INHERITANCE IN CROSSES BETWEEN *NICOTIANA LANGSDORFFII* AND *NICOTIANA ALATA*

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INTRODUCTION

In a rather intensive genetic study of the genus Nicotiana including some sixty inter-specific crosses, the writer has found very few fertile crosses between species whose status would not be questioned by taxonomists. Of these, the one showing the most perfect fertility is to be described in this paper.

Nicotiana Langsdorffii Weinm. and *Nicotiana alata* Lk. and O. are so different from each other in their characters that they were placed by GEORGE DON in the different sections of the genus, that he called Rustica and Petuniodes, and have been kept there by COMES, the most recent monographer of the Nicotianas. The writer agrees with the suggestion of LOCK (1909) that *N. Langsdorffii* should be removed from the Rustica section to the Petuniodes section on the basis of its genetic behavior when crossed with *N. alata*, but the very fact that taxonomists without access to genetic data have seen fit thus to separate them is an indication of a specific distinction not to be questioned except by those who would fuse all types giving fertile hybrids.

Nicotiana Langsdorffii was described by WEINMANN (ROEM. &

SCHULT. Syst. iv. p. 323) from Brazil. It probably has a wide distribution in South America as it has been found in Chile (COMES 1899). The immediate sources of my plants were SETCHELL (1912, his $\frac{22}{02}$) and A. SPLENDORE, Scafati, Italy. I do not know where SETCHELL obtained his plants, and the two strains may be from the same stock. At any rate they are practically identical, both corresponding with the plate in the BOTANICAL MAGAZINE (1825 pl. 2555).

FIGURE 1

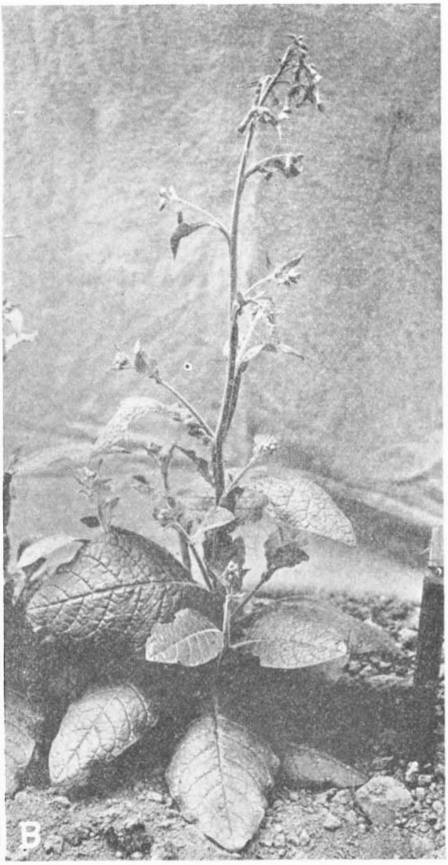

FIGURE 2

FIGURE 1. A young flowering plant of *Nicotiana alata* Link and Otto, var. *grandiflora* Comes.

FIGURE 2. A young flowering plant of *Nicotiana Langsdorffii* Weinm.

The plants are from 120—145 cm in height, vigorous, profusely branched, the branches erect. The basal leaves are 20—30 cm long, obtuse, ovate, sessile, narrowed and decurrent at the base. Upper leaves are lanceolate, and all are extremely rugose above. Inflorescence racemo-paniculate. Flowers are about 20 mm long, very uniform in size; corollas funnel-shaped, a gibbous ring above, the limb concave, spreading, and very slightly notched; greenish yellow, pendulous. The pollen is blue.

Nicotiana alata as described by LINK and OTTO (see Ic. Pl. Rar. I, 63, t. 32. DC. Prodr. XIII. I. p. 567. GARTENFLORA tab. 1010. COMES 1899, p. 35) from Brazil (found in Uruguay and Paraguay according to COMES), I have never seen. The type used in these experiments is the common *N. affinis* Moore (GARDN. CHRON., 1881, p. 141) referred by COMES to the variety *grandiflora*. The variety seems to have no points by which it can be distinguished from the species. It is described as having larger flowers with more perfume, more zygomorphism and less gibbosity than the species, but these are very indefinite and inconstant qualities.

The strain with which our crosses were made has plants 110-130 cm high, appearing shorter because of the loosely spreading habit. Basal leaves are acute, ovate, quickly narrowed to a slightly decurrent base, slightly rugose; upper leaves lanceolate to linear. Inflorescence is a raceme. Flowers are 75—95 mm long, tube gradually enlarging toward the limb and slightly gibbous at the top, light greenish yellow faintly lined with purple; limb broadly expanded into obtuse, ovate lobes the lower two being distinctly smaller than the other three and giving the flower a decidedly zygomorphic form. The corolla limb is pure white on the inside and cream with sometimes a tinge of purple on the outside. One anther is usually somewhat shorter than the others. The pollen is white or yellowish. Some plants are self-fertile, others are completely self-sterile.

EARLY WORK

These two species were crossed and studied by at least three of the earlier hybridizers, NAUDIN, GODRON and FOCKE. Concerning their results, I quote FOCKE (1881):

"*N. alata* Lk. × *Langsdorffii* Weinm. GÄRTNER found no foreign species with which he was able to fertilize *N. Langsdorffii*. Reciprocal crosses between *N. alata* and *N. Langsdorffii* are not difficult, however; NAUDIN obtained especially good, well filled capsules by fertilizing *N. Langsdorffii* with pollen from *N. alata*, and although only one pollination of *N. alata* with *N. Langsdorffii* pollen was successful, in this case also a large capsule full of seeds matured. I found no difficulty with either cross. Of *N.*

Langsdorffii ♀ × *N. alata* ♂ (*N. Persico-Langsdorffii* Naud. l. c. p. 74) NAUDIN produced 118, and of *N. alata* ♀ × *N. Langsdorffii* ♂ (*N. Langsdorffii-Persica* Naud.) 53 examples; all of which were exactly like one another. They were 130—160 cm high (*N. Langsd.* ca. 100; *N. alata* 60 cm) and because of their spreading branches more nearly resembled *N. Langsdorffii.* The blossoms were medium large, greenish white, with the limbs distinctly rounded. Pollen bluish gray. Fruitfulness perfect. I have made the same crosses with like results. Pollen was plentiful and the grains well-formed. The capsules contained in the neighborhood of 500 seeds.

"NAUDIN'S hybrids were to be distinguished from *N. commutata* by their higher stature, their larger and more greenish flowers, and their darker leaves.

"*Later generations.* Through continued self-fertilization, NAUDIN'S hybrid plants gradually returned toward the condition of the parent species, although this was never fully reached. GODRON received from ALEX. BRAUN of Berlin, seed of *N. alata-Langsdorffii* (as well as of *N. Langsdorffii* ♀ × *N. alata* ♂) and raised many forms from it; among others were varieties with yellow, with cream, and with pure white flowers. The leaves were variable, the decurrence at the stem being sometimes very pronounced, sometimes just traceable and sometimes lacking.

"Two varieties in which crossing had been prevented by gauze produced fruits whose seeds reproduced the mother form exactly."

From this extract, it is clear that FOCKE was familiar with the facts that in the cross under consideration—as well as in other crosses—the F_1 generation is more vigorous than either of the parent species, that the population is uniform and the individual plants fertile, and finally that the F_2 and following generations are variable and may produce plants having a striking resemblance to the original parents. In this he was merely copying NAUDIN. Both NAUDIN and GODRON perceived the essential facts of inheritance in hybrids much more clearly than other contemporary hybridizers, and we may be assured that had NAUDIN had an opportunity of reading MENDEL'S paper, as did NÄGELI, he would have appreciated its significance. He came very close to an enunciation of what we now know as the Mendelian laws, but either he lacked the ability for mathematical analysis that characterized MENDEL because of the latter's training in physics, or was prevented from making such an analysis by the greater complexity of the hybrids he studied.

NAUDIN (1865) says of his cross, *Nicotiana Persico-Langsdorffii:*

"The two plants here united, although very different at first sight, have distinct analogies in their habit of growth, the form of their leaves, their general aspect, and up to a certain point, in their long, tubular, pendent flowers. One feels these analogies more strongly if he remembers that there exists a form exactly intermediate between the two (*N. commutata,* Fisch.), of which I shall speak later. As well as I can judge by the descriptions, *N. Persica,* of which there is a pretty good figure in the BOTANICAL REGISTER, pl. 1592, appears to be identical with the *N. alata* of DUNAL;

not being sure, however, I have preserved the name that it carried at the Museum."

NAUDIN wished to see whether *N. commutata* was a natural hybrid and if he could reproduce it by this cross. He obtained 118 plants from his cross, "all of the most uniform appearance" and from 130-160 cm high. He concludes that *N. commutata* is not the hybrid *N. Persico-Langsdorffii,* "at least of the first generation." Since he made this conclusion solely on account of the greater height and vigor of his artificial hybrids, it must be that he suspected that he might have duplicated *N. commutata* when by inbreeding his plants had lost their hybrid vigor. I have duplicated plants of *N. commutata* grown from seed received from Dr. SPLENDORE of Scafati, Italy, several times in my own crosses and have obtained F_3 families that bred as true to the form (intermediate between *N. alata grand.* and *N. Langsdorffii*) of the so-called *N. commutata* as did the species (?) itself.

NAUDIN found that the reciprocal cross was so nearly like the other that "without the labels the two lots would have been taken the one for the other." Unfortunately, however, although these crosses were perfectly fertile, NAUDIN did not self them and continue his observations. On the other hand, he did obtain some information regarding later generations by a consideration of the volunteer seedlings that appeared during the next few years on the plat that had borne the original cross. He says:

> "Without having given these hybrids of the second and the third generations the attention they merited, I have noticed that their forms became more and more divergent, some approaching *N. Persica* and others distinctly tending toward *N. Langsdorffii.*"

Some of these plants he potted, and obtaining seed from one that resembled *N. Langsdorffii* he grew a population that bred true to a type that could scarcely be distinguished from *N. Langsdorffii.* These experiments were continued, and from seed of this generation, he raised in 1863, fifty plants nearly all of which "had returned to the type well known as *N. Langsdorffii.*"

Thus it is seen that NAUDIN observed nearly all the essential facts of Mendelian heredity in this one cross,—a uniform F_1 generation, a segregating F_2 generation, and a later generation which showed that certain of the extreme segregates bred true. But the observations on this particular cross are not so important as the general conclusions to which NAUDIN was led by his broad experience as a hybridizer. Under the heading "Physionomie des hybrides," he says:

"In order to have a correct idea concerning the phenomena presented by hybrids, it is essential to distinguish between the first generation and those that follow.

"I have always found, in the hybrids I have made myself or of whose origin I was certain, a great uniformity of aspect between individuals of the first generation and originating from the same cross no matter what their number. This fact we have seen exemplified in *Petunia violaceo-nyctaginiflora, Datura Tatulo-Stramonium* and *D. Stramonio-Tatula, D. Meteloido-Metel, D. Stramonio-lœvis,* etc., *Nicotiana Texano-rustica* and *rustico-Texana, N. Persico-Langsdorffii,* etc.; having already emphasized these resemblances it is useless for me to dwell upon them."

"In fact, one may say that hybrids of the first generation resemble each other as much or nearly as much as the individuals that come from a single legitimate species."

(It is well to note that the hybrids with which NAUDIN supports his thesis here are all between solanaceous species that are generally self-pollinated naturally, and may be presumed to approach homozygosis.)

"Beginning with the second generation, the aspect of hybrids is changed in a remarkable manner. Ordinarily, the perfect uniformity of the first generation is succeeded by a regular medley of forms, some approaching the specific type of the father, others that of the mother, a few returning suddenly and entirely to the one or the other form. At other times, this progress toward the original types is by degrees and slowly, and sometimes one sees a whole collection of hybrids incline toward the same side. The important fact, however, is, that it is the second generation, in the great majority of cases (and perhaps in all), that starts this dissolution of the hybrid forms, a phenomenon recognized by many investigators, doubted by others, but which appears to me to-day to be established beyond argument. We shall explain the cause in the following paragraph."

"All of the hybrids of which I have studied the second generation with some care, have shown these changes in appearance and have manifested this tendency to return to the forms of the original species, and this when conditions have been such that the pollen of the species themselves could not have been the cause. We have seen striking examples of it in *Primula officinali-grandiflora,* in all of the hybrids of *Datura Stramonium,* in *D. Meteloido-Metel,* in the reciprocal hybrids of *Nicotiana angustifolia* and *macrophylla, N. Persica* and *Langsdorffii, Petunia violacea* and *nyctaginiflora,* in *Luffa acutangulo-cylindrica,* and further in *Linaria purpureo-vulgaris.* In the second generation of several of these hybrids there has been a complete return to one or the other or to both of the parent species together with individuals approaching each species in varying degrees; in other cases also we have seen intermediate forms continued at the same time that other specimens from the same family have effected the return of which I speak. Moreover, we have observed cases (*Linaria purpureo-vulgaris* of the third or fourth generation) of actual retrogression toward the hybrid form, sometimes a plant that had apparently returned entirely to one of the two species, has even given rise to individuals that very nearly resembled the other species. All of these facts are explained naturally by the *segregation* (disjunction) *of the two specific essences* in the pollen and the ovules of the hybrid."

Space may not be claimed to show just how NAUDIN'S views differed from those of MENDEL, except the bare statement that he did not grasp the idea of a unit-character inheritance. Our quotations are already somewhat lengthy. They may well be pardoned, however, since they are taken from a paper not readily available to most geneticists, and have a considerable theoretical and historical interest. But it was not for this alone that I have used them, nor because they contain observations upon the particular cross that is the subject of this paper. And in passing let me say that there is scarcely a doubt but that NAUDIN'S *Nicotiana Persica* and *N. Langsdorffii* are the same as our own *N. alata grandiflora* and *N. Langsdorffii*. The particular reason for the citations is this: While it is to be hoped that with the fruitful hypotheses of modern biology as guides, contemporary genetic research is to be more productive than that of the early nineteenth century hybridizers, it must not be forgotten that very often we are merely repeating more carefully, more quantitatively and with a better idea of relative values, the experiments of these pioneers. The observations of such men as NAUDIN have been confirmed and as far as they go are usually correct. For this reason I think that we may accept their facts until the same experiments have been repeated more carefully and have given us more precise data. This being true, there is no question but that these numerous observations on hybrids between species belonging to so many different groups, showing as they do all the essential phenomena of Mendelian inheritance, go far toward proving Mendelian heredity in quantitative characters.

The only recent work upon *N. Langsdorffii-alata* hybrids is that of LOCK. LOCK made a number of crosses between species of Nicotiana in the years 1906-8, but published only one paper (1909) on the subject. He crossed *N. alata* and *N. Langsdorffii* reciprocally, made several back-crosses, and studied the selfed progeny of the F_1 generation. He noted the uniformity of the F_1 generation and the variability of the F_2 generation and reported a few measurements of the flowers. He established the dominance of blue pollen over yellow and of yellow corollas over white. He also believed that the facts indicated the dominance of gibbous over funnel-shaped corollas.

INHERITANCE OF POLLEN COLOR AND OF FLOWER COLOR

In a cross such as this between two distinct species it is important to know whether any distinct qualitative difference shows a Mendelian behavior. I found only two such differences, pollen color and flower color, and have corroborated the results of LOCK in regard to them. I was not able to corroborate his conclusions in regard to corolla shape, as F_2 plants all showed some development of the gibbous condition. And it seems to me that this was to be expected for it is characteristic of both species.

N. alata has yellow pollen and *N. Langsdorffii* blue pollen. No matter which way the cross is made the pollen is blue. The pollen of the heterozygotes is often lighter than that of the pure *N. Langsdorffii,* but not invariably so. Microscopical examination showed no distinctly yellow grains on the F_1 plants so that, like pollen shape in Lathyrus, the color is a sporophytic character. This is less astonishing than the phenomenon in the sweet pea, for it is well known that pollen color is a tapetal deposit. One F_2 consisted of 342 plants with blue pollen and 100 plants with yellow pollen. Counts of smaller segregating populations corroborated these results, though there was an excess of blues in all but one case, an F_3 family consisting of 39 blue and 22 yellow. Just what this excess of blue-pollened plants means, I am unable to say. It may be only a technical difficulty, as the anthers of both species are blue. At any rate, there seems to be no possibility of other factors being concerned directly. Yellow-pollened plants have never given blues.

The flower of *N. Langsdorffii* is greenish yellow both outside and inside the corolla. *N. alata,* on the other hand, though slightly greenish yellow with sometimes a faint tinge of purple on the outside of the corolla, is pure white on the inside of the limb. Apparently the cells just beneath the epidermis on the inside of the limb of these flowers contain no colored chromatophores and very few plastids of any sort. The flowers of the F_1 hybrids (made either way) are cream-colored, but appear to be variable because the old flowers are so light as to be sometimes mistaken for whites. Further the smaller-flowered plants appear to be a darker yellow owing to a concentration of chromoplasts which show through the upper two layers of cells. There is no question but that the inheritance of these differences is Mendelian, but it is not certain that only one factor is involved. Three F_2 populations gave ratios of 196 yellows to 61 whites, 50 yellows to 15 whites, and 57 yellows to 15 whites respectively. A heterozygous F_3 family also gave a ratio of 112 yellows to 29 whites, but one of our F_2 families produced 70 yellows to 6 whites. This constant excess of yellows leads one to suspect complications, but it can be said that no white ever produced yellows after self-pollination, though a number of such families were grown.

It is possible that there is a correlation between small flowers and yellow color though this could not be established.

FERTILITY OF THE HYBRIDS

These slightly distorted ratios give some cause for the surmise that differential fertility exists among the gametes and the zygotes. That all the possible gametic constitutions mature at spermatogenesis cannot be asserted without a cytological study of the early stages. The capsules on the F_1 plants were well filled, however, and the germination of the seeds was between 90 percent and 100 percent.[1] This seems a fair proof that the ovules were all functional and that there was no selective elimination of zygotes.

On the other hand, all of the pollen produced by the F_1 plants and of the plants of later generations was not well formed. An examination of the pollen of 20 F_1 plants after having simply shaken it out on slides showed both when dry and in glycerin or in sugar solutions that apparently functional pollen grains existed in percentages varying from 70 to 96. Of course one cannot say that all of these seemingly well-formed pollen grains are functional, as DORSEY (1915) has shown that in certain Vitis species they sometimes contain no generative nuclei; but since in nearly all the plants there are around 85-90 percent perfectly formed pollen grains one may be fairly certain that if much selective elimination of gametes occurs it occurs before the pollen grains are formed, for the parent species themselves show only from 80-90 percent of well formed grains.

HEIGHT

Nicotiana Langsdorffii (328) and *N. alata* (321) are nearly the same height,—about 132 cm and 120 cm respectively,—but they are very difficult to measure owing to their becoming so profusely branched during

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[1] GOODSPEED (1913) has criticized a table published by EAST and HAYES (1912, p. 28) entitled "Condition of hybrids in crosses between species of Nicotiana" because a number of Nicotiana hybrids were tabled as showing 100 percent germination. This table was published to indicate the general type of certain hybrids with regard to vigor, and I think served its purpose. It was distinctly stated, however, (p. 29) that "the voluminous data that have been collected on these hybrids have been condensed and approximated so that they include only facts germane to the matter in hand." It would seem that it might have been clear to GOODSPEED from this statement that these germinations were only classes. Possibly it would have been better to have said germination "high, medium, low and failing," but it does not seem to me that the readers were led far astray. As a matter of fact the germinations tabled as 100 percent, included all hybrids that tested over 90 percent.

the latter part of the season. The F_1 generation was as uniform as either parent and showed distinct evidence of hybrid vigor. The plants varied from 140 cm to 160 cm. In the second hybrid generation there was no evidence of segregation into distinct types, either as regards height or general habits of growth but plants varied from below the height of No. 321 (extremes about 100 cm) to that of the F_1 generation.

RAPIDITY OF GROWTH

Though both of these species continue flowering until frost, *N. Langsdorffii* commences flowering earlier than *N. alata*, when planted at the same time, and owing to the multitude of ripe capsules formed, takes on a more mature appearance in September. The variation in time of flowering within each species is very slight. In seasons with normal rainfall, sunlight and heat, plants of *N. Langsdorffii* planted in the greenhouse at the same time and set in the field on the same day, come into blossom within three days of each other. Plants of *N. alata* treated in the same manner, show greater variation, sometimes a week elapsing between the time that extremes begin blossoming. The F_1 plants are as uniform in this respect as *N. Langsdorffii* and are slightly earlier. The F_2 plants, on the other hand, are more variable than those of *N. alata*, and this variability is not wholly an effect of environment as is beautifully demonstrated by the F_3 cultures. Ten progeny rows from different F_2 plants showed a difference of 25 days in the time the plants began to flower. Four of the families were variable like the F_2 population, but the remainder were very uniform within the family. On the fifteenth of July two families were in full blossom without an exception, one family had just begun to bloom, one family had the central stalks well advanced and two families were in the rosette stage.

LEAVES

In general the shape of the leaves of both of these species is the same. The basal leaves of *N. alata*, however, are acute, with redundant, folded margins, while those of *N. Langsdorffii* are obtuse and not redundant at the margins. *N. Langsdorffii* is much more rugose than *N. alata*. Both species are decurrent. The tips of the leaves of the F_1 plants are intermediate, but in other qualities the leaves are like those of *N. alata*. The F_2 plants run the whole gamut of these variations. There are plants, the rugosity of whose leaves is like *N. Langsdorffii*, that are like *N. alata* in other respects (except that the flowers are smaller).

TABLE I

Frequency distribution of corolla length in the families of cross 328 × 321.

Pedigree Number	Size in parent	Class Centers in millimeters																										No.
		19	22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	
328, (1914)		10	41																									51
328, (1911+1914)		13	56																									69
321, (1911)																				3	8	10	11	8	5	3	1	49
(328×321) F_1							1	4	24	16	1																	46
(328×321)—1 F_2	41		1	1	5	35	52	58	45	35	13	6	2	1	0	1	1											256
(328×321) total F_2			2	3	13	65	110	132	107	82	33	15	6	7	0	4	2											581
(328×321) 1—1 F_3	23		40	10																								51
(328×321) 1—2 F_3	37					1	24	25																				50
(328×321) 1—3 F_3	40						4	11	26	6	1																	48
(328×321) 1—4 F_3	63								1	3	14	10	17	11	7	5	3											71
(328×321) 1—41 F_3	60							1	1	5	12	17	9	11	7	2	1	0	0	0	1							67
(328×321) 1—5 F_3	54							2	0	7	5	2	1	3	1	2	1	1										25
(328×321) 1—6 F_3	60							2	2	9	27	29	29	17	28	15	3	3	1	3								168
(328×321) 1—7 F_3	21	11	39																									50

Those plants that have returned to the *N. Langsdorffii* habit and size of flower, however, always have rugose leaves though they may be acute.

In all of these respects the reciprocal crosses are so nearly alike that it is impossible to distinguish between them.

COROLLA LENGTH

As I have explained in other papers (East 1916 a, b), corolla length is an excellent character upon which to make genetic studies because of the very slight effect produced by environmental differences. Corolla measurements of single flowers when taken with due precautions as to uniformity of age of plant, age of flower, position of flower, etc., well represent the phenotypes of the plants concerned.

Table 1 gives the frequency distributions of such measurements upon populations of the pure species, the cross when *N. Langsdorffii* was used as the female, a single F_2 population, the total of several F_2 populations and eight F_3 families. Only three of these distributions contain as many individual measurements as I should like, and one—(328 × 321)1-5—must be discarded entirely on account of the small number of plants.

The statistical constants for these distributions are shown in table 2.

Table 2

Statistical constants for the frequency distributions of corolla length shown in table 1.

Pedigree Number	Size in parent	Mean	S. D.	C. V.	No.
328, (1914)		21.41 ± .11	1.19 ± .08	5.56 ± .37	51
328, (1911 + 1914)		21.43 ± .10	1.17 ± .07	5.46 ± .31	69
321, (1911)		81.76 ± .49	5.08 ± .35	6.21 ± .42	49
(328 × 321) F_1		40.78 ± .22	2.20 ± .15	5.39 ± .38	46
(328 × 321) — 1 F_2	41	37.77 ± .24	5.63 ± .17	14.91 ± .45	256
(328 × 321) total F_2		38.30 ± .17	5.99 ± .12	15.64 ± .32	581
(328 × 321) 1 — 1 F_3	23	22.65 ± .12	1.24 ± .08	5.47 ± .37	51
(328 × 321) 1 — 2 F_3	37	35.44 ± .15	1.62 ± .11	4.57 ± .31	50
(328 × 321) 1 — 3 F_3	40	39.31 ± .25	2.54 ± .17	6.46 ± .44	48
(328 × 321) 1 — 4 F_3	63	52.04 ± .44	5.52 ± .31	10.61 ± .61	71
(328 × 321) 1 — 41 F_3	60	51.02 ± .51	6.16 ± .36	12.07 ± .71	67
(328 × 321) 1 — 5 F_3	54	49.24 ± 1.09	8.05 ± .77	16.35 ± 1.60	25
(328 × 321) 1 — 6 F_3	60	52.79 ± .35	6.79 ± .25	12.86 ± .48	168
(328 × 321) 1 — 7 F_3	21	21.34 ± .12	1.24 ± .08	5.81 ± .39	50

As may be seen, *N. Langsdorffii* (No. 328) has a very low variability. This is to be expected, for *N. Langsdorffii* is practically always self-fertilized naturally. *N. alata* is often self-fertilized, but evidence of considerable cross-fertilization has been found by observing the actions of Sphingidae in the evening, by isolating plants, and by self-sterility studies. Though the coefficient of variability (6.21 ± .42 percent) is almost as low as that of No. 328, therefore, it is probable that No. 321 is not so nearly homozygous. Furthermore, the number of individuals measured is small. On the other hand, since a single plant of No. 321 was used in the cross, it is possible that the true variability of this "blood" introduced, is somewhat smaller than that represented by the frequency distribution.

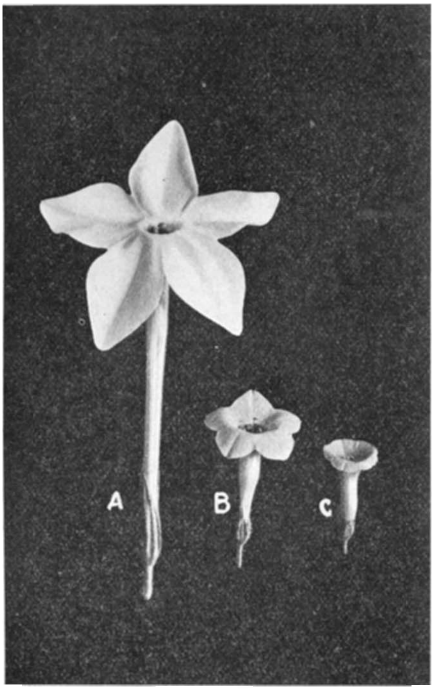

FIGURE 3

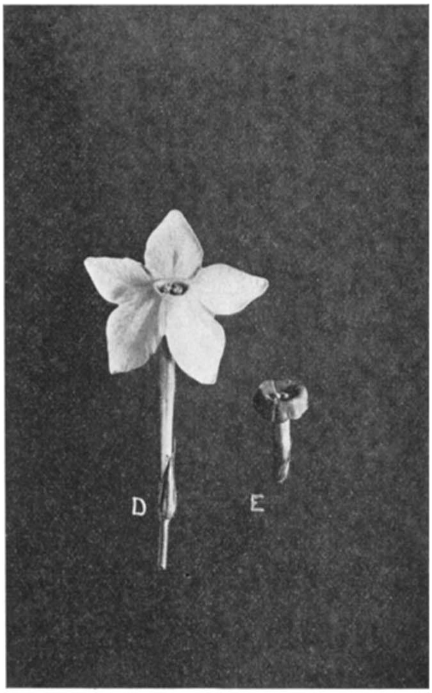

FIGURE 4

FIGURE 3. A, *N. alata grandiflora;* B, F_1 of *N. Langsdorffii* × *N. alata grandiflora;* C, *N. Langsdorffii* (1911) × ½.
FIGURE 4. D and E, extremes of the F_2 generation (1912) × ½.

Curiously enough the mean of the F_1 population is smaller than the average of the two parents. Thus there apparently is no effect of heterosis on the flowers. The square root of the F_1 mean is more nearly that of the average of the square roots of the means of the two parents, but I do not feel justified in attaching any significance to the fact.

The coefficient of variation of the F_2 generation is nearly three times that of the F_1 generation. Though extremes like each parent were not produced, it is hardly possible to see any other cause for this great difference in variability than segregation and recombination of Mendelian factors. From the theory of probability one might expect to recover both parents with a comparatively small number of F_2 plants, but the variability of F_1 is so small that even the plants obtained in F_2 could not be expected in the F_1 if the whole of New England were planted with them.

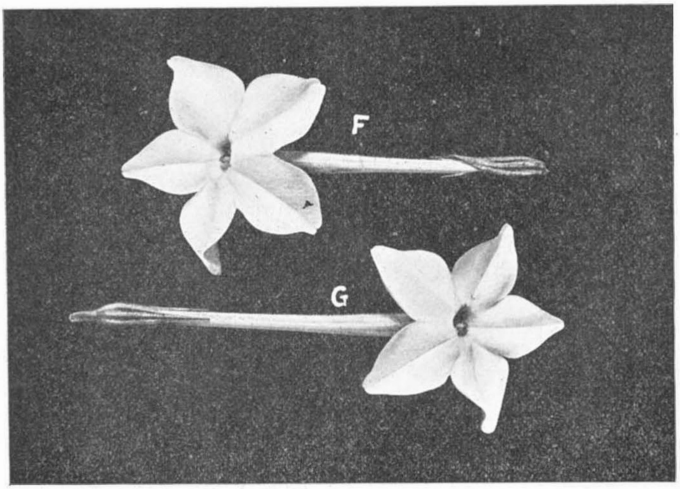

FIGURE 5. F, extreme of the F_3 generation, and G, pure *N. alata grandiflora* (1913) × ½.

In the F_3 generation there was regression toward the mean of the F_2 population in six out of seven cases (excluding No. 1-5 on account of the small number of plants), *but the greatest extremes gave the least regression.* The coefficients of variability were lower than that of F_2 in every family, and three of them bred as true as the parental species. F_3 family No. 1-7 reproduced *N. Langsdorffii* exactly.

The Mendelian theory calls for the production of the same type of F_2 population no matter what F_1 parent is selected, when the original individuals entering the cross are homozygous. Critics of the use of the Mendelian terminology in crosses involving size characters have maintained, however, that small F_1 individuals will give F_2 populations

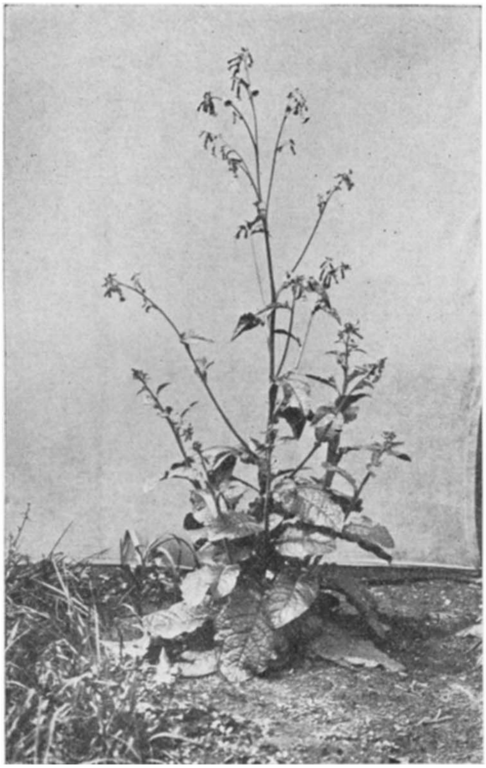

FIGURE 6

FIGURE 7

FIGURE 6. Individual plant produced by an F_2 segregate that was like *N. Langsdorffii* in every detail (E of figure 4).

FIGURE 7. Progeny row showing uniformity of F_3 family to which the plant shown in figure 6 belonged.

with lower means than will large individuals. As there are many indications that the plants entering into this cross were very nearly true homozygotes I have endeavored to test this proposition. Of course, as might be expected by pro-Mendelians, in such a cross the variability of the F_1 population is so low that the extremes selected differed by only 8 mm. Nevertheless five F_2 frequency distributions from different F_1 parents are presented in table 3. The statistical constants shown in table 4 emphasize the fact that the means and the standard deviations

TABLE 3

Frequency distributions of corolla length in five F_2 families of cross 321 × 328 — 1.

Pedigree Number	Size in parent	Class centers in millimeters															No.
		22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	
(328 × 321) — 1	41	1	1	5	35	52	58	45	35	13	6	2	1	0	1	1	256
(328 × 321) — A	44	1	1	3	9	27	25	24	11	2	3	1	1	0	1		109
(328 × 321) — 2	40			1	6	8	18	11	11	7	1	2	0	0	2		67
(328 × 321) — 3	36		1	2	7	13	18	16	12	6	1	0	1				77
(328 × 321) — 4	39			2	8	10	13	11	13	5	4	1	4	0	0	1	72

TABLE 4

Statistical constants of the corolla length in the five F_2 families reported in table 3

Number	Size in parent	Mean	S. D.	C. V.	No.
(328 × 321) — 1	41	37.77 ± .24	5.63 ± .17	14.91 ± .45	256
(328 × 321) — A	44	37.55 ± .36	5.65 ± .26	15.05 ± .70	109
(328 × 321) — 2	40	39.73 ± .52	6.32 ± .37	15.91 ± .95	67
(328 × 321) — 3	36	38.21 ± .40	5.21 ± .28	13.63 ± .75	77
(328 × 321) — 4	39	40.08 ± .56	7.11 ± .40	17.24 ± 1.00	72

very nearly overlap. In other words the curves are very nearly identical, and it can be shown mathematically that the probability is very high that they are all samples of the same population. The similarity of the curves is shown graphically in figure 9. The points of the theoretical curves of these five F_2 populations were calculated and are shown in comparison with the theoretical curve of the total distribution of all F_2 observations. The extreme classes are so nearly identical that curves could not be distinguished when drawn super-imposed, so that only the points are indicated. Where no points for a particular curve are given it is understood that they lie on the single curve of total observations which is drawn.

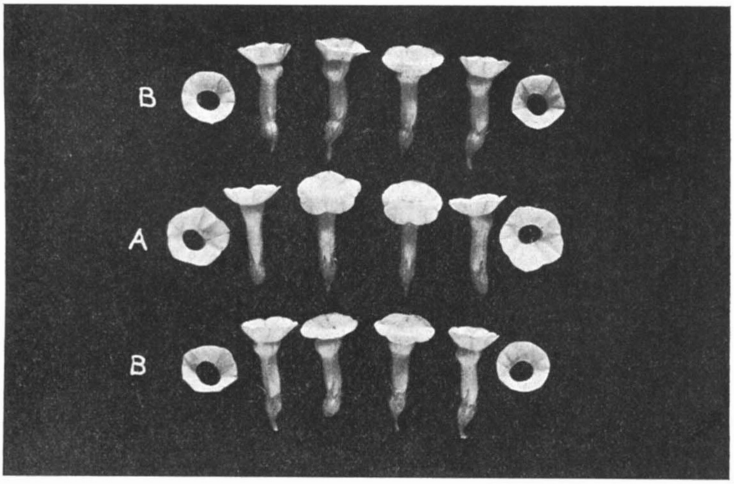

FIGURE 8. A, a random sample of *N. Langsdorffii* flowers from six different plants; B, a random sample of flowers from twelve different plants from the progeny row shown in figure 7 (× ½).

Certainly no one can well maintain that these curves show any decided difference when the probable errors are taken into consideration. There is no dissimilarity in variability like that shown by F_3 populations from different points on the F_2 curve. The mean of the population from the 36-mm parent is higher than that from the 44-mm parent. This fact is not to be taken as significant; it is merely a coincidence. It is very evident that the only just conclusion is that selection has no effect.

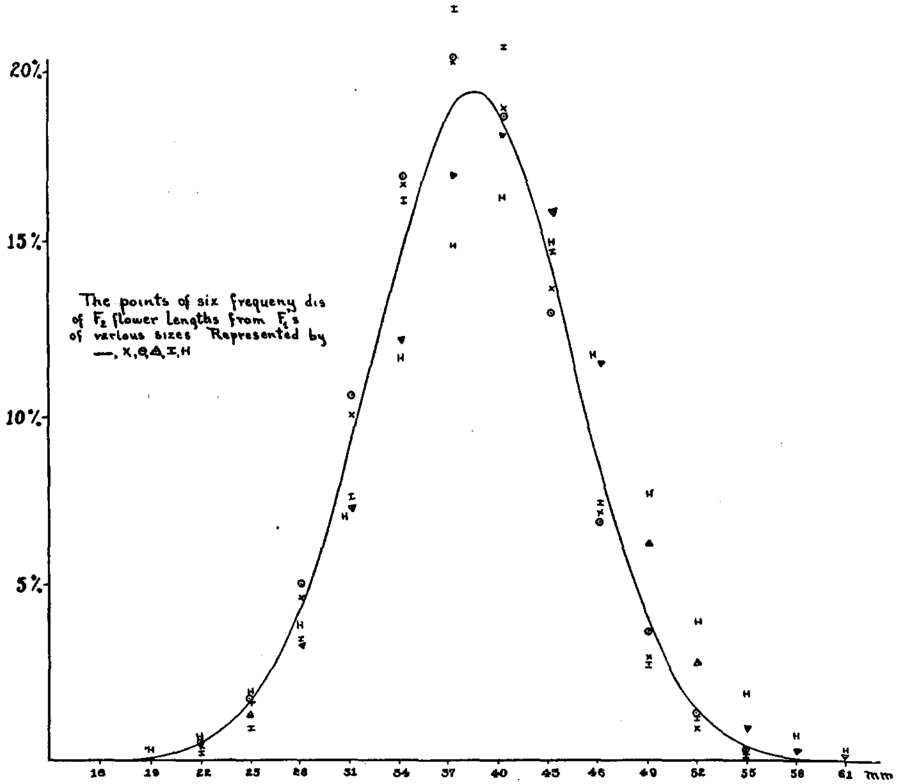

FIGURE 9. The points of the theoretical curves for flower length, calculated from the data obtained from growing F_2 populations from F_1 individuals of various sizes in cross 328 × 321. The curve drawn in full is that calculated from the combined observations.

In tables 1 and 2 one may notice an F_3 family, No. (328 × 321)1—7, that seems to have repeated the small parent, *N. Langsdorffii*. This was indeed the case. Several F_2 plants duplicated *N. Langsdorffii* in every feature and two of them were selfed and their progeny grown. Family No. (328 × 321)1—1 from the larger of these two plants as regards flower size was very uniform, but the flowers were slightly larger than those of the *N. Langsdorffii* strain used, and the plants differed from it slightly in other ways. In short, it could hardly be maintained that the great-grandparent had been duplicated. On the other hand family (328 × 321)1—7 was exactly like a family from a selfed plant of No. 328. I could not find a distinguishing trait by the most minute examination. Figures 6, 7 and 8 show this fact plainly, but since photographs of the other small family would appear to show the same thing, so small are the differences between it and our strain of No. 328, a table of corolla measurements has been introduced in order to demonstrate the matter quantitatively.

Since it is obvious that the use of 3-mm classes in table 1—classes as small as can be treated conveniently in connection with such great size difference—obscures somewhat the true distribution of the corolla length of No. 328, table 5 gives the distribution of the corolla lengths of the same plants in 1-mm classes. This brings out the wonderful uniformity of the populations of both No. 328 and No. (328 × 321)1—7 and the marvelous similarity between the two families. Furthermore, it shows how similar are two populations of No. 328 grown from the same seed but in different years.

TABLE 5

Frequency distribution of No. 328 and of an F_3 family that bred true to the characters of this species.

Pedigree Number	Class centers in millimeters					Mean	S. D.	C. V.	No.
	19	20	21	22	23				
328, (1911)		3	12	1	2				
328, (1914)	1	9	33	7	1	20.96 ± .06	0.69 ± .05	3.27 ± .21	51
328, total	1	12	45	8	3	21.00 ± .06	0.72 ± .04	3.43 ± .20	69
(328 × 321) 1 — 7 F_3		11	33	6		20.90 ± .05	0.57 ± .04	2.75 ± .19	50

THE RECIPROCAL CROSS

The cross in which No. 321 was used as the mother, was not a true reciprocal of the other in that the same individuals were not used. In fact a different strain of *N. Langsdorffii* known as No. 328—1 was used, which had flowers slightly smaller than No. 328. For this reason as well as that each generation of this cross was grown a year later than the

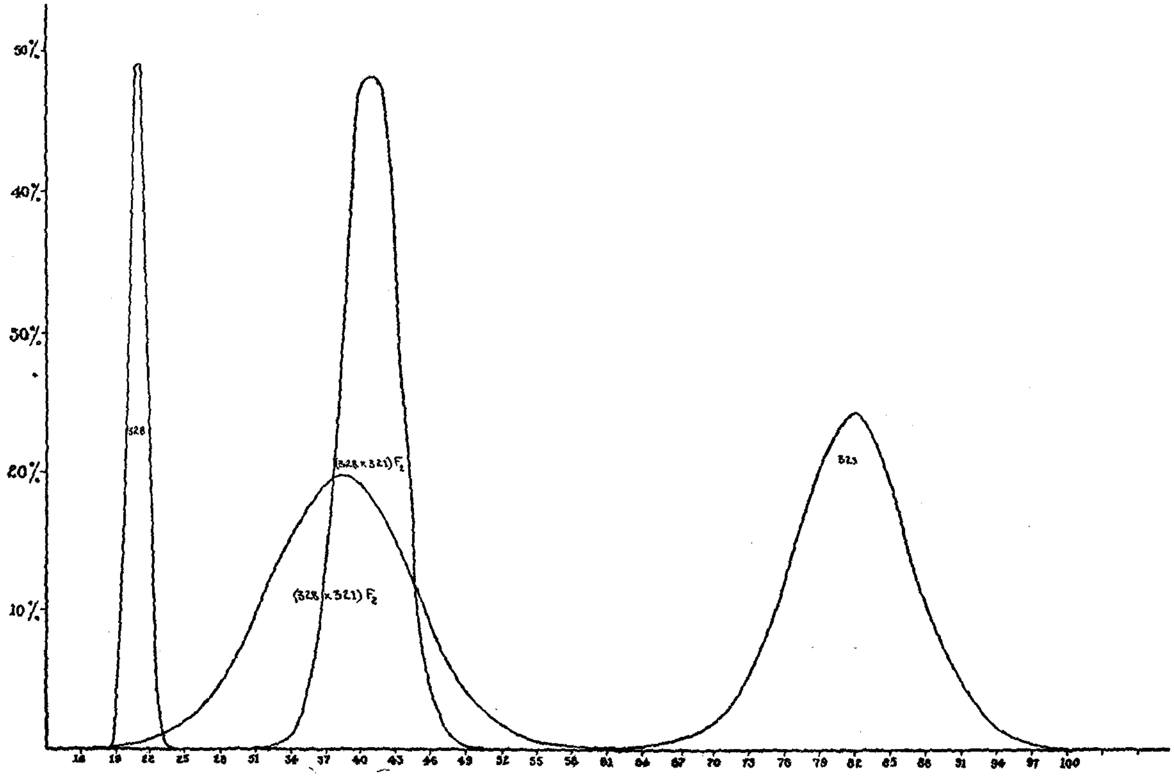

FIGURE 10. The theoretical curves of flower length for population of *N. Langsdorffii* (328), of *N. alata grandiflora* (321), and of the F_1 and F_2 of the cross 328 × 321. The curve of 328 cannot be compared with the others because it is shown in classes of one millimeter instead of classes of three millimeters.

TABLE 6

Frequency distribution of corolla length in the families of cross 321 × 328.

Pedigree Number	Size in parent	Class centers in millimeters																											No.
		16	19	22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	
328—1, (1910)			26	4																									30
328—1, (1912)		2	20	4																									26
328 - 1, total		2	46	8																									56
321, (1911)																					3	8	10	11	8	5	3	1	49
(321×328—1) F_1										8	21	2																	31
(321×328—1) F_2				1	0	9	16	21	53	25	23	6	6	3															163
(321×328—1)1—1 F_3	22	8	75	18																									101
(321×328—1)1—2 F_3	49								7	17	25	18	12	2															81
(321×328—1)1—3 F_3	51						1	2	4	8	30	31	19	6	1	2	0	1											105
(321×328—1)1—4 F_3	49								6	8	19	15	18	1															67

other, the slight differences between the two can not be attributed to the different way of making the cross. The cross with No. 321 as the mother was more difficult to make but this is probably due to the greater length of the style of No. 321.

Cross 321 × 328 is notable for the extreme uniformity of the first hybrid generation and the great increase in variability in the second hybrid generation, as is shown in tables 6 and 7. *N. Langsdorffii* was again reproduced in F_2 and plant (321 × 328) 1—1 bred true to its characters. There was no nearer approach to No. 321, however, than there was in cross 328 × 321. The cross appeared to be fully fertile and the seeds germinated well though in general not so perfectly as those of the reverse cross. It does not seem as if the slight infertility shown, however, could be the explanation of the failure to reproduce the larger parent.

TABLE 7

Statistical constants for the frequency distributions of corolla length shown in table 6.

Pedigree Number	Size in parent	Mean	S. D.	C. V.	No.
328 — 1, (1910)		19.40 ± .13	1.02 ± .09	5.26 ± .46	30
328 — 1, (1912)		19.23 ± .19	1.42 ± .13	7.38 ± .69	26
328 — 1, total		19.32 ± .11	1.23 ± .08	6.37 ± .41	56
321, (1911)		81.76 ± .49	5.08 ± .35	6.21 ± .42	49
(321 × 328 — 1) F_1		42.42 ± .19	1.60 ± .14	3.77 ± .32	31
(321 × 328 — 1) F_2		37.79 ± .28	5.36 ± .20	14.18 ± .54	163
(321 × 328 — 1) 1 — 1 F_3	22	19.30 ± .10	1.50 ± .07	7.77 ± .37	101
(321 × 328 — 1) 1 — 2 F_3	49	43.63 ± .28	3.73 ± .20	8.55 ± .45	81
(321 × 328 — 1) 1 — 3 F_3	51	45.34 ± .32	4.80 ± .22	10.59 ± .50	105
(321 × 328 — 1) 1 — 4 F_3	49	44.52 ± .32	3.85 ± .22	8.65 ± .50	67

Again the coefficients of variability of the four F_3 families grown are below that of the F_2 generation. Considering them together with the other later generations previously reported it would seem as if the case for Mendelian inheritance were pretty clearly proven.

CONCLUSION

A fertile cross between two distinct species, *Nicotiana Langsdorffii* and *Nicotiana alata grandiflora,* each uniform in its characters, has been reported here with the following results, no matter which way the cross was made.

1. The F_1 populations are as uniform as the parents.

2. The F_2 generations are nearly three times as variable as the F_1 generations.

3. Individuals reproducing the smaller species were found in the F_2 generation.

4. Certain of these F_2 individuals reproduced *N. Langsdorffii* populations in the F_3 generation.

5. No F_2 individuals reproducing *N. alata grandiflora* were found, but F_3 plants approaching such a type were produced.

6. Galtonian regression occurred, but selected extremes regressed no more than those deviating moderately from the parental mean.

7. Individuals from the same point on the F_2 curve showed different variabilities in F_3.

8. The variabilities of F_3 families were invariably smaller than those of F_2 families.

9. The above conclusions are based upon corolla length measurements but apparently are true for other characters, except that in other characters, *N. alata grandiflora* types were reproduced.

10. Mendelian inheritance of corolla color and pollen color is shown.

11. Mendelian inheritance seems to be the *only* logical interpretation of the other phenomena.

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