalk develops. The corolla tube is extremely long and slender. The broad, spreading limb is deeply divided into five moderately broad, blunt-pointed lobes. During the first metaphase (figure 30) and the first anaphase I counted 10 chromosomes.

15. From *N. plumbaginifolia* Viv., a species closely related to *N. longiflora*, I have obtained good preparations of the root tips in which 20 well separated chromosomes of different shapes and sizes could be clearly distinguished (figure 13).

16. *N. alata* Lk. et Otto. The plants which I have investigated came from commercial seed, and showed great variability. Concerning their origin I know nothing. They may have been segregates from

crosses between *N. alata* and *N. Forgetiana*, since it is supposed that the different colored varieties of *N. Sanderae* have originated by crossing *N. alata* with *N. Forgetiana* (HEMSLEY 1905). Some of these plants, however, seem to be the pure *alata* type. I have investigated the pollen mother cells of about 25 plants differing in habit, shape and corolla color, making both acetocarmine preparations and permanent slides. In some plants I found 8 chromosomes in all of the stages. In other plants I obtained irregular results. It was doubtful whether the number was 8 or 9, because one of the chromosomal masses could be considered as two small chromosomes lying close together, or as end portions of a single chromosome. Since I found the somatic chromosomes to be very different in shape and size, I am inclined to accept the second hypothesis. In the cells of the root tips I could distinguish 16 chromosomes; one pair very long and S-shaped, one pair long and straight, 4 pairs U-shaped and of different sizes, one pair very short and rod-shaped, and a small pair almost spherical (figure 14). Since the difference in size is so extreme in the somatic cells, the relative size proportions ought not to disappear in the pollen mother cells even though the contraction of the chromosomes there is very great. Some spindles of the first metaphase show clearly that the chromosomes are mostly long and dumb-bell-shaped, though in polar view they appear almost spherical. I am inclined to believe, therefore, that the position of some of the 4 U-shaped chromosomes may be such as to present only the tips which could appear as separate chromosomes lying close together.

It may be possible, also, that this occurrence is due to an earlier split of some of the chromosomes as described by WINGE (1917) for *Chelidonium majus* and *C. majus* var. *laciniatum*. WINGE explained BÖNICKE's determination of the chromosome number in *Chelidonium majus* as eight instead of six by the observation that the chromosomes in the pollen mother cells of this species have a marked power of precocious division. He found that "in the early anaphase, the six chromosomes moving towards the pole are not only divided into two, but into four, thus resembling a *Sarcina*, and, indeed, it would seem as if still finer subdivisions were present, which renders it difficult to ascertain the true number of chromosomes." The splitting phenomenon he found to be still more frequent in var. *laciniatun* than in the typical *Chelidonium majus*, and concluded: "The fact is of considerable interest from a theoretical point of view, as the distinct division in the heterotypic anaphase shows that the chromosome must not be regarded as an indivisible unit, but as a complexity, in which an inner differentiation or duality is already present. And in all probability,

it is also actually existent even when no outward and visible sign of division is discernible."

From the many plants in which I have studied chromosome number in the pollen mother cells I shall present some figures drawn from temporary slides made by the acetocarmine method. By this method I was able to distinguish the rod shape of the chromosomes not only in side view on the spindles, but also in polar view, for the acetocarmine fluid included between the slide and the cover glass allowed the chromosomes to move slightly and to take different positions during the observation. In cases in which questionable numbers were found, the final conclusion was drawn only after exact observations of the questionable chromosomes in different positions. In figure 34, one plate of the second metaphase was in polar view, and appeared to have 9 chromosomes; the other was not exactly in polar view, and only 7 chromosomes could be counted with certainty. After standing, however, the position of the material was changed, the plate with the 7 chromosomes appearing as a regular spindle in which the chromosomes were more or less indistinguishable, and the other plate showing clearly 8 chromosomes.

In all of the other cells counted the number of the chromosomes was always 8 (as in figure 35). Figure 36 shows the shape of the chromosomes of the first metaphase in side view. In figure 32 all chromosomes clearly show splitting, and are similar in shape to those in figure 21*b*, *d*, and *h* presented by WINGE for *Chelidonium majus*. Figure 33 is very similar to figure 21*i* of WINGE. The chromosomes can be counted as 10, but since in two places chromosomes lie so close together, I am inclined to believe that they are two long chromosomes instead of four.

GOODSPEED in a preliminary note (1923) has reported for different Nicotiana species the haploid numbers 9, 12, and 24, but for some of them he makes the following reservation.

> Three of these counts are open to some question. In the case of *N. alata*, homotypic anaphase plates in the fixed material show 10 chromosomes in some cases, although the predominating number in such stages is 9. Similarly, *N. longiflora* can be counted as 9 or 10, but in this case the predominating number is 10. Only a small amount of fixed material of *N. suaveolens* was available, and there is some doubt as to whether the number is not larger than 12, possibly 18.

Later, (1924) he describes a very interesting case of *N. alata* in which, after the second division, two of the anaphase plates have 8, and the other plates 10 chromosomes. Failure of conjugation in one chromosome pair, he believes, will account for this condition, because in other cases he found

9 chromosomes in three nuclei of the tetrads. For *N. suaveolens*, he decides that the number is 18. At the IVth International Congress of Botany at Ithaca, I showed Dr. GOODSPEED on one of my slides a single section in which both the plates of the homeotypic anaphase contained only 16 chromosomes. In a recent publication (GOODSPEED and CLAUSEN 1927), this number is admitted to be the correct one.[1]

17. *N. Langsdorffii* Weinm. This is a small plant with sessile, elliptical-lanceolate leaves, narrowed and decurrent at the base. The corolla is funnel-shaped below with a gibbous ring above and a concave, spreading limb slightly notched into five broad, shallow lobes, greenish yellow and pendent.

At the various stages, 8 chromosomes were observed (figure 31), though it is not always easy to count them for reasons similar to those discussed in the case of *N. alata*. In the root tips (figure 15), I found 16 chromosomes similar in shape to the chromosomes found in the root tips of *alata* (figure 14).

Polydiclia section

18. *N. quadrivalvis* Pursh. This is a small plant somewhat similar to *Bigelovii*, except that the leaves are not elongate deltoid but broadly deltoid. The corolla is shaped somewhat similarly to that of *Bigelovii*, but is much larger. The capsule is spherical and twice as large as that of *Bigelovii*. During the various stages of the reduction division in the pollen mother cells, 24 chromosomes were easily counted (figure 17).

[1] Since this paper was finished, Miss RUTTLE has published an interesting study of the chromosomes in *N. alata* var. *grandiflora* (Univ. of California Pub. Bot. **11**: 159–176, 1927). She has gone about her investigation in a very workmanlike manner, making extensive counts and even endeavoring to identify the individual chromosomes in various plates. She concludes that there are 18 chromosomes in the somatic cells, the usual distribution being 9–9, though 8–10 distributions occur in about nine percent of the cases. She believes that in my previous work (Jahrb. d. Univ. Sofia, Agric. Fac. **3**: 37–86, 1925) where a diploid number 16 was reported for the species, I incorrectly figured two chromosomes as one—a very long one. It is possible that Miss RUTTLE'S view is correct. But I must call attention to two points which should be weighed carefully before a decision is made. First, Miss RUTTLE figures 3 pairs of U-shaped chromosomes in her Figure 1a plus 2 pairs the ends of which lie very close together (upper center). This latter configuration I believe to be a single pair of chromosomes. Second, and this is my critical point, in certain hybrids with species having 10 chromosomes as the haploid number, the reduction division of the pollen mother cells shows the number of univalents properly to be expected if the chromosome number for *N. alata* is $x=8$. For example, in heterotypic spindles of *N. longiflora*×*Sanderae*, *N. longiflora*×*alata*, *N. plumbaginifolia*×*alata*, *N. plumbaginifolia*×*Langsdorffii*, *up to two lagging univalent chromosomes* occur and are often to be seen approaching the poles, whereas the bivalents are still at the equator (see figure 37); and *regularly more than two lagging chromosomes* appear during the homeotypic division.

19. *N. multivalvis* Lindl. This is a plant very similar to *quadrivalvis*, only larger, with very large corolla and capsules. Counts were made only from temporary slides with actetocarmine staining and 24 chromosomes were counted in the pollen mother cells.

HYBRIDIZATION RESULTS

In the hybridization experiments the following species of Nicotiana were used:

1. *N. alata*
2. *N. Sanderae*
3. *N. longiflora*
4. *N. plumbaginifolia*
5. *N. acuminata*
6. *N. trigonophylla*
7. *N. sylvestris*
8. *N. suaveolens*
9. *N. Bigelovii*
10. *N. nudicaulis*
11. *N. Langsdorffii*
12. *N. paniculata*
13. *N. glutinosa*
14. *N. rustica*
15. *N. Tabacum macrophylla*

The technique of hybridization has already been described. Crosses were made between nearly all of these species in both directions, pollinating five (or more) flowers in each case. The few exceptions were the omission of *N. Sanderae* as female, of nine combinations with *N. nudicaulis*, and of one combination with *N. rustica*. The results obtained are recorded in table 1.

Two weeks after the capsules were harvested the seeds were sown. For every cross several sowings were made according to the quantity of seed harvested. If all of the flowers which were pollinated produced capsules, 100 seeds were first taken for the germination test, the rest being sown in sterilized soil for the production of F_1 plants. For crosses from which one or two capsules only were harvested, or in which only a few seeds developed, the material was used in the germination experiment. If germination occurred, the seedlings were transferred to pots of sterilized soil. If no germination occurred after three weeks, successive sowings were made until all the seed was used. The germination experiment was made in the usual way on blotting paper, the papers being kept in an incubator at a temperature of 27° to 30° C. Sometimes it happened that not a single seed germinated in the soil while a few germinated in the germination test; but it also happened that a few germinated in the soil though none appeared in the germination test.

A prospectus of all the crosses is given in table 1. In the horizontal rows are given the species used as female, and in the vertical row those used as male. The chromosome number for each of the species follows the name. The crosses in which the flowers have dropped after pollination are de-

noted by *o*, those in which the harvested capsules contained no seeds or non-germinative seeds by *d*, those which produced defective seedlings by *s*, those in which mature hybrids were produced by *h*.

TABLE 1.

Results of the hybridization experiments.

SPECIES USED AS MALES		SPECIES USED AS FEMALES														
		N. alata	*N. Sanderae*	*N. Langsdorffii*	*N. longiflora*	*N. plumbaginifolia*	*N. acuminata*	*N. sylvestris*	*N. trigonophylla*	*N. paniculata*	*N. glutinosa*	*N. suaveolens*	*N. Bigelowii*	*N. nudicaulis*	*N. rustica*	*N. Tabacum*
		X=8	8	8	10	10	12	12	12	12	12	16	24	24	24	24
N. alata	X=8	..	..	h	h	h	d	d	d	s	o	s	d	d	..	h
N. Sanderae	8	h	..	h	o	o	o	d	d	h	d	o	d	..	h	h
N. Langsdorffii	8	h	..	..	o	h	o	o	s	h	s	o	s	d	h	s
N. longiflora	10	o	..	o	..	h	d	o	d	s	d	h	s	..	d	s
N. plumbaginifolia	10	o	..	o	h	..	d	o	d	s	d	h	s	..	d	s
N. acuminata	12	o	..	o	o	o	..	o	o	o	d	d	d	..	d	o
N. sylvestris	12	o	..	o	o	o	o	..	o	d	h	o	s	o	o	h
N trigonophylla	12	o	.	o	o	o	o	o	..	d	o	o	o	h	d	o
N. paniculata	12	o	..	o	o	s	o	o	d	..	d	o	s	o	h	o
N glutinosa	12	o	..	o	o	o	o	o	d	h	..	h	h	..	d	h
N. suaveolens	16	o	..	o	o	o	o	o	d	s	d	..	h	d	s	d
N. Bigelovii	24	o	..	o	o	o	o	o	d	d	h	h	..	..	d	d
N. nudicaulis	24	o	..	o	..	..	o	..	d	d	h	o	h	..	d	d
N. rustica	24	o	..	o	o	o	o	o	d	d	o	o	o	d	..	o
N. Tabacum	24	o	..	o	o	o	d	h	d	d	d	s	h	s	h	..

Combinations marked .. were not made
Crosses marked o produced no capsules
Crosses marked d produced capsules without viable seeds
Crosses marked s produced defective seedlings
Crosses marked h produced mature hybrids

Thus *N. alata*, used as female, gave hybrids with *Langsdorffii* and *Sanderae*; but when pollinated by other species it dropped its flowers. When used as male, however, *alata* produced hybrids not only with *Langsdorffii*, but also with *longiflora*, *plumbaginifolia*, and *Tabacum*. *Langsdorffii* as female shows the same results as *alata*, but as male exhibits some differences.

Trigonophylla, which is placed by DUNAL (1852) in the *Rustica* and by COMES in the Petunioides section, produced defective seedlings when pollinated by *Langsdorffii* of the Rustica section, and with *alata* and *Sanderae* of the Petunioides section it did not even give viable seeds. *Glutinosa*

which is in the Rustica section, gave the same results when pollinated with these species. In general, the species with 12 haploid chromosomes show a very weak power of hybridization, *acuminata* and *trigonophylla* producing no hybrids with any of the species used in these experiments.

In species with 24 chromosomes the hybridizing ability is somewhat greater than in those with lower chromosome numbers, but compatibility between species shows no significant relation to taxonomic status.

Out of 186 crosses 84 were made reciprocally, and 67 of these crosses were between species differing in their chromosome number. In 56 of the latter no mature hybrids were obtained when the species with the lower chromosome number was used as female, but a higher degree of compatibility was shown when the species with the higher chromosome number was used as female. In 8 crosses where the species with the lower chromosome number was used as female the pollinated flowers dropped, while from the reciprocals mature hybrids were raised. In 4 other crosses where the species with the lower chromosome number was used as female, defective seeds were produced; while in the reciprocals mature hybrids were obtained. In 13 crosses the pollinated flowers dropped from the species with the lower chromosome number, while the reciprocals produced defective seedlings. In 19 crosses the pollinated flowers of the species with the lower chromosome number also dropped, although the reciprocals produced defective seeds. In one cross both types of crossing resulted in defective seedlings, and in another only defective seeds were produced. In 9 crosses the incompatibility between the species was so great that the pollinated flowers dropped in both the direct and reciprocal crosses. In one cross defective seeds were produced by using the species with the smaller number of chromosomes as female, and defective seedlings by the reciprocal.

In 8 reciprocal crosses the degree of compatibility appeared to be greater when the species with the lower chromosome number was used as female, but none of these crosses produced mature hybrids. In 6 of these crosses the flowers of the species with the lower chromosome number produced defective seeds, while the flowers of the reciprocals dropped after pollination. In 2 crosses defective seedlings were produced when the species with the lower chromosome number was used as female; in one of the reciprocals defective seeds were produced, in the other the flowers dropped.

Mature hybrids were obtained in both directions from only three of the 67 crosses involving species differing in their chromosome number, but in two of them (*glutinosa*×*Bigelovii* and *sylvestris*×*Tabacum*) a much

larger number of plants per capsule were obtained when the female parent had the higher chromosome number.

Mature hybrids were obtained in both directions from only two (*alata*×*Langsdorffii* and *longiflora*×*plumbaginifolia*) of the 17 reciprocal crosses between species having the same chromosome number. Of 10 reciprocally made crosses between species with 12 haploid chromosomes, 4 did not produce seeds, no matter which parent was used as female. In 3 crosses the flowers dropped after pollination, while their reciprocals produced defective seeds. In 1 cross defective seeds were produced by both parents. From 2 crosses (*glutinosa*×*sylvestris* and *paniculata*×*glutinsoa*) a few very weak mature hybrids were raised, although their reciprocals produced respectively defective seeds and nothing.

Out of 5 reciprocally made crosses between species with 24 haploid chromosomes, 2 produced very vigorous hybrids, while their reciprocals produced defective seedlings and nothing. In a third cross defective seedlings were produced in one direction and defective seeds in the other. In the fourth cross both female parents produced defective seeds. In the last cross the seeds were defective in one female parent and the flowers dropped in the other.

DESCRIPTION OF THE SPECIES HYBRIDS

A. Species hybrids which did not reach maturity.

Of the various crosses made, 19 produced hybrids which died in early seedling stages.

a. Hybrids obtained by crossing species having 12 with species having 8 as the haploid chromosome numbers are:
   1. *N. trigonophylla*×*Langsdorffii*
   2. *N. glutinosa*×*Langsdorffii*
   3. *N. paniculata*×*alata*.

b. Hybrids obtained by crossing species having 12 and 10 as the haploid chromosome numbers are:
   4. *N. paniculata*×*longiflora*
   5. *N. paniculata*×*plumbaginifolia*
   6. *N. plumbaginifolia*×*paniculata*.

c. Hybrids obtained by crossing species having 16 and 8 as the haploid chromosome numbers are:
   7. *N. suaveolens*×*alata*.

d. Hybrids obtained by crossing species having 12 and 16 as the haploid chromosome numbers are:
   8. *N. paniculata*×*suaveolens*.

e. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers are:
   9. *N. Bigelovii* × *Langsdorffii*
   10. *N. Tabacum* var. *macrophylla* × *Langsdorffii*.
f. Hybrids obtained by crossing species having 24 and 10 as the haploid chromosome numbers are:
   11. *N. Bigelovii* × *longiflora*
   12. *N. Bigelovii* × *plumbaginifolia*
   13. *N. Tabacum* var. *macrophylla* × *longiflora*
   14. *N. Tabacum* var. *macrophylla* × *plumbaginifolia*.
g. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers are:
   15. *N. Bigelovii* × *sylvestris*
   16. *N. Bigelovii* × *paniculata*.
h. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers are:
   17. *N. rustica* × *suaveolens*
   18. *N. suaveolens* × *Tabacum* var. *macrophylla*.
i. Hybrids obtained by crossing species having 24 and 24 as the haploid chromosome numbers are:
   19. *N. nudicaulis* × *Tabacum* var. *macrophylla*.

Of these crosses, only *N. suaveolens* × *Tabacum* var. *macrophylla* (germination 82 percent), *N. Tabacum* var. *macrophylla* × *plumbaginifolia* (germination 3 percent), and *N. nudicaulis* × *Tabacum* var. *macrophylla* (germination 16 percent) yielded a great number of seedlings. In all other crosses the germination was very low. In the cross *N. Tabacum* var. *macrophylla* × *plumbaginifolia*, the seedlings all died after developing the fourth or fifth true leaf. In most of the crosses the seedlings died before developing the second true leaf.

B. Species hybrids which reached maturity.
   a. Hybrids obtained by crossing species having 8 as the haploid chromosome number.

I. *N. alata* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 81 percent. The ten hybrids raised were sterile. They were vigorous but variable, which may indicate that *Sanderae* is heterozygous for various genes, since in other species crosses where *Sanderae* was used the hybrids were also variable.

Nearly all of the hybrids resembled *N. Sanderae* in habit of growth. The leaves were an intermixture of the characteristics of both parents. In corolla color the *Sanderae* parent was dominant; but in size and shape the flowers were nearly intermediate.

II. *N. alata* × *Langsdorffii*

The germination of the seeds obtained was 80 percent (93 percent in the reciprocal). The ten hybrids raised were fertile, vigorous, uniform, and in habit of growth resembled *Langsdorffii*. The leaves were nearly intermediate in color, size and shape. The flowers also were intermediate in color, size and shape. The pollen-grains were intermediate in color—slightly bluish.

III. *N. Langsdorffii* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 62 percent. The ten hybrids raised were fertile, vigorous, and variable in habit of growth, though resembling *Langsdorffii*. The leaves were different in size and shape in the different individuals. In respect to corolla color, *Sanderae* was dominant. In size and shape the flowers were different in the different individuals. The pollen-grains were intermediate in color—light bluish.

b. Hybrids obtained by crossing species having 10 and 8 as the haploid chromosome numbers.

IV. *N. longiflora* × *alata*

The germination of the seeds obtained was 95 percent. The fifty hybrids raised were sterile. They developed slowly into vigorous, uniform plants, in habit of growth an intermixture of the characteristics of both parents. The leaves were slightly rugose, like those of *longiflora*. The flowers, which remained slightly closed during the day, were intermediate in size and shape, though with the corolla lobes bluish externally as in *longiflora*.

V. *N. longiflora* × *Sanderae*

I was unsuccessful in this cross; but ten hybrids were raised from seeds obtained by DR. A. J. MANGELSDORF. The plants were sterile; and though variable in size and in habit of growth, were quite vigorous. The leaves of each individual were slightly rugose like those of *longiflora*. The flowers, although somewhat variable, resembled the two parents in size and shape. They remained slightly closed during the day like those of *longiflora*.

VI. *N. plumbaginifolia* × *alata*

The germination of the seeds obtained was 49 percent. The fifty hybrids raised were sterile. They developed slowly to vigorous, uniform plants, in habit of growth an intermixture of the traits of both parents. The leaves were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, bluish externally like *plumbaginifolia*, and remained slightly closed during the day.

VII. *N. plumbaginifolia* × *Langsdorffii*

The germination of the seeds obtained was 35 percent. The thirty hybrids raised were sterile. They were vigorous, uniform, and in habit of growth intermediate between the parents. The leaves resembled *Langsdorffii* in color, but were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, shape and color. The corolla lobes were yellowish on the upper side, but slightly bluish underneath. The pollen-grains were intermediate in color—slightly bluish.

c. Hybrids obtained by crossing species both having 10 as the haploid chromosome number.

VIII. *N. longiflora* × *plumbaginifolia*

The germination of the seeds obtained was 96 percent (81 percent in the reciprocal). The ten hybrids raised were fertile, very vigorous, uniform, and in habit of growth intermediate between the parents. The leaves and the flowers were also intermediate in their characteristics.

d. Hybrids obtained by crossing species having 12 and 8 as the haploid chromosome numbers.

IX. *N. paniculata* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 28 percent. The ten hybrids raised were sterile. They varied markedly in vigor, from very weak to very vigorous; they also varied in habit of growth. The leaves were variable in the different individuals, but were nearly all petiolate as in *paniculata*. The flowers in all were as red as those of the *Sanderae* parent, varying in size but more closely resembling *paniculata* in shape.

X. *N. paniculata* × *Langsdorffii*

The germination of the seeds obtained was 12 percent. The ten hybrids raised were sterile. The plants were uniformly very weak, in habit of growth resembling *Langsdorffii*. The petiolate leaves resembled *paniculata*

rather closely. The flowers in size and shape resembled *Langsdorffii*. The pollen-grains were intermediate in color—slightly bluish.

e. Hybrids obtained by crossing species both having 12 as the haploid chromosome number.

XI. *N. paniculata*×*glutinosa*

The germination of the seeds obtained was less than 1 percent. The five hybrids raised were sterile. The plants were very weak at the beginning, but quite vigorous at the end. They exhibited little variablity, and were an intermixture of the characteristics of both parents. The first leaves were oblong, the later ones cordate, thus resembling *glutinosa*. The flowers were yellow, tinged with pink, and were intermediate in size and shape.

XII. *N. glutinosa*×*sylvestris*

The germination of the seeds obtained was 5 percent. The two hybrids raised were sterile. They developed slowly and were very weak, though uniform and in habit of growth intermediate between the parents. The leaves were nearly petiolate as those of *glutinosa*, but oblong like those of *sylvestris*. The flowers were pink as in *glutinosa*, but in size and shape intermediate.

f. Hybrids obtained by crossing species having 16 and 10 as the haploid chromosome numbers.

XIII. *N. suaveolens*×*longiflora*

The germination of the seeds obtained was 22 percent. The thirty hybrids raised, though sterile, were very vigorous and in habit of growth resembled *suaveolens*. The leaves also resembled *suaveolens*. The flowers were more like *suaveolens* in size and shape; but in corolla color *longiflora* was dominant, the corolla lobes being bluish underneath.

XIV. *N. suaveolens*×*plumbaginifolia*

The germination of the seeds obtained was 2 percent. The fifteen hybrids raised, though sterile, were very vigorous, uniform, and in habit of growth resembled *suaveolens*. The leaves and flowers resembled *suaveolens*, though in corolla color *plumbaginifolia* was dominant, the corolla lobes being bluish underneath.

g. Hybrids obtained by crossing species having 16 and 12 as the haploid chromosome numbers.

XV. *N. suaveolens*×*glutinosa*

The germination of the seeds obtained was 88 percent. The five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *suaveolens*. In size and shape the leaves were intermediate, but petiolate as those of *glutinosa*. The flowers were pink like those of *glutinosa*, but in size and shape resembled *suaveolens*.

h. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers.

XVI. *N. Tabacum, Cuba*×*alata*

The germination of the seeds obtained was less than 1 percent. The two similar hybrids raised were sterile, very weak, and in habit of growth resembled *Tabacum*. The characteristics of both leaves and flowers were more nearly like those of *Tabacum*.

XVII. *N. Tabacum*, white tobacco×*Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. Three hybrids were raised from a cross in which *Sanderae* Cl_{10} was used. All of them were sterile, quite weak, and though different, were more nearly like *Tabacum*. The leaves and the flowers resembled *Tabacum* in size and shape, but the corolla color of *Sanderae* was dominant. From another cross, where *Sanderae* N16 with bluish corolla was used, the four hybrids obtained showed the result of segregation in corolla color, three being bluish and one white. These hybrids also were sterile, weak, and variable in habit, though resembling *Tabacum*.

XVIIII. *N. rustica*×*Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. The four hybrids raised were sterile. The plants varied markedly in vigor and size, although in habit of growth they resembled *rustica*. The leaves of all were petiolate, and in size and shape were like those of *rustica*. The flowers although different in size and shape in the different individuals, resembled *rustica*, except that the red corolla color of *Sanderae* was dominant.

XIX. *N. rustica*×*Langsdorffii*

The germination of the seeds obtained was less than 1 percent. The only hybrid raised was sterile, very weak and in habit of growth like *rustica*. The leaves were petiolate, resembling *rustica*, in size and shape. The

flowers also resembled *rustica* in size and shape. The pollen grains were intermediate in color—slightly bluish.

i. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers.

XX. *N. nudicaulis*×*trigonophylla*

The germination of the seeds obtained was 46 percent. The twenty hybrids raised were sterile, though quite vigorous, uniform and with a habit of growth like *nudicaulis*. The leaves were also more like those of *nudicaulis*. The corolla limb was curved as in *nudicaulis*, but the tube intermediate between those of the parents in length.

XXI. *N. glutinosa*×*nudicaulis*

The germination of the seeds obtained was 10 percent. The twenty-eight hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the characteristics of both parents. In color, the leaves were like those of *nudicaulis*, but petiolate as in *glutinosa*. The flowers were like those of *glutinosa* in color and shape, and like those of *nudicaulis* in size.

XXII. *N. Bigelovii*×*glutinosa*

The germination of the seeds obtained was 98 percent, but less than 1 percent in the reciprocal. The fifty hybrids raised were sterile, vigorous, uniform and in habit of growth resembling *Bigelovii*. The leaves were petiolate like those of *glutinosa*, but in size, shape and color resembled *Bigelovii*. The flowers were slightly pink, but in size and shape resembled *Bigelovii*.

XXIII. *N. Tabacum* var. *macrophylla*×*sylvestris*

The germination of the seeds obtained was 50 percent. The seventy-five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Tabacum*. The leaves and the flowers resembled *Tabacum*, but the corolla tube was more slender, the corolla limb smaller and the corolla lobes narrower than those of *Tabacum*. The reciprocal cross was made by using another *Tabacum* variety as the male parent. Here the germination of the seeds obtained was less than 1 percent. The three hybrids raised behaved in respect to the parental characters as in the cross *macrophylla*×*sylvestris*.

XXIV. *N. Tabacum* var. *macrophylla*×*glutinosa*

The germination of the seeds obtained was 25 percent. The twelve hybrids raised were sterile, very weak, variable in size and habit, an intermixture of the traits of both parents. The leaves were petiolate, small and ovate, more like those of *glutinosa*. The flowers were as red as those of *macrophylla*, but in size and shape were intermediate.

XXV. *N. rustica*×*paniculata*

The germination of the seeds obtained was 35 percent. The fifteen hybrids raised were partially fertile, quite vigorous, uniform and in habit of growth resembled *rustica*. The leaves and the flowers approached closely to those of *rustica*, but the corolla tube was somewhat intermediate in length.

j. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers.

XXVI. *N. Bigelovii*×*suaveolens*

The germination of the seeds obtained was 84 percent (98 percent in the reciprocal). The thirty-three hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Bigelovii*. The leaves and flowers were more like those found in *Bigelovii*.

k. Hybrids obtained by crossing species having 24 as the haploid chromosome number.

XXVII. *N. Bigelovii*×*nudicaulis*

The germination of the seeds obtained was 15 percent. The twenty hybrids raised were sterile, vigorous, uniform and in habit of growth an intermixture of the traits of both parents. The leaves resembled *Bigelovii*. The flowers were intermediate in color, but were small with curved corolla as in *nudicaulis*.

XXVIII. *N. Bigelovii*×*N. Tabacum* var. *macrophylla*

The germination of the seeds obtained was 15 percent. The twenty-five hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the traits of both parents. The leaves and flowers were also intermediate.

XXIX. *N. rustica*×*N. Tabacum* white tobacco

The germination of the seeds obtained was less than 1 percent. The two hybrids raised were slightly fertile, vigorous, and in habit of growth inter-

mediate. The leaves were petiolate, and in shape were more nearly like *rustica*; but were intermediate for size. The flowers were intermediate in color, size and shape.

CYTOLOGICAL OBSERVATIONS ON CERTAIN NICOTIANA SPECIES HYBRIDS

A. *N. alata*×*Langsdorffii*

The heterotypic and homeotypic divisions in the pollen mother cells are normal.

B. *N. longiflora*×*Sanderae*

The reduction division shows a picture very similar to that of the Drosera hybrids (ROSENBERG 1909). There are eight bivalents and two univalents. Sometimes it appears that in an earlier stage of the heterotypic metaphase the univalents are in the same plane as the bivalents, but frequently one observes spindles with two lagging chromosomes (figure 37). During the second metaphase (figure 38), two univalent chromosomes regularly lag outside both equatorial plates. In figure 39, one of the univalents has already split. During the first and second divisions the univalents are usually distributed at random to the poles in a normal manner, but cases in which the 4 univalent chromosomes are lagging (figure 40) are frequently observed.

C. *N. Tabacum*×*alata*

The reduction division here is quite irregular. In the first division as many as 8 gemini can be distinguished (figure 41 and 43). Figure 41 shows the chromosomal arrangement at the multipolar spindle immediately after diakinesis. Figure 42 shows the next stage with the chromosomes aligned on the equatorial plate. Such figures are rather infrequent, however, for soon after this stage, the univalents pass to the poles. The first metaphase spindle most commonly seen is shown in figure 43. Eight bivalents and three univalents lie in the equatorial plate, the rest of the univalents being on the way to the poles. It seems that soon after some of the univalents have reached the poles, the bivalents begin to divide (figure 44). Figure 45 represents a situation occasionally occurring in which there has been precocious division of some of the chromosomes either during the final stages of the first division or at the beginning of the second division, the chromosomes of both plates plus the lagging one are $18+19+1=38$ instead of 32. Presumably 6 chromosomes have already divided. Such chromosomes, that is to say those which have gone through the equational division at the end of the first division, seem to behave during the

second anaphase much as do those of the Triticum hybrids (SAX 1922, KIHARA 1924, THOMSON 1926). I think that the fairly large number of lagging chromosomes found during the second division (figure 46) are not merely descendants of the few chromosomes which lagged during the first division, but also of those which have not undergone the equational division at the same time as the majority.

D. *N. Tabacum*×*Sanderae* (Cl_{10})

The reduction division in these hybrids is similar to that found in the hybrids of *N. Tabacum*×*alata*. Diads were frequently found.

E. *N. paniculata*×*Langsdorffii*

No bivalent chromosomes were observed during the various stages from early prophase to the second division. In figure 47, a heterotypic spindle, four of the chromosomes are very long and slender; but it is clear from the preparation that they are attached at both ends to spindle fibers. In a slightly later stage (figure 48), two such chromosomes are shown so securely fastened to a fiber that they seem to be pulled apart. At the end of the first division the chromosomes are regularly distributed between both poles, and during the second metaphase the number of lagging chromosomes is usually small (figure 49). An exceptional case is illustrated in figure 50 where all chromosomes lie in the same plane. The second division is virtually an ordinary equational division (figure 51).

F. *N. rustica*×*paniculata*

In the heterotypic metaphase of this hybrid the 12 bivalent chromosomes are easily distinguished from the 12 univalents. The bivalents are about twice as large and somewhat longer than the univalents, and are always found on the equatorial plate. The spherical univalents are scattered. Figure 52 shows the 12 bivalents in the center, 5 univalents on one side, 6 univalents on the other side, and one univalent overlapping one of the bivalents. The group of 5 lies in one plane, the group of 6 in another plane, while the equalorial plate with the 12 bivalents lies between them.

The arrangement of the chromosomes during the heterotypic anaphase is illustrated by figure 53. The bivalents, which divide soon after the univalents approach the poles, are found together with the univalents near the poles. Figures 54 and 55 show two sets in the second metaphase. In the first figure the chromosome number of the two plates is $18+18=36$, in the second $16+20=36$. This indicates that no univalent chromosomes

had split during the first division. This is the case for all counts made at this stage. (Cases of lagging chromosomes were also found). The second division is usually very regular (figure 56) though figures with lagging chromosomes have occasionally been observed.

G. *N. suaveolens*×*glutinosa*

During the first metaphase the chromosomes of both parents, 16+12 = 28, are scattered throughout the spindle, with no bivalents visible (figure 58). At the heterotypic anaphase the chromosomes are distributed at random to the two poles, lagging chromosomes being quite common. Figure 57 illustrates a second metaphase with 9 chromosomes in one equatorial plate and 17 in the other, while outside there are 2 lagging chromosomes. Figure 59, of the same stage, shows 4 chromosomes on a small spindle, 2 chromosomes outside, and 22 chromosomes on the equatorial plate. The chromosomal distribution during this stage is quite variable. The second division is chiefly equational. The chromosomes which lag during this division seem to be descendants of univalent chromosomes which, at the beginning of the second division, failed to reach the equatorial plates. The tetrads show a great deal of degeneration. Very often, in acetocarmine preparations, they stuck together, appeared to be shrunken, and failed to stain.

H. *N. suaveolens*×*Bigelovii*

During the heterotypic division it is very difficult to distinguish the metaphase from the anaphase, nevertheless it is easy to see that the chromosomes do not pair. The chromosomes of both parents (16+24) are scattered along the spindle. Figure 60a-b shows 37 chromosomes lying in one section and three in the other. Because the chromosome distribution in the first division is so variable the second metaphase looks quite different in different cells. Figure 61 shows 21 chromosomes lying on one plate, 16 on the other plate, and 3 lagging. Figure 62 shows the same stage in which the chromosomes are so distributed that 38 chromosomes lie on one plate, while 2 are arranged as a small spindle. The spindle fibers in this case are not visible, but the position of the chromosomes indicates a spindle. Figure 63 presents a second metaphase in which the chromosomes are distributed among three very well formed spindles, with 2 chromosomes lagging in a spindle position. The second division is regular. At this stage the chromosomes of the different spindles normally undergo the equational split simultaneously, and any lagging chromosomes which are found during and after the second division are presumably descendants

of the lagging chromosomes seen during the first division. In figure 64, showing the end of the second division, there are 8 nuclei visible. In three of the small nuclei there are 6 chromosomes; in another nucleus there are 5 chromosomes; in two other nuclei there are 10 chromosomes; while in the two very large nuclei there are 19 and 13 (?) chromosomes respectively. It is very difficult to count accurately in such a stage since the prochromosomes lie in so many different planes. I am inclined to believe that in this case, however, both of the very large nuclei really contain 19 chromosomes, the two next largest contain 10, two of the smaller ones have 6, and the other two only 5 chromosomes, originating through a chromosome distribution during the first division in which the formation of four spindles containing $19+10+6+5=40$ chromosomes took place at the beginning of the second division. The degeneration begins at the second telophase. The protoplasm is almost always very poorly fixed, the cross walls being shrunken and the nucleus itself taking the stain irregularly. It may be that the disperison of non-homogeneous chromatin between so many nuclei is due to the acceleration of the degeneration.

I. *N. rustica*×*Tabacum*, white tobacco

The first reduction division is very irregular. Although I examined many slides showing the heterotypic division there is so much variation that I could not be certain whether gemini were formed or not. In the two serial sections of figure 65 a and b, a very common situation is illustrated in which the 48 chromosomes scattered on the spindle can be easily distinguished. In some cases I have been unable to count 48 chromosomes, which I assume to be due to the presence of loose gemini.

During the second metaphase the chromosomes are distributed irregularly to both poles, some of them lagging on either side of the equatorial plates. In figure 67 is illustrated the most unequal distribution found; 36 chromosomes in one plate, 11 in the other plate, and 1 chromosome lagging between them. In anthers showing different stages of the second division, especially of the second metaphase, fairly normal single spindles were visible which were twice as large as ordinary second division spindles. Similar cases are reported by Miss LJUNGDHAL (1922) in the cross *Papaverum somniferum*×*orientale*. Such cases were thought by her to have originated through the fusion of two second metaphase spindles. Figure 66 corresponds roughly to figure 5d of her plates, though the two cases are somewhat unlike because of the different type of division. Figure 68 illustrates a case in which a cell wall has already developed between the two spindles. It is not impossible that the peculiar chromosome

distribution caused the development of the cell wall thus halting the second division just as it was starting, the result being the formation of the diads frequently found in the pollen mother cells of this hybrid. Normally the second division is very irregular, with many lagging chromosomes scattered over the two spindles. In figure 69a,b,c, the chromosomes lying in the plane of one spindle are represented in solid black, those of the other spindle in stipple, the lagging chromosomes in outline.

DISCUSSION

Inheritance in species hybrids

The gradual growth of the chromosome theory of heredity has led geneticists to interpret the anomalous distribution of characters in certain species crosses to an abnormal distribution of the chromosomes. Where the hybrids are fertile and the chromosome behavior is that which is ordinarily found in varietal crosses, the normal type of inheritance obtains (*Vide Mirabilis jalapa*×*M. longiflora*, CORRENS 1909; *Antirrhinum majus*×*A. molle*, BAUR 1911, LOTSY 1912; *Nicotiana Langsdorffii*×*N. alata*, EAST 1916). In a few cases, even partially fertile hybrids have shown a behavior similar to that found in intervarietal crosses (*Godetia Whitneyi*×*G. amœna*, RASMUSSON 1920). In general, however, one cannot go very far in a pedigree culture analysis of the results of species hybridization; though it appears probable, following STURTEVANT's work on *Drosophila melanogaster* and *D. simulans* (1920, 1921) that related species may have genes in common and may produce similar mutants.

Lack of sufficient analysis in intervarietal crosses prevents any examination of Nicotiana species hybrids after the manner used for Drosophila. SACHS-SKALINSKA (1921), however, has demonstrated Mendelian segregation for corolla color in the fertile hybrid, *N. Langsdorffii*×*Sanderae*. The colored corolla of *N. Tabacum* var. *macrophylla* which is dominant in intervarietal crosses with white tobacco has also proved dominant in the cross with *N. sylvestris*, and in somewhat diluted form in the cross with *N. Bigelovii*. The red corolla color of a *N. Sanderae* variety proved dominant over the white of *N. alata* and white tobacco. This corolla color of *N. Sanderae* was also dominant over the yellow-greenish corolla color of *N. Langsdorffii*, *N. paniculata*, and *N. rustica*. An intermediate corolla color occurred in the hybrid *N. Bigelovii*×*glutinosa* as well as in *N. Tabacum* var. *Cuba*×*alata* and *Cuba*× white tobacco. In intervarietal crosses of *Tabacum* it has been found (SETCHELL, GOODSPEED, and CLAUSEN 1922) that the petioled leaf shape is more or less dominant over

the sessile. Similarly, the petioled leaf character of *N. rustica*, *N. paniculata*, and *N. glutinosa* proved dominant in the various interspecific crosses involving these species.

The various hybrids derived by crossing species having the same chromosome number show a mixture of the parental characters due, presumably, to lack of dominance or to a similar number of dominants contributed by each parent. Where, on the other hand, the hybrids are obtained by crossing species widely differing in their chromosome number, as *N. Tabacum*×*Sanderae*, *N. Tabacum*× *sylvestris*, *N. rustica*×*Langsdorffii*, *N. suaveolens*×*longiflora*, *N. suaveolens*×*plumbaginifolia*, the hybrid resembles the parent having the larger chromosome number except in the cross *N. paniculata*×*Langsdorffii* in which the latter species appears to be dominant apart from the petioled condition of the leaves. It is not impossible that this resemblance to *N. Langsdorffii* is due to a few striking dominant characters contributed by it.

The characteristics of the sterile hybrid *N. Tabacum*×*sylvestris*, in which *N. Tabacum* is better represented, led GOODSPEED and CLAUSEN (1916) to conclude that distinct reaction systems are involved in species crosses, and that the phenomenon must be viewed in the light of a contrast between systems rather than between specific factor differences. Although they have, in some instances, recognized the influence of *N. sylvestris* on the hybrid, these authors have questioned the plate in BAUR's textbook in order to establish the complete dominance of *N. Tabacum*. KLEBS (1917), however, claims an intermediate character for the flower of this hybrid, and the intermediate corolla length of the hybrid can be distinguished in plates given by GOODSPEED and CLAUSEN (1917). Although the calcyina flower is almost completely recessive in intervarietal *Tabacum* crosses (SETSHELL, GOODSPEED, and CLAUSEN 1922), it appears in the *N. Tabacum*×*sylvestris* hybrid, albeit in not so extreme a form as in the parental types. The probability is that the twelve chromosomes of *sylvestris* do not carry the factors necessary to limit the appearance of this character, and that in general, the great resemblance to *Tabacum* is due to the transmission to the hybrid of a larger number of dominant factors with the larger number of chromosomes. The high degree of sterility, on the other hand, depends (EAST 1915, 1921) on the interrelation between the chromosomes.

A species hybrid parallel to that cited by Goodspeed and Clausen is found in crosses between white tobacco and two different *N. Sanderae* plants. The hybrids obtained show a high degree of resemblance to the *N. Tabacum* parent, but corolla color of the *Sanderae* parent is dominant.

Non-viability of the gametes in species hybrids

In the matter of sterility through the production of non-viable gametes, it is necessary to distinguish between completely sterile and partially sterile hybrids. The first category is the one most frequently found in *Nicotiana*; the second I have found only in *N. rustica*×*paniculata*. From cytological studies of different hybrids of the first category, such as *N. Bigelovii*×*suaveolens*, *N. suaveolens*×*glutinosa*, and *N. paniculata*× *Langsdorffii*, complete sterility seems to be the logical expection, since no pairing of chromosomes occurs during the first division. The chromosomes are distributed irregularly between the two poles of the heterotypic spindle, some being left behind; or, as in *N. Bigelovii*×*suaveolens*, they are distributed among three or four homeotypic spindles. In *N. rustica*×*paniculata*, on the other hand, there is distinct pairing of the chromosomes. Presumably this should afford a greater expectation of the production of viable gametes, though there are cases known where a seemingly normal reduction does not lead to viable gametes.

Of some interest is the relation between the chromosome behavior and the morphological appearance of the hybrids. *N. paniculata* and *N. Langsdorffii* both belong to the *rustica* section, but they produced very weak hybrids with no pairing of chromosomes. It seems that the mode of the reduction division is not correlated with the vigor of the hybrid, nor even with the relationship between the species, although this case is somewhat questionable as the accepted taxonomic position of *Langsdorffii* is open to criticism (Lock 1909). *N. suaveolens* and *Bigelovii*, both of the *Petunioides* section, must have some degree of genetic compatibility as demonstrated by the extreme vigor of their hybrids, but again there was no pairing of chromosomes in the reduction division, In the hybrid *N. suaveolens*×*glutinosa* I again found hybrid vigor and non-pairing of chromosomes, but here the parents are not closely related taxonomically.

It is to be noted that regularly in species hybrids of Triticum and other genera, the longitudinal split of the univalent chromosomes occurs during the first division (KIHARA 1924, SAX 1922, THOMSON 1926); but in Nicotiana in all the species hybrids studied no longitudinal split of the univalent chromosomes occurred during the first metaphase.

Non-viability of the zygote in species hybrids

Sterility in plant and animal hybrids is sometimes due to the fact that the zygote dies during its development. Such cases are found very frequently in Drosophila, where they are due to definite lethal or semi-

lethal factors (BRIDGES, MORGAN and STURTEVANT 1925). I have observed some interesting cases of zygote mortality in certain Nicotiana hybrids, such as *N. suaveolens*×*Tabacum* var. *macrophylla*. The cross is easily made and the germination of the seeds is nearly normal. After germination the seedlings start their growth at a rapid rate with a tendency to hybrid vigor until the first true leaves appear. At this stage the seedling growth stops, and after a time the plants die. This phenomenon was confirmed by three or four successive sowings. The leaves of the seedlings were a normal green as if no disturbance had occurred in the chlorophyll development. The root system, however, which was quite normal at the beginning, began to degenerate rather early although the leaves remained green a long time after. This was characteristic of all hybrids which died during vegetative development. This same cross was successful for SAGERET and for GÄRTNER, and their plants reached maturity. It is rather peculiar that in the reciprocal cross the pollinated *Tabacum* flowers dropped soon after pollination, although crosses were almost always successful when using species with the higher chromosome number as female.

The hybrid *N. Tabacum* var. *macrophylla*×*glutinosa* stopped its growth in a later stage, after from 5 to 10 leaves had developed. The root system which was very well developed degenerated with the cessation of growth, but long after that the plant was still green. I was inclined to believe that some pathological organism was responsible for the death of the roots, although the neighboring plants of other crosses were normal. I cut away the old root of several plants, disinfected them in Uspulun, and grew them in sterilized soil under artificial light 3–4 hours during the night, as the day was very short at that time. The plants recovered, and after only one week I was able to see a new root developing normally. New leaves were formed, and in three weeks the plants came into flower. Sister plants growing without artificial light never came to flower. It seems that for some reason metabolism was abnormal, and that this defect was partially nullified by increased exposure to light. This success encouraged me to try defective plants of hybrid combinations under similar conditions, and I was especially hopeful of the hybrid *N. Tabacum* var. *macrophylla*×*plumbaginifola*; but had no success. Thirty plants from two sowings were observed. In both cases the seedlings started with a very rapid growth, but stopped after the 3d or 4th leaf developed.

The single seedlings obtained from *N. Tabacum* var. *macrophylla*×*Langsdorffii* and from *N. Tabacum* var. *macrophylla*×*longiflora* had deformed cotyledons and no visible growing point. The same *N. paniculata*

plant was pollinated by three different *alata* plants and in two of the crosses the seedlings died after developing the fourth true leaf. In this case I am not sure whether they died because of their genetic constitution or because of the greenhouse conditions during the winter, or, perhaps, a combination of both. One plant of *N. Tabacum* var *Cuba* was pollinated by two different *alata* plants and by a *Sanderae* plant. With one of them, *alata*, No. 20, three capsules were obtained, but not a single seed germinated. With *alata*, No. 25, the seedlings produced died in different stages during their development and only two finally reached maturity. With *Sanderae*, No. 16, four seedlings reached maturity. The chromosomes were counted in the root-tips of some of the hybrids seedlings of *cuba*×*alata* No. 25, which died after developing the fourth true leaf and the number was found to be over 30.

From the cross *N. Tabacum* var. *macrophylla*×*nudicaulis* I obtained plump seeds having endosperms, but which did not germinate. I do not know whether the seeds contained embryos or not, as I did not make a histological investigation.

Incompatibility between species in Nicotiana

The degree of compatibility between different species is supposed to be indicated by their taxonomic relationship. This study has shown that, with some exceptions, hybridization is successful more frequently when the species with the higher chromosome number is used as female. While these results are not entirely in accord with those of other investigators, I believe that this correlation holds in Nicotiana. The literature of genetics affords no clearly parallel case. From the hybridization results between the various Triticum species as reported by different investigators, it is not clear in which direction the cross is easier, but it is evident that the species with the lower chromosome number is generally used as the female parent. Of the 26 crosses reported (TSCHERMAK 1914, SAX 1922, KIHARA 1924) in only 4 is the species with the higher chromosome number used as the female. KIHARA reports successful pollination of a species in the Emmer group ($n=14$) by one of the *monococcum* group ($n=7$). The other three cases are reported by SAX who successfully pollinated three species of the *vulgare* group ($n=21$) by three of the Emmer group. Similar results have been reported for Papaverum species crosses, where 4 out of 17 hybrids (FOCKE 1881, LJUNGDAHL 1924) were produced by using pollen of species with the higher chromosome number. Unfortunately, these investigations do not make clear just what crosses were attempted, and which were successful.

CONCLUSION

The chromosome numbers of the different Triticum species (SAKAMURA 1918, SAX 1918) have been found to be what should be expected from the taxonomic arrangement of the species, the interspecific hybridization results, the serological studies, and resistance to parasites. The three sections of the genus are represented by three different numbers, each found only among the species of a given section, and the three numbers establish the polyploid series 2x, 4x, 6x, with the basic number 7. In Fragaria (LONGLEY 1926, ICHIJIMA 1927) the situation is similar. In Nicotiana, however, as in Chrysanthemum (TAHARA 1921), and Rosa (TÄCKHOLM 1922), each taxonomic section contains more than one characteristic chromosome number. Species with an aberrant number of chromosomes in a polyploid series, as in *longiflora* and *plumbaginiolia* in Nicotiana are frequently observed in various genera. KUWADA (1919) assumed for maize that transverse segmentation of some of the chromosomes is a genetically fixed phenomenon. HEILBORN (1924), however, believes that in Carex the higher chromosome number in various related species arose practically always through non-disjunction of single whole chromosomes rather than through fragmentation; and that hybridization was an important factor in the perpetuation of the new chromosome number. If transverse division is to be taken as the explanation of the origin of some Nicotiana chromosome numbers, one may explain the 10 haploid chromosomes of certain species as arising by the occasional transverse division of two long U-shaped chromosomes, and the 12 chromosome type by the transverse division of four long U-shaped chromosomes of certain 8 chromosome species. It is to be noted, however, that the 12 chromosome species have a large number of U-shaped and very long chromosomes.

WINGE's (1917) theoretical interpretation of the origin of new forms by the reduplication of all the chromosomes of two species after hybridization is probably important in connection with Nicotiana species (CLAUSEN and GOODSPEED 1925). The morphological appearance of certain related Nicotiana species indicates that they have a common origin. *Rustica* and *paniculata* differ noticeably only in respect to corolla length and shape, for example. *Nudicaulis* and *trigonophylla* are also similar to each other, and also show the same chromosome relation, 24:12. The three species *glutinosa*, *tomentosa*, and *Rusbyi* show a gradation in the morphological characteristics of the two sections *Tabacum* and *Rustica*, *glutinosa* showing largely *Rustica* characters, *Rusbyi* largely *Tabacum* characters, with *tomentosa* standing in between. Such instances point to hybridization as a possible origination of species.

The possibility that the species have originated as multiples from a type having the basic number 4, might be indicated if the reduction division of any of the hybrids showed this number of bivalents among the univalents or *vice versa*, as has been found in Triticum (SAX, K., and SAX, H., 1924); but no such cytological situation has been found.

SUMMARY

1. Nineteen species of Nicotiana have been studied cytologically, and species with the following haploid chromosome numbers were found: 8, 10, 12, 16, 24. All of these numbers have occurred among the species belonging to the Petunioides section. Among the species of the *Rustica* section, the haploid numbers found were 8, 12, and 24. Differences in shape and size were found among the chromosomes of the different species and in certain cases among the chromosmes of a single species. Counts made in the root-tips confirmed the numbers found in the pollen-mother cells. There are great differences in size and shape between the chromosomes of the somatic cells and those of the pollen-mother cells, the first being very long, entwined, and generally U-shaped, while the latter spherical or bean-shaped.

2. Fifteen species were crossed with the view of discovering if any parallelism exists between their taxonomic relationship and their ability to hybridize. Four degrees of compatibility between the species were distinguished according to the results obtained in the hybridization experiments: (1) Crosses in which the flowers pollinated dropped after the pollination; (2) those in which capsules were produced without seeds, or with seeds which did not germinate; (3) those in which the seedlings died before reaching maturity, and finally (4) those which produced mature hybrids. It was found further that the species with 8 haploid chromosome numbers produced fertile hybrids (as did species with 10 haploid chromosomes); but the ability to hybridize was very low between the species with 12 chromosomes, though less difficult between the species with 24 chromosomes. In crosses between species differing in their chromosome number, success was rare when species with a lower chromosome number were used as female; and in the cases where the cross succeeded in both directions, the percentage of successes was less when the female parent had the lower chromosome number.

3. A morphological description of the species hybrids obtained is presented. In crosses between species with the same chromosome number, the fertile hybrids, generally speaking, were intermediate, but

the great variability of the F_2 plants and the obvious Mendelization of the striking qualitative differences leads one to believe that the genes are distributed as in ordinary varietal crosses. In hybrids between species differing in their chromosome number, the parental type with the greater number of chromosomes was better represented morphologically.

4. In nine of the species hybrids, the reduction division in the pollen-mother cells was studied. Four types of division were found. In *N. alata*×*Langsdorffii* (8×8), the reduction was normal, no univalents being found. In *N. longiflora*×*Sanderae* (10×8), *N. Tabacum Cuba*×*alata* (24×8), *N. Tabacum* white×*Sanderae* Cl_{10} (24×8), and *N. rustica*×*paniculata* (24×12), the number of bivalent chromosomes was equal to the haploid chromosome number of the parent with the lower chromosome number. In *N. rustica*×*N. Tabacum* white tobacco (24×24) a few loose pairs appeared to be present. In *N. paniculata*×*Langsdorffii* (12×8), *N. suaveolens*×*glutinosa* (16×12), and *N. suaveolens*×*Bigelovii* (16×24), no pairing of chromosomes occurred.

In cases in which a regular pairing of chromosomes was observed, the univalents were usually distributed to both poles without splitting during the heterotypic division, though occasionally lagging chromosomes were found. In cases where no pairing was observed, the chromosomes were distributed irregularly to the poles; and in some few cases, particularly in hybrids between species with high chromosome numbers the occurrence of three spindles during the second metaphase was observed. The reduction division of those hybrids in which some few loose pairs occurred during the heterotypic metaphase or before, was most complicated and irregular; and this type of irregularity was observed in a hybrid where the total number of chromosomes was high (24×24); here diads were observed among the tetrads.

The second division was always a regular equational division.

5. The bearing of these results on certain theoretical problems is discussed. Where the reduction division is normal, inheritance appears to be normal. Where the number of chromosomes contributed by the two parents is identical and the reduction division is abnormal, the hybrids show an intermixture of the characteristics of the two parents, presumably because of lack of dominance or because an approximately equal number of dominants is contributed by each parent. Where the number of chromosomes contributed by the two parents is unequal, the hybrids tended to resemble the parent with the higher chromosome number, with but few exceptions. In these exceptional cases, perhaps the con-

tribution of a few striking characters by the species having the lower chromosome number tends to distort the result.

6. The hybrids which reached maturity were completely sterile, with the exception of *N. rustica*×*paniculata*; and this hybrid was partially sterile. Non-viability of the gametes seems to parallel irregular reduction division.

7. The compatibility of the species shows a greater relation to the chromosome number than to the taxonomic status, but the mere fact that two species have the same chromosome numbers does not mean that they will cross. In general a cross can be made more easily when the species with the higher chromosome number is used as the female parent.

8. There is no particular correlation between the vigor of the hybrid and the mode of the reduction, except that in cases where the reduction division is normal, the plants are never weak. Cases were found in which the hybrid was weak although chromosome pairing occurred, as in *N. Tabacum*×*alata*. On the other hand, in *N. Bigelovii*×*suaveolens*, where no chromosome pairing occurred, the hybrid was very vigorous.

9. Weak hybrids can sometimes be aided in their development by a change in external conditions, such as an increase in the relative amount of light.

10. Though the Nicotiana species probably did not originate by multiplication of 4 chromosomes, it is possible that they did originate from one or more 8-chromosome species by a reduplication of certain chromosomes or of whole sets of chromosomes.

After this paper was completed, there was an opportunity for studying cytologically the following two species of the genus:-

N. caudigera Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised are large, branched, with long petioled leaves, cordate below and lanceolate above, having spine-like glandular hairs on the surface and on the margin. The corolla is white with broad cylindrical tube and short, shallow rounded lobes on the limb. In the pollen-mother cells, stained with acetocarmine, the dumbbell shaped chromosomes are constricted so that their number in some stages appears higher than the real one—$x=12$. This was clear on permanent slides where, presumably due to the fixation, the chromosomes were shortened, less closely arranged, and their constriction invisible in the deep hematoxylin stain. The root tip cells contain 24 slender, straight or U-shaped chromosomes

N. solanifolia Wolf–*N. cardiophylla* Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised developed slowly to a short non-branched stem, with many long petioled, broadly ovate and highly undulate leaves. The corolla resembled that of *N. paniculata*, except for being larger and of cream yellow color. The pollen-mother cells stained with acetocarmine, show 12 chromosomes and the root tip sections on the permanent slides show 24 straight or U-shaped chromosomes. Under the name *N. solanifolia* were raised plants from seeds obtained from a commerical source which were quite different from those described above, and resembled more closely *N. rustica* var. *humilis*, except that the corolla was even smaller, somewhat different in shape and slightly violet-tinged on the tube. Their chromosome number was $x = 24$ or $2x = 48$.

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Description of Plates

The drawings except figures 60, 65, 69, were made from single sections with the aid of a camera lucida. All figures in plates I and II were drawn by using the large Zeiss microscope with ocular 10 and oil immersion 1/16. Those of plates III and IV by using compensating ocular 12 instead of 10. As reproduced, all figures have a magnification of 1500 diameters.

Plate 1

Figures 1–15 represent the polar view of the mitotic metaphase of the following *Nicotiana* species:

$2x = 48$

Figure 1.—*N. Tabacum*, var. *macrophylla*.
Figure 2.—*N. rustica*, var. *brasilia*.
Figure 3.—*N. Bigelovii*.
Figure 4.—*N. nudicaulis*.

$2x = 32$

Figure 5.—*N. suaveolens*.

$2x = 24$

Figure 6.—*N. glutinosa*.
Figure 7.—*N. sylvestris*.
Figure 8.—*N. paniculata*.
Figure 9.—*N. glauca*.
Figure 10.—*N. tomentosa*.
Figure 11.—*N. trigonophylla*.
Figure 12.—*N. Palmeri* (?)

$2x = 20$

Figure 13.—*N. plumbaginifolia*.

$2x = 16$

Figure 14.—*N. alata*.
Figure 15.—*N. Langsdorffii*.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 1

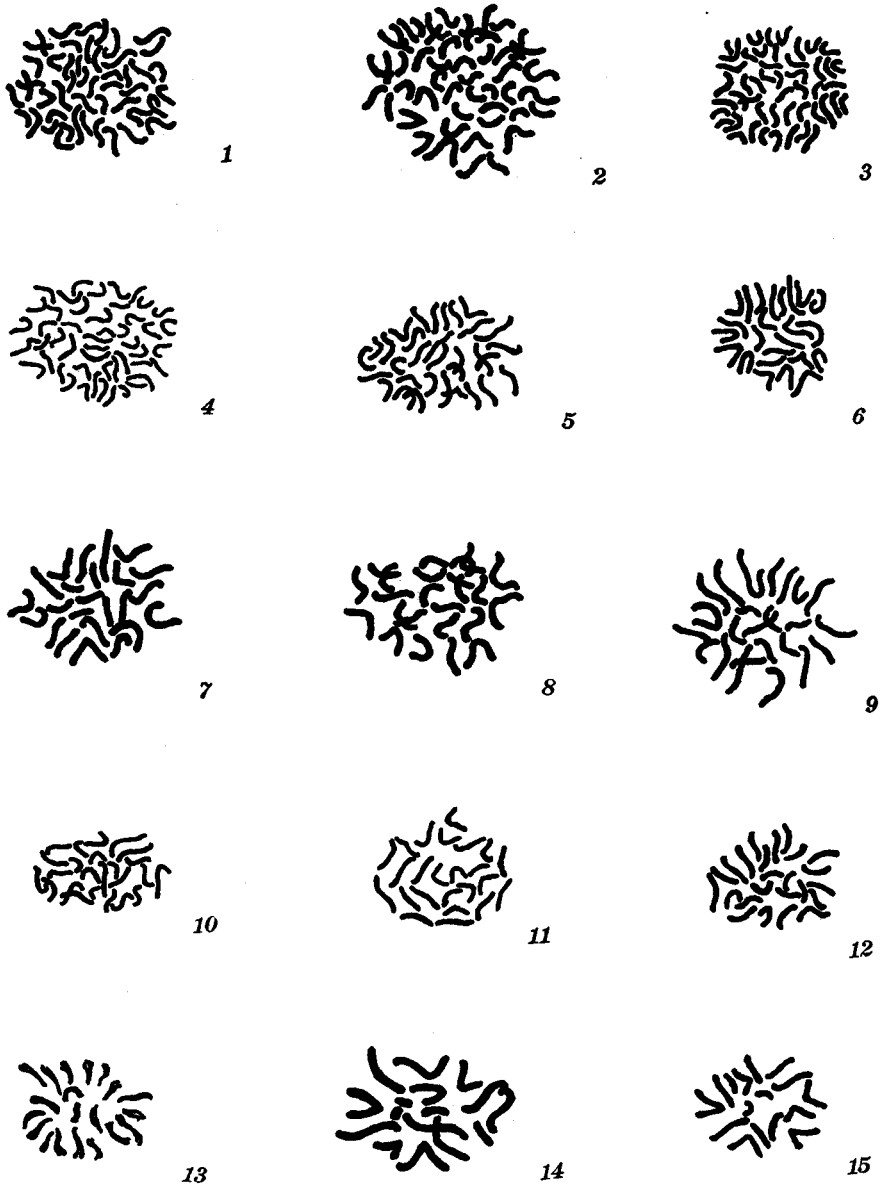

PLATE 2

Figures 16–33 represent the polar view of the heterotypic metaphase of the following Nicotiana species: $x=24$ Chromosomes

FIGURE 76.—*N. Bigelovii.*
FIGURE 17.—*N. quadrivalvis.*
FIGURE 18.—*N. rustica*, var. *humilis.*
FIGURE 19—*N. Tabacum*, var. *macrophylla.*
FIGURE 20.—*N. viscosa* (?)
FIGURE 21.—*N. nudicaulis.*
$x=16$ chromosomes
FIGURE 22.—*N. suaveolens.*
$x=12$ chromosomes
FIGURE 23.—*N. glauca.*
FIGURE 24.—*N. sylvestris.*
FIGURE 25.—*N. paniculata.*
FIGURE 26.—*N. glutinosa.*
FIGURE 27.—*N. acuminata.*
FIGURE 28.—*N. trigonophylla.*
FIGURE 29.—*N. Palmeri* (?)
$x=10$ chromosomes.
FIGURE 30.—*N. longiflora.*
$x=8$ chromosomes.
FIGURE 31.—*N. Langsdorffii.*
FIGURE 32.—*N. alata.*
FIGURE 33.—*N. alata.*
FIGURE 34.—*N. alata*—homeotypic metaphase.
FIGURE 35.—*N. alata*—homeotypic metaphase.
FIGURE 36.—*N. alata*—heterotypic metaphase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 2

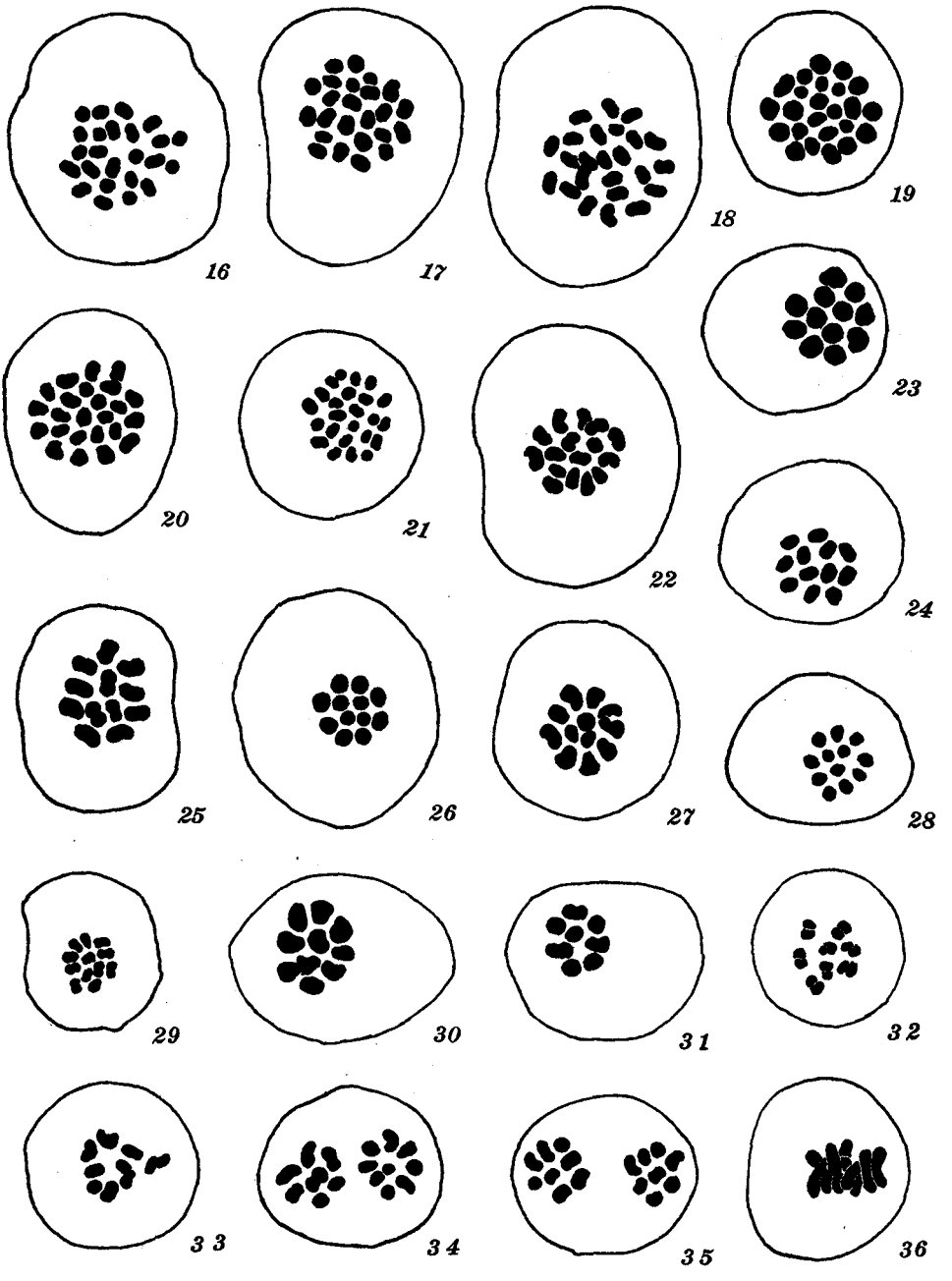

PLATE 3

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. longiflora ($x=10$)×*Sanderae* ($x=8$)

FIGURE 37.—heterotypic metaphase, side view.

FIGURE 38.—homeotypic metaphase.

FIGURE 39.—homeotypic metaphase.

FIGURE 40.—homeotypic anaphase.

N. Tabacum ($x=24$)×*alata* ($x=8$)

FIGURE 41.—very early heterotypic metaphase, polar view.

FIGURE 42.—very early heterotypic metaphase, side view.

FIGURE 43.—heterotypic metaphase, side view.

FIGURE 44.—late heterotypic metaphase.

FIGURE 45.—homeotypic metaphase, polar view.

FIGURE 46.—homeotypic anaphase, side view.

N. paniculata ($x=12$)×*Langsdorffii* ($x=8$)

FIGURE 47.—heterotypic spindle.

FIGURE 48.—heterotypic anaphase.

FIGURE 49.—homeotypic metaphase, polar view.

FIGURE 50.—homeotypic metaphase, polar view.

FIGURE 51.—homeotypic anaphase, side view.

N. rustica ($x=24$)×*paniculata* ($x=12$)

FIGURE 52.—heterotypic metaphase, polar view.

FIGURE 53.—heterotypic anaphase, side view.

FIGURE 54.—homeotypic metaphase, polar view.

FIGURE 55.—homeotypic metaphase, polar view.

FIGURE 56.—telophase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 3

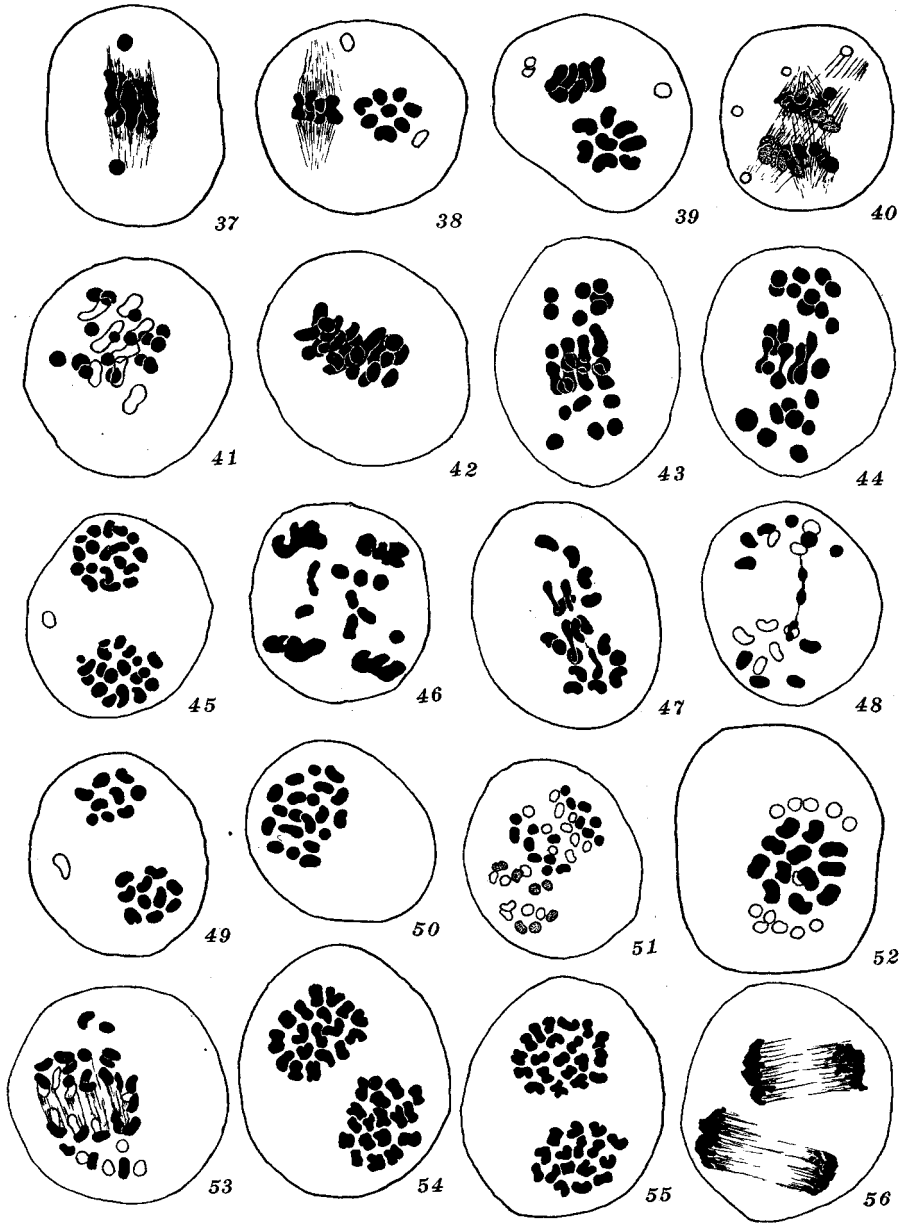

PLATE 4

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. suaveolens ($x=16$)×*glutinosa* ($x=12$)
FIGURE 57.—homeotypic metaphase, polar view.
FIGURE 58.—heterotypic metaphase,side view.
FIGURE 59.—homeotypic metaphase.
N. suaveolens ($x=16$)×*Bigelovii* ($x=24$)
FIGURES 60a & b.—heterotypic spindle.
FIGURE 61.—homeotypic metaphase, polar view.
FIGURE 62.—homeotypic metaphase, polar view.
FIGURE 63.—homeotypic spindles.
FIGURE 64.—octad.
N. rustica ($x=24$)×*Tabacum* var. *alba* ($x=24$)
FIGURE 65a & b.—heterotypic spindle.
FIGURE 66.—homeotypic anaphase.
FIGURE 67.—homeotypic metaphase, polar view.
FIGURE 68.—homeotypic metaphase, side view.
FIGURE 69, a, b, c.—homeotypic anaphase, polar view.

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